Central parasympathetic activation assessed by cardiac vagal motoneuron recordings in rat

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ACTIVATION PARASYMPATHIQUE CENTRALE MISE EN EVIDENCE PAR ENREGISTREMENT DES MOTONEURONES CARDIAQUES VAGAUX CHEZ LE RAT

CENTRAL PARASYMPATHETIC ACTIVATION ASSESSED BY CARDIAC VAGAL MOTONEURON RECORDINGS IN RAT

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Glossary of abbreviations

5HT	5-hydroxy-triptamine
Ach	Acetylcholine
ANS	Autonomic nervous system
AP	Arterial pressure
BJ	von Bezold-Jarisch reflex
BP	Blood pressure
BNP	Brain natriuretic peptide
CHF	Congestive heart failure
CNP	C-type natriuretic peptide
CNS	Central nervous system.
CVLM	Caudal ventrolateral medulla
CVM	Cardiac vagal motoneurons
DLH	DL homocysteic acid
DMN	Dorsal motor nucleus
HBP	High blood pressure, hypertension
HR	Heart rate
HRV	Heart rate variability
HRP	Horseradish peroxidase
MI	Myocardial infarction
NA	Nucleus ambiguus
NAvl	Ventrolateral nucleus ambiguus
NTS	Nucleus tractus solitarius
PNS	Parasympathetic nervous system
PBG	Phenyl biguanide
PTCA	Percutaneous transluminal coronary angioplasty
RSA	Respiratory sinus arrhythmia
RVLM	Rostral ventrolateral medulla
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
SUA	Single unit activity
VCPN	Vagal cardioinhibitory preganglionic neurons

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INTRODUCTION

The format of this document is not intended to provide a thorough coverage of the literature. Thus, many lapses will appear in this brief review. By contrast, an attempt has been made to provide the reader only with salient facts and key figures to help understanding the data. Legends will be provided accordingly.

Blood pressure regulation

Blood pressure (BP) and heart rate (HR) are fluctuating on a beat-by-beat basis. They are controlled, at different levels, by long-, short- and ultra-short-term regulations. Long-term regulation is ensured by the kidney, while short-term consists in sympathetic, renin-angiotensin-aldosterone and vasopressin systems (*AVP)*. Ultra short-term regulation is performed by the parasympathetic cardiac system (cardiac vagal activity). Ultra-short-term regulation involves two systems: *arterial baroreceptor – heart rate reflex (cardiac baroreflex)* and *the von Bezold-Jarisch (BJ) cardiopulmonary chemoreflex*.

A) The *cardiac baroreflex* is a key mechanism involved in BP regulation. The cardiac baroreflex is continuously active. The cardiac baroreflex can be ascribed as a regulation loop of a dynamic system: i). sensors i.e. the baroreceptors detect BP variations; ii). BP is the regulated variable to be maintained around a fixed value (set-point); iii). the minute changes in HR are the output of this regulatory system. HR rectifies the detected variations of BP. The cardiac baroreflex acts as an effective buffer of short-term BP fluctuations and prevents excessive BP swings (65).

B) The *BJ reflex* is activated in certain specific clinical and pathological conditions, like ventricular overstretch or myocardial ischemia[1]. Nerve endings located within the ventricular myocardium sense different substances released during cardiovascular emergency situations. By reducing BP and HR, the BJ reflex is presumed to have an unloading effect on the left ventricle.

BP regulation mechanisms dysfunction and therapy

BP lability: In mammals, BP level fluctuates around a mean BP level value (set point): the beat by beat changes in systolic BP (SBP) are called **BP variability**. HR is also variable. These spontaneous fluctuations on a beat-by-beat basis of BP and HR over time are called respectively **BP variability** and **HR variability**. BP variability and HR variability are related by an inverse relationship (39). The BP

[1] Myocardial infarction (MI) is a disease that occurs when the blood supply to a part of the heart is interrupted, causing death of myocardial tissue.

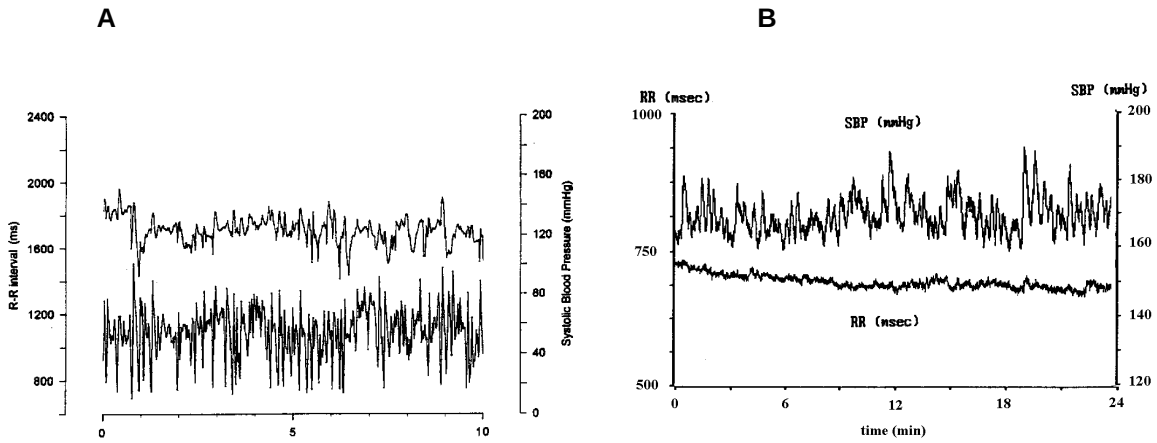


Figure 1. Pressure variability and sinus arrhythmia in a healthy volunteer and in a hypertensive patient

A. Raw traces of systolic blood pressure (SBP, top line) and RR interval (bottom line) of a healthy volunteer at rest. Note a SBP fine tuned around 120 mmHg and a large sinus arrhythmia. Sinus arrhythmias are beat-by-beat variations of HR, which are linked, at least partially, to ventilation ("respiratory sinus arrhythmia"). A key question is how to explain the large changes in HR, within one beat, given the minimal changes in pressure (see Perspectives and Appendix).

B. High blood pressure (BP) variability, or BP lability, in a hypertensive patient. Note that: i). the mean SBP (set point) is around 170 mmHg; ii). the SBP rises last many beats (breakthroughs) due to the ineffective cardiac baroreflex and/or prolonged sympathetic activation. Indeed, the sinus arrhythmia is suppressed, and thus the RR interval is almost constant ("fixed heart rate"). The mechanism that buffers the BP rises within one or two beats is defective. (Gratadour, unpublished data)

fluctuations represent normal BP variability. In pathological conditions, the amplitude of the BP variability is important, and is called **BP lability** (**figure 1**). Studies in the ambulatory setting show that outcome is linked to beat-by-beat BP lability, independently from the **mean** level of pressure (73; 120). BP lability is a phenomenon which is underemphasized despite its association with increased perioperative mortality (7). A possible mechanism for such an outcome is through *plaque* rupture. BP lability has also been demonstrated in awake–paralysed volunteers during attempts at voluntary efforts (42).

Cardiovascular diseases: The decrease in *c*ardiac baroreflex sensitivity is a characteristic feature of a number of cardiovascular diseases (15; 35; 92; 109). An altered baroreflex control of HR may carry an adverse prognosis in cardiac patients (81; 110). Increased sympathetic drive and reduced vagal activity are associated with reduced cardiac baroreflex sensitivity and carry a prognostic of poor outcome in cardiovascular disease (18; 81; 132). A low baroreflex sensitivity is associated with a high mortality after a myocardial infarction (MI) (81). The same association was observed in heart failure patients (102). Interventions that improve the sensitivity of the cardiac baroreflex, such as physical training, are known to reduce the risk of cardiovascular events (55; 57).

Emergence from anesthesia during the postoperative period may induce cardiovascular instabilities. Many of the circulatory modifications that occur during recovery from general anesthesia and surgery seem to result from sympathetic activation (44) and parasympathetic deactivation (113).

Parasympathetic drive to the heart: a common pathway for regulatory systems

The cardiac baroreflex and the BJ reflex share the same common projection that is responsible for beat-to-beat changes in HR: the vagal parasympathetic supply to the heart. This cardiac parasympathetic activity is also called cardiac vagal activity. Although there is no cause-effect relationship between cardiac vagal activation and successful therapy, cardiac vagal activation is considered as an index of successful treatment of cardiovascular disease (131). Although many studies have shown the benefits of reducing sympathetic activity in cardiovascular disease (58), activation of the vagal arm of autonomic nervous system has been under-exploited. However, efferent vagal stimulation can improve outcome after MI in dogs (132). Thus, enhancing vagal drive to the heart may be beneficial. Mechanisms that may increase vagal tone are therefore of interest for their therapeutic potential in cardiovascular disease and will be the core of this thesis.

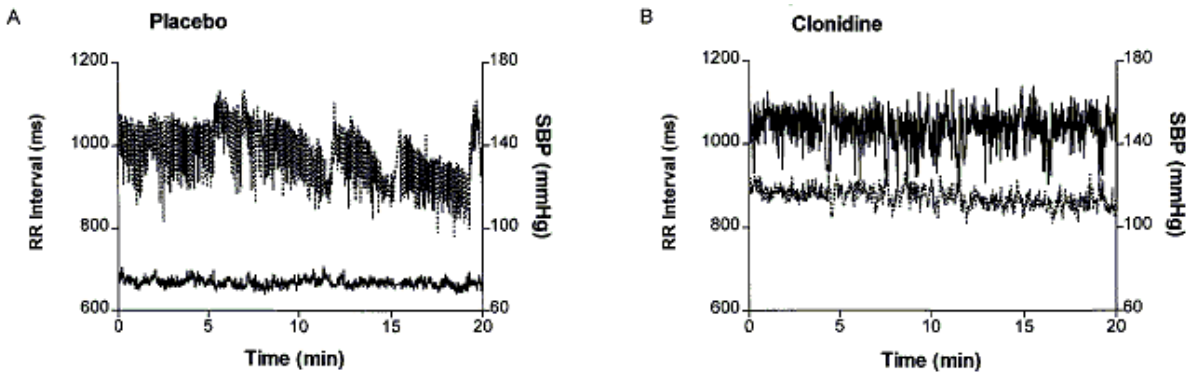


Figure 2. Pressure lability and sinus arrhythmia in the peri-operative setting, after placebo or clonidine

Raw traces of RR interval (solid line) and SBP (dotted line) in two representative hypertensive patients receiving placebo (A) or an α-2 agonist, clonidine (B). Data were recorded 3 h postoperatively after major abdominal surgery. The clonidine patient (B) exhibited a lower BP lability compared with the placebo patient (A).Note that HR variability is lower in the placebo patient, as compared to the clonidine patient. Figure adapted from Parlow (114).

Cardiac vagal motoneurones (CVM)

Although vagal actions on HR variability have been studied extensively at a peripheral level, there is little direct information about the behaviour of the neural structure responsible for cardiac vagal activation, the cardiac vagal motoneurons (CVM). This is especially true in rats, now favoured for invasive studies on the neural control of autonomic function (16). The limited availability of such information may be linked to the difficulty in obtaining *in-vivo* single-unit recordings from CVM (101). Indeed, CVMs are scattered in the external formation of the ventrolateral nucleus ambiguus (NAvl). Recent use of carbon-fiber electrodes increased the success rate of this experiment in rat (119) as opposed to conventional methods (101). Indeed, these electrodes "see" the neurone over a much larger area than conventional glass micropipettes, at the expense of a higher background noise. Thus, single unit activity (SUA) recordings of the CVMs done in rat demonstrated the possibility to assess the vagal parasympathetic supply to the heart (119).

Pharmacology

In the treatment of congestive heart failure (CHF) and other cardiovascular disorders, different approaches are available. Vasodilators are used in an attempt to decrease the BP and/or the ventricular load. However, vasodilators cause sympathetic activation and other compensatory processes that counteract the therapeutic goal. Therefore, using drugs that act directly on the regulatory reflex loop seems to be another approach to the management of cardiovascular diseases which may be closer to physiology. Thus, cardiac vagal activation may present an interest in cardiovascular therapy.

A centrally-acting agent and an α-2 agonist, *clonidine*, is used as an anti-hypertensive agent. Selective stimulation of α-2 adrenoceptors with clonidine elicits hypotension and enhances cardiac baroreflex gain in human studies (123) and in rabbits (46). However, other studies in rats (135) and in humans (104) suggested that clonidine does not improve the cardiac baroreflex sensitivity. Species differences and different study protocols including different anesthetics may contribute to this controversy. Different methods of data analysis (few seconds vs. minutes, HR vs. RR interval) might also be a source of discrepancy. Clonidine is also known to reduce the BP lability in hypertensive subjects. One hypothesis for this reduced BP lability is through increased HR variability (**figure 2,** (113)). The mechanism of action of clonidine on the CVM themselves is unknown.

In addition, the action of clonidine has been ascribed to imidazoline receptors in the RVLM (14). As this thesis restricts itself to the cardiac vagal activity but not to the sympathetic premotoneurons, this will not be further discussed.

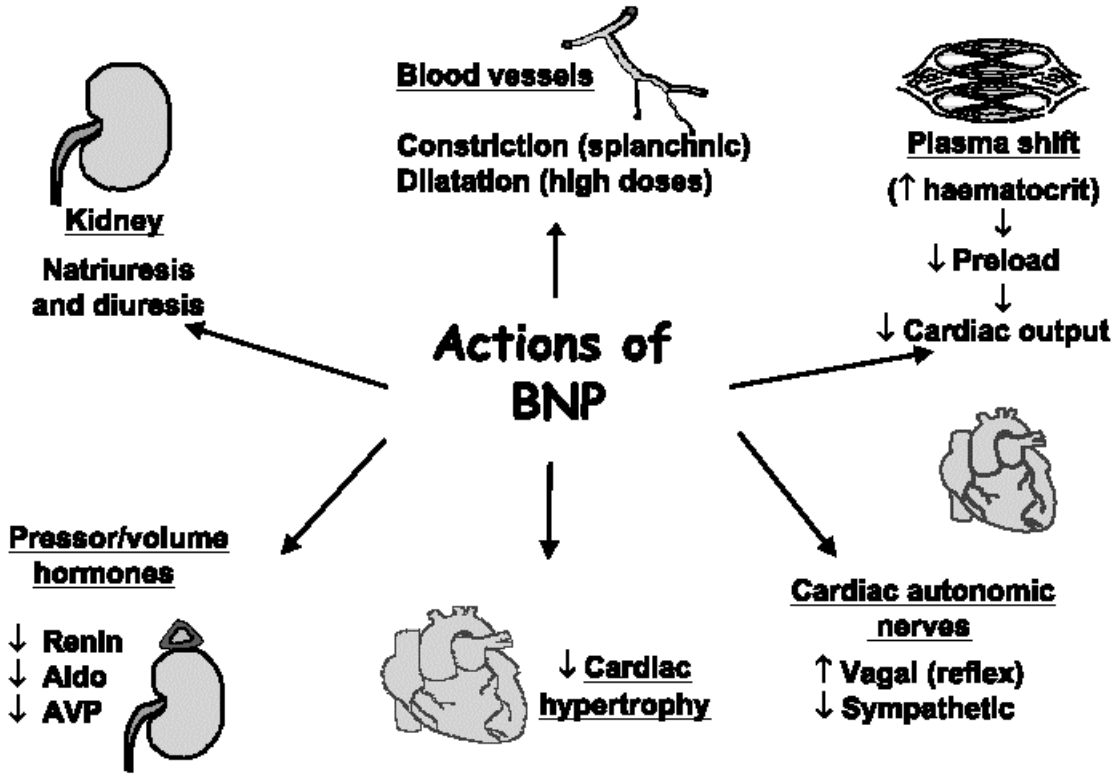


Figure 3. Schema of multiple synergistic cardioprotective actions of circulating brain natriuretic peptide

BNP main effects can be divided in three types: a) short-term action on the sympathovagal balance. BNP influences the autonomic control of the heart itself, by inhibiting the cardiac sympathetic nerve activity and enhancing the cardiac vagal activity (cf here: ms2); b) medium-term actions on circulation and hormonal status: i). renal natriuresis and diuresis; ii). inhibition of other pressor systems, i.e. renin-angiotensin-aldosterone system, vasopressin, catecholamines; iii). vasodilatation (at high doses or in CHF); iv). Plasma sequestration from the vascular compartment. c) Long-term action on the cardiac hypertrophy. Figure adapted from Woods (138).

AVP: arginine vasopressin; aldo: aldosterone.

The *cardiac natriuretic peptides* present actions which may be interpreted as cardioprotective (**figure 3**) (27). Indeed, brain natriuretic peptide (BNP, Neseritide®) has been proved clinically useful in the treatment of CHF (27). One of these presumed cardioprotective actions is to enhance bradycardic reflexes such as the BJ (cardiopulmonary) chemoreflex and the 'ramp' (cardiac) high pressure baroreflex. This action has been shown in several species. Other natriuretic peptides are also active in this respect. However, BNP is the most potent (53; 130). However, despite the fact that BNP is believed to improve the cardiac vagal activity, the site of action of BNP is not yet deciphered.

Thus, both clonidine and BNP improve two of the cardiovascular protective reflexes, the BJ reflex and the cardiac baroreflex respectively. The present study analyses the action of clonidine and BNP on the cardiac vagal activity. This approach opens the baroreceptor-HR reflex loop, allowing us to distinguish mechanisms of action before and after its efferent arm. Using the latest methods of CVM activity recording in rat (119), the present study will focus on the influence of these substances on the cardiac vagal supply to the heart, the CVM.

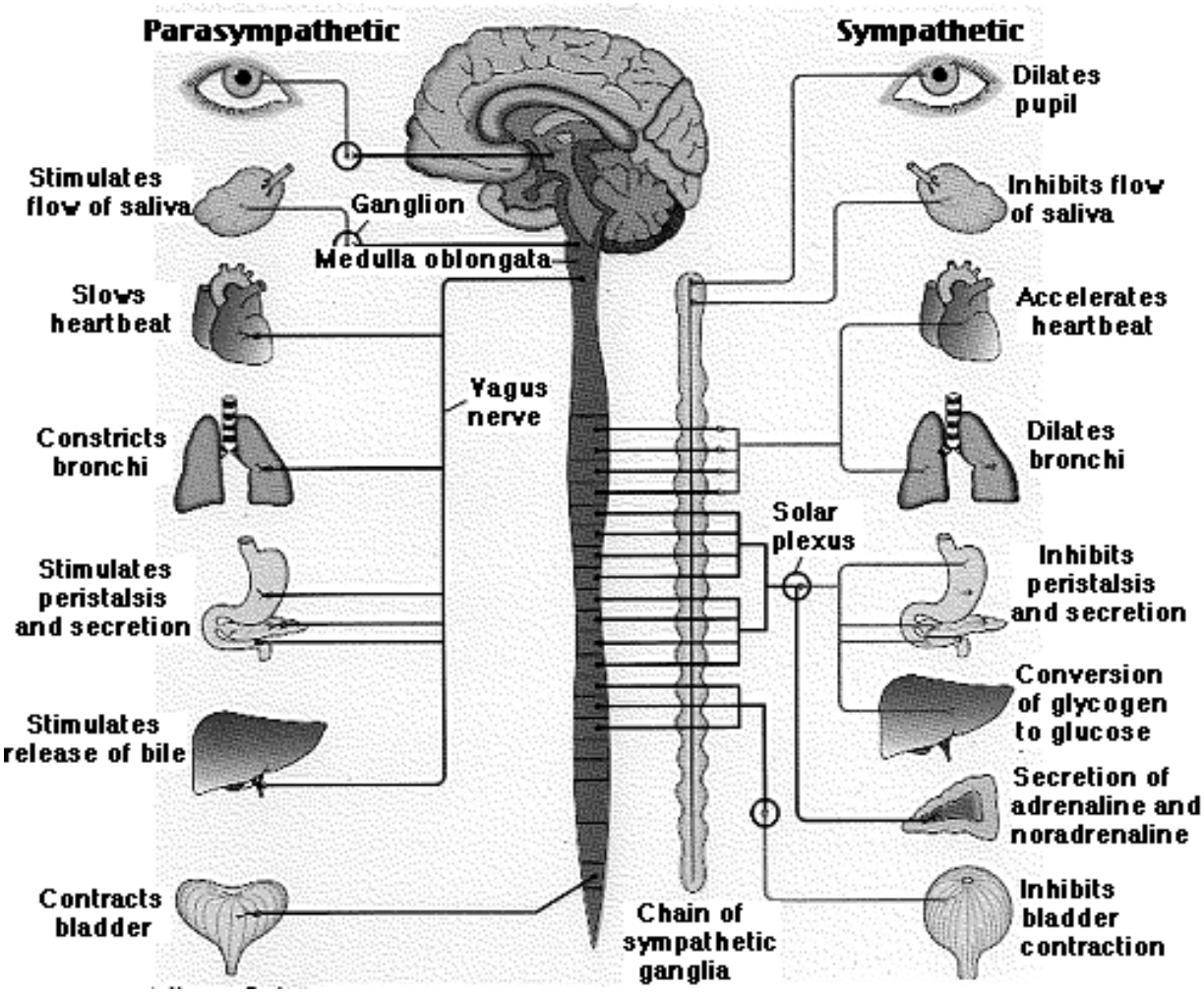


Figure 4. Sympathetic and parasympathetic divisions of the autonomic nervous system

Sympathetic (right) and parasympathetic (left) systems project to all organs. Preganglionic motor neurons are located in the central nervous system: i) with respect to the sympathetic system within the spinal cord and ii) with respect to the parasympathetic system within the brain stem. The efferent sympathetic and parasympathetic pathways from the preganglionic motor neurons to the target organs, are *disynaptic*: a synapse in the autonomic ganglion is interposed between the preganglionic motor neuron in the central nervous system and the target organ in the periphery. In the parasympathetic system, autonomic ganglia are very close to visceral targets, or imbedded in them (e.g. cardiac ganglion apposed to atrium). Figure adapted from http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/autonomic.gif.

CARDIAC CONTROL BY THE AUTONOMIC NERVOUS SYSTEM

I. The autonomic nervous system

The autonomic nervous system (ANS) is a visceral and largely involuntary motor system that controls smooth muscle, heart muscle, and exocrine glands. The body is regulated by the ANS towards a steady state. This process is called *homeostasis.* ANS has three major divisions: sympathetic, parasympathetic and enteric (33). The enteric system, which innervates the gastrointestinal tract, the pancreas, and the gall bladder, is of no interest to this study. Sympathetic and parasympathetic systems have a primary role in adapting the internal parameters to changes in the external or internal environment. Sudden changes, such as physical exercise, temperature variation, emotional changes, blood loss etc. generate changes within the body. Upon emergency situations, the sympathetic system increases the cardiac output, alters the body temperature, dilates the pupils, in order to respond to potentially disturbing external conditions. This adaptation to the environment conditions via the sympathetic system is the nervous output of the “stress response”, also called *the fight and flight reaction (defence reaction)*. In contrast, the parasympathetic system is called upon under normal conditions to regulate internal parameters. Maintenance of basal HR, respiration, and metabolism through negative feedback regulation is called *rest and digest reaction*.

The efferent pathway of both sympathetic and parasympathetic systems is *disynaptic* (33). A first set of neurones, located within the central nervous system (brain stem nuclei and spinal cord), project their axons on the autonomic ganglia (**figure 4**). The sympathetic ganglia form a chain parallel to the spinal cord, while the parasympathetic ganglia are embedded within the target organs.

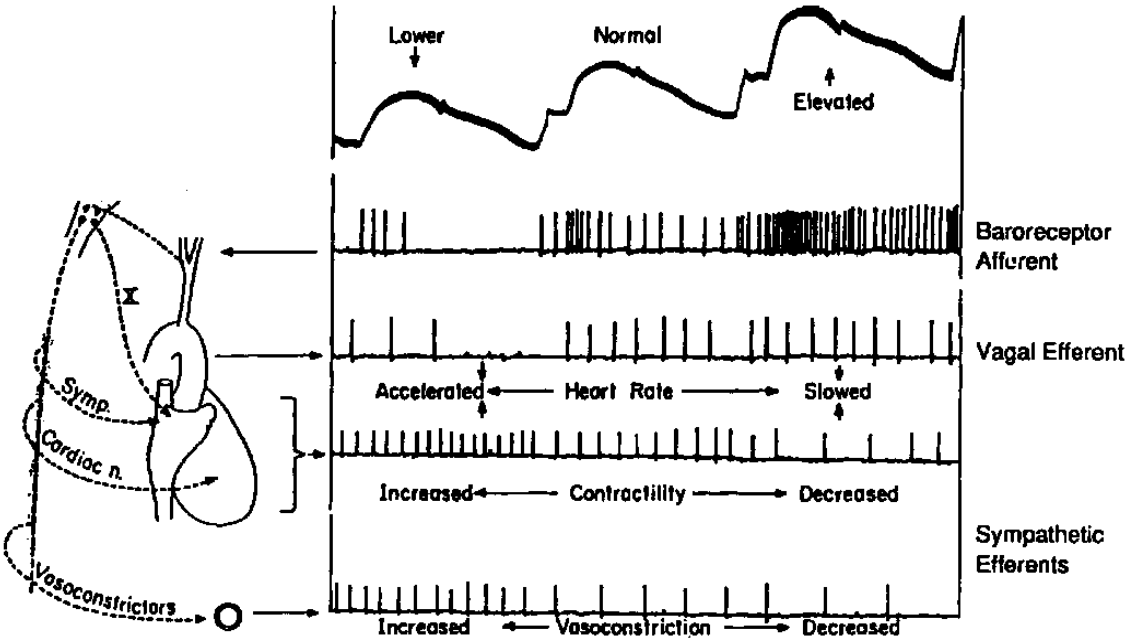


Figure 5. Sympathetic and parasympathetic components of the cardiac and vasomotor baroreflex

Baroreceptors transduce BP variations through the afferent pathways toward the central nervous system. Increases in BP result in the increase of the afferent baroreflex input, which leads to an increase of the parasympathetic limb and a decrease in the sympathetic limb. The efferent parasympathetic limb has an *active* and fast bradycardic effect on the heart (within one beat in healthy humans). The efferent sympathetic limb has two components: the cardiac component and the vascular component. Increases in BP lead to a decrease of the cardiac and vascular sympathetic efferent traffics. The decrease of the cardiac sympathetic outflow decreases the HR and contractility. The decrease of the vascular sympathetic outflow generates a *passive* vasodilatation, which is a slow process and decreases vasomotor tone. Consequently, increases in BP lead to i) a decrease of the sympathetic outflow, ii) a passive slowing of the heart, iii) a passive vasodilatation and thus, iv) to a decrease of the BP. Figure reproduced from Spyer (125).

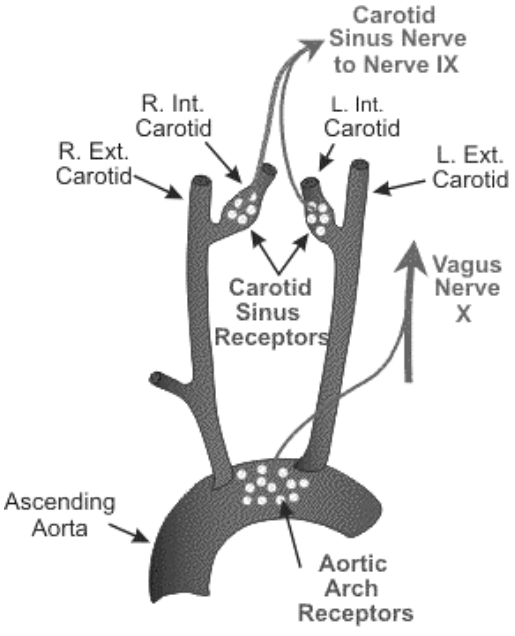


Figure 6. Location of arterial baroreceptors

Arterial baroreceptors are located in the carotid sinus, at the bifurcation of external and internal carotids, and in the aortic arch. The carotid sinus nerve, a branch of the glossopharyngeal nerve (IXth cranial nerve), innervates the carotid sinus. The carotid sinus nerve synapses at the level of the NTS. The aortic arch baroreceptors are innervated by the aortic depressor nerve (ADN), which then travel closely apposed to the vagus nerve (Xth cranial nerve) to the NTS. Arterial baroreceptors are sensitive to the stretching of the walls of the vessels in which the nerve endings lie. Increased stretching augments the firing rate of the receptors and nerves (figure II.1), and recruits additional afferent nerves. Figure adapted from http://www.cvphysiology.com /Blood%20Pressure/bp012%20aroreceptor%20anat.gif.

II. The baroreflex loop

The cardiovascular division of the ANS regulates the BP, which is not constant over time. Thus, i) occupational factors as emotion, physical exercise, sleep, etc., ii) cardiac and vascular modification exerted by the baroreflex and cardiopulmonary reflex, iii) mechanical factors as the ventilation and iv) thermoregulatory factors, evoke continuous fluctuations of the BP and of the HR. To each spontaneous BP variation, a reflex change in the cardiac and vascular functions generates an opposite BP variation. This is called *negative feedback regulation*. The BP regulation is performed either by modifying the HR, through the *cardiac baroreflex*, or by adjusting the vasomotor tone, through the *vasomotor baroreflex* (**figure 5**). The vasomotor baroreflex is mediated by the sympathetic system and will not be further considered. The cardiac baroreflex loop is divided in three parts: afferent, central and efferent.

A. The *afferent* pathways are common to both sympathetic and parasympathetic limbs of baroreflex. Changes in BP are sensed by *mechanoreceptors*, also called *baroreceptors*. These receptors detect changes in stretch (33). Baroreceptors are tonically active. A rise in BP activates the baroreceptors. An increase in the baroreceptor activity results in increased baroreflex afferent traffic. Baroreceptors are divided into two categories, **high pressure mechanoreceptors** and **low pressure mechanoreceptors**, which are respectively localized either in a *high* or *low* pressure zone of the circulatory system.

High pressure baroreceptors are located mainly on the carotid sinus and on the aortic arch (**figure 6, 7**). Some high pressure mechanoreceptors are also located in ventricles (93) and, possibly, the coronary arteries. The *high pressure ventricular baroreceptors* are activated upon large and fast pressure changes (more than 50-60 mmHg). By contrast, carotid and aortic baroreceptors are activated by minimal pressure changes (a few mmHg).

Low pressure baroreceptors, also known as atrial receptors, are found mainly in the walls of the left and right atria. They are also extending in the large vessels attached to them. The low pressure baroreceptors monitor blood volume and have two main actions: i) inhibition of vasopressin release by the neurohypophysis; ii) specific inhibition of renal sympathetic nerve activity. Stretching the atria also releases ANP, directly from the atrial myocytes. However, ANP's effect as a circulating hormone is weaker than the effect of the neural pathway from atrial receptors.

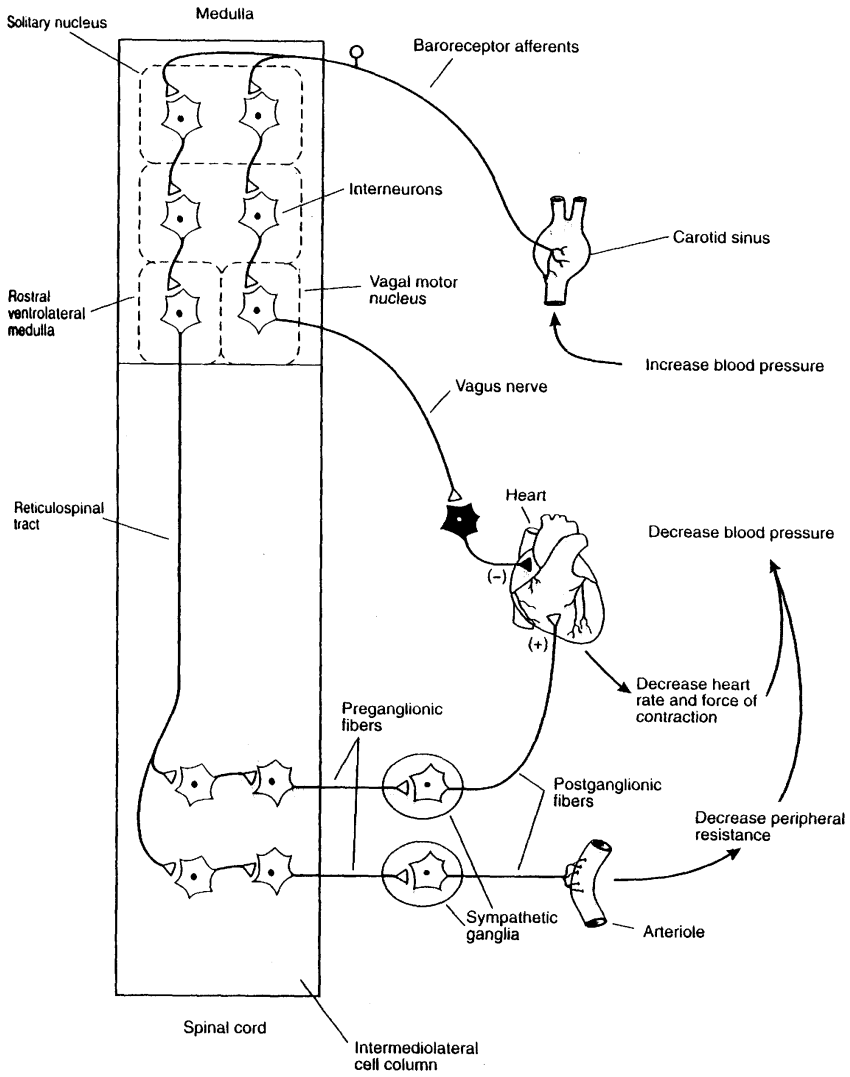


Figure 7. The cardiac and vasomotor baroreflex

The cardiac baroreflex loop is composed of three parts: afferent, central and efferent. At the efferent level, the baroreflex loop is composed of two limbs: sympathetic and parasympathetic. By extension and for simplicity, at the central level, the cardiac and vasomotor baroreflex arc will be considered also as composed of the sympathetic and parasympathetic central limbs. Changes in BP are sensed by baroreceptors. Afferent baroreflex information is sent from baroreceptors to the central nervous system. The central parasympathetic pathway is composed of i) the NTS and ii) the motor preganglionic neurons (or preparasympathetic motoneurons) in the ventrolateral nucleus ambiguus (NAvl; external formation of the NA), called *cardiac vagal motoneurons* (CVM). The efferent parasympathetic pathway is represented by the axons of CVM. These axons project through the vagus nerve (X). CVMs project to the cardiac ganglion located in close apposition to the atrium. The postganglionic neurons innervate the heart. The central sympathetic pathway is composed of i) the NTS, ii) the neurons in the CVLM, iii) the neurons in the rostral ventrolateral medulla (RVLM barosensitive bulbospinal neurons: RVLMbb neurons, sympathetic premotor neurons or presympathetic mortoneurons) and iv) the preganglionic motor neurons in the spinal cord. Beginning from the level of the motor preganglionic neurons and continuing at the efferent level, the sympathetic baroreflex limb is split in two divisions: cardiac and vascular. Both cardiac and vascular sympathetic preganglionic fibers project to their postganglionic motor neurons. The sympathetic postganglionic cardiac motor neurons project to the heart. The sympathetic postganglionic vascular motor neurons project to the vessels. Figure adapted from Kandel (33).

Afferent *arterial high pressure baroreceptor* information is sent to the central nervous system (CNS) through: i) the aortic depressor nerve, which projects toward the brain next to the vagal nerve (X), for the aortic baroreceptors and ii) the carotid sinus nerve which merges with the glossopharyngeal nerve (IX), for the carotid baroreceptors. Afferent *low pressure baroreceptor* information is sent through the vagus nerve.

B. The *central* core of the *baroreflex* loop is located in the *medulla oblongata* (**figure 7**). The sympathetic and the parasympathetic circuitries of the cardiac baroreflex are distinct within the CNS. Sympathetic and parasympathetic baroreflex pathways segregate already at the level of the NTS (115). As this work restricts itself to the ventrolateral part of the medulla, the physiology and anatomy of the NTS will not be delineated. Most investigators consider the ventrolateral medulla, including the NA external formation, as the final integrative structures of the PNS and SNS systems.

1). The **central sympathetic pathway** begins within the nucleus tractus solitarius (NTS). Neurons within the NTS project to the rostral ventrolateral medulla (RVLM) by two pathways. The first pathway is made of direct inputs (1). This direct pathway actually does not carry baroreflex, but other reflex signals (29). The second pathway is indirect and mediate baroreceptor reflex signals. It includes an excitatory projection from the NTS to the caudal ventrolateral medulla (CVLM). The CVLM exerts an inhibitory effect over the RVLM (3). Thus, through the second pathway, the NTS exerts an inhibitory effect on the RVLM (4). The RVLM is the location of sympathoexcitatory neurons (sympathetic premotoneurons, presympathetic neurons, RVLM barosenstive bulbospinal neurons) which control the activity of sympathetic preganglionic neurons in the intermediolateral nucleus of the spinal cord (*tractus intermediolateralis*) (51). Neurons from RVLM innervate the motor preganglionic neurons in the spinal cord via the reticulospinal tract. At the level of motor preganglionic neurons, the sympathetic baroreflex limb is already split in two subdivisions (**figure 7**). One subdivision sends efferent innervation to the heart. This subdivision is called *cardiac*. The other subdivision innervates the vessels by multiple efferent pathways, each innervating the blood vessels in a different tissue. This subdivision is called *vascular*. Both cardiac and vascular motor preganglionic sympathetic neurons are tonically active. An increase in BP results in a decrease of the cardiac and vascular sympathetic activity at the level of motor preganglionic neurons (**figure 5, 8**).

2). The **central parasympathetic pathway** also begins with the NTS (**figure 7**). Neurons within the NTS project to motor preganglionic neurons in the ventrolateral nucleus ambiguus (NAvl), also called the external formation of the NA. These motor preganglionic neurons are called *cardiac vagal motoneurons* (CVM) or *vagal cardioinhibitory preganglionic neurons* (VCPN). CVM are tonically active. An increase in BP results in an increase of CVM unit activity (**figure 5**).

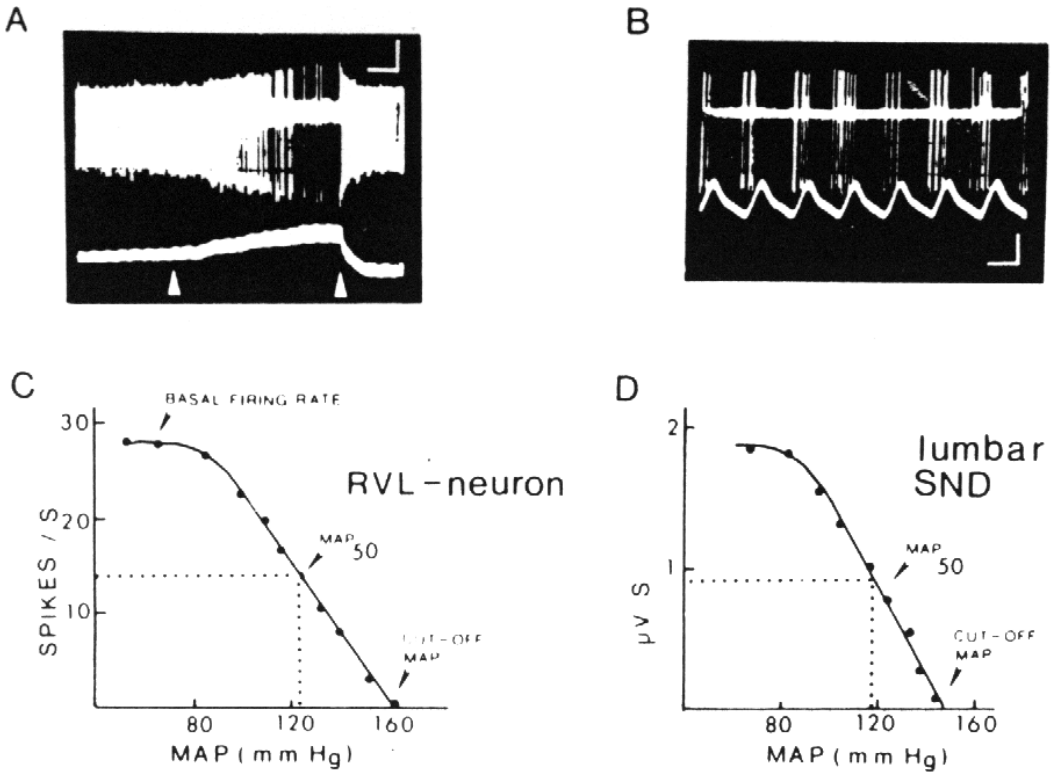
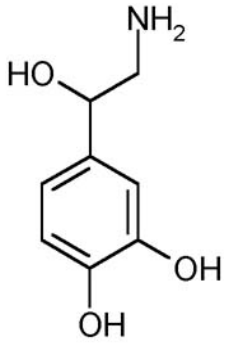


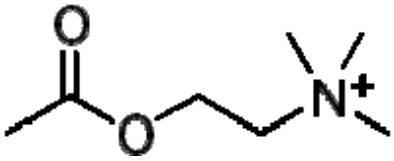
Figure 8. Properties of RVLM presympathetic vasomotor and cardiac neurons

Unit recordings of neuron identified as reticulospinal by antidromic stimulation from the thoracic spinal cord (conduction velocity: 4m/s). A. Baroreflex inhibition of neural discharges. AP (lower trace) was gradually increased by restricting flow through descending aorta (between arrows) resulting in baroreceptor activation and time-locked inhibition of unit discharge (calibration: 0.2mV, 2 s). B. When examined at a faster time scale, the unit activity is distinctly pulse synchronous due to phasic nature of arterial baroreceptor discharges relayed multisynaptically to the rostral medulla (calibration: 0.5mV, 120 ms). C. Relationship between mean discharge rate of vasomotor neuron and MAP. Note the high basal firing rate below the baroreceptor threshold, linear reduction thereafter, and abrupt well-defined cut-off pressure beyond which the cell is silent. This cut-off does not represent the saturation of baroreceptor inputs but the point at which this input hyperpolarizes the cell below firing threshold. The cut-off MAP thus depends on the presence or absence of excitatory inputs to these neurons and can be raised by hypothalamic stimulation for example. D. the lumbar sympathetic nerve discharge in the same preparation exhibits the same relationship to MAP as that of RVLM neurons. Figure reproduced from Guyenet (51).

C. *Baroreflex efferents*: The **efferent sympathetic pathways** are divided in cardiac and vascular pathways (**figure 7**). Both cardiac and vascular efferent preganglionic fibers project to their postganglionic motor neurons. The cell bodies of sympathetic postganglionic motor neurons are located in the autonomic sympathetic ganglia. The sympathetic postganglionic motor neurons release a catecholamine, *norepinephrine* (noradrenaline, 4-(2-Amino-1-hydroxyethyl)benzene-1,2-diol, [$C_8H_{11}NO_3$]). The sympathetic postganglionic cardiac motoneurons project to the heart, where norepinephrine increases the HR and contractility. This effect of norepinephrine is mediated through the *β-adrenergic receptors* and intracellular increase of the Ca^{2+} current into the myocardial muscle cells. The sympathetic postganglionic vascular motor neurons project to the vessels, where norepinephrine provokes a vasoconstriction, i.e. increases the vasomotor tone[2] through the *α-adrenergic receptors*. An increase in BP reflexly generates decrease of the cardiac and vascular sympathetic outflows. The decrease of the cardiac sympathetic outflow occurs very quickly (150ms approx). However, sympathetic nerve actions on the heart are slow (5-10s) and consist in a *passive* i) slowing of the heart and ii) decrease of the heart contractility. The decrease of the vascular sympathetic outflow results in a *passive* vasodilatation (2-3s delay). The decrease of the HR and contractility on one hand and the vasodilatation on the other hand, lead to a passive and slow decrease in BP.



Noradrenaline



Acetylcholine

The **efferent parasympathetic cardiac pathway** is represented by the axons of CVM. These axons project through the vagus nerve (**figure 7**). CVM project to the cardiac ganglion, that is embedded in the fat of the right atrium. The postganglionic neurons innervate the heart and release acetylcholine (Ach, 2-acetoxy-N,N,N-trimethylethanaminium, [$C_7H_{16}NO_2$]). Ach slows the heart. This effect is

[2] Vasomotor tone cannot be measured. To approach quantitatively the vasomotor tone, the systemic vascular resistance is calculated using the formula R=P/Q, which supposes a non pulsatile flow.

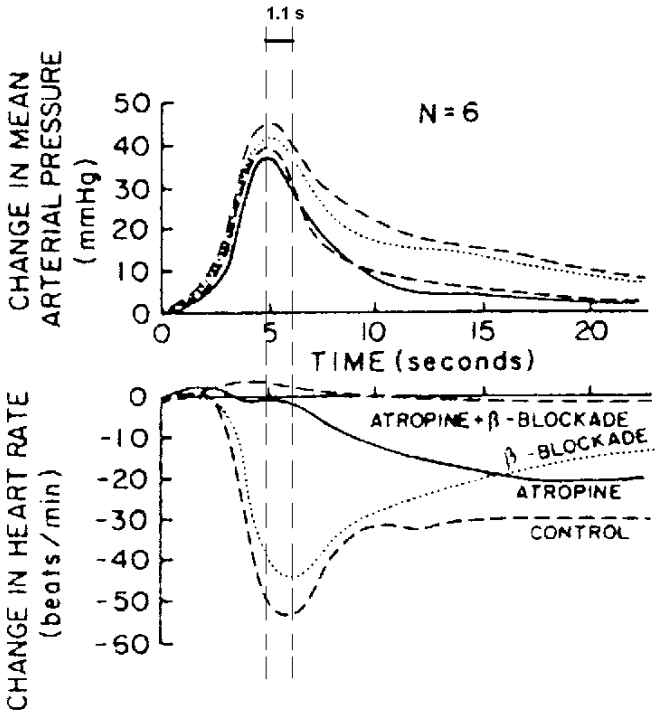


Figure 9. Time course of BP and HR changes following pressure rises in rats

The HR response (bottom) in the untreated group (control) reflects the baroreflex reaction of both sympathetic and parasympathetic limbs. In another group (β-blockade), β-adrenergic blockade was performed with a β-blocker, propanolol. In this group, the sympathetic limb of the cardiac baroreflex was inhibited. Thus, by a subtractive logic, the HR response in the β-blockade group represents the time course of the parasympathetic limb of the cardiac baroreflex only. The reaction of the parasympathetic limb of the baroreflex is rapid. Indeed, the delay between the *maximum* in BP and the *maximal* bradycardia is circa 1.1 s. In the atropine group, the parasympathetic limb of the cardiac baroreflex was inhibited. Thus, the HR response in the atropine group represents the time course of the sympathetic limb of the cardiac baroreflex only. The reaction of the sympathetic limb of the baroreflex is slower. Indeed, the delay between the maximum BP and the greatest change in the HR is circa 14.5 s. This figure also shows that in the atropine group the beginning of the bradycardia was observed after more than 5 s following increases in BP. In the last group (atropine+β-blockade), both sympathetic and parasympathetic limbs of the cardiac baroreflex were inhibited with propanolol and atropine. In this group, atropine+propanolol, the HR response was minor. This suggests that the HR response to short term BP responses is primarily under the parasympathetic, but not sympathetic control. Figure adapted from Coleman (24).

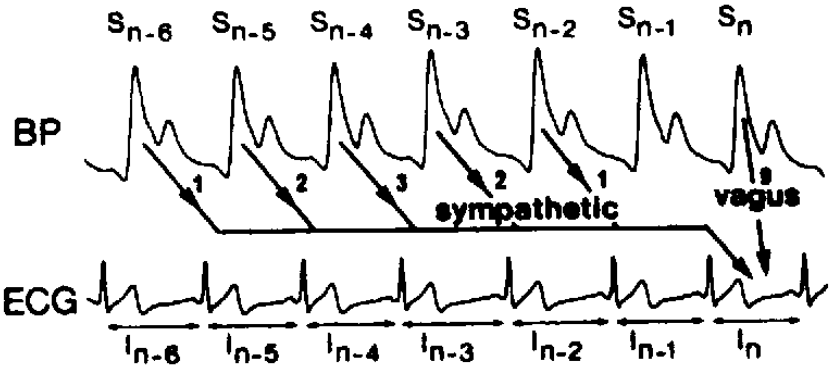


Figure 10. Control of pulse interval by parasympathetic and sympathetic systems

The pulse interval I_n is affected by the systolic pressure S_n through the rapid effect of the parasympathetic system (vagal activity). The pulse interval I_n is also affected by the systolic pressures $S_{n-2},\ldots,S_{n-6}$, through the slow effect of the sympathetic system. Figure reproduced from DeBoer (30).

mediated via the M2 subtype of *muscarinic* receptors (**table1)**. The muscarinic receptors are located in the cardiocytes of the sinoatrial and atrioventricular nodes, and mediate Ach-induced increase of the resting K^+ conductance. An increase in BP results in the increase of the parasympathetic outflow. The increase of the parasympathetic outflow is *fast* and results in an *active* i) slowing of the heart and ii) mostly an indirect decrease of heart contractility. The decrease of the HR and contractility lead to an active and *fast* decrease in the BP.

Table 1. Agonists and antagonists of muscarinic and nicotinic receptors

Receptor type	Agonist	Antagonist
M2 muscarinic	Ach	atropine
nicotinic	Ach, nicotine	trimetaphan

In humans, the maximum bradycardia due to the parasympathetic drive is observed within 250 ms-2 s after the peak in BP (12; 34; 86). Similarly, in rats, the maximum bradycardia due to the parasympathetic drive is observed circa 1.1 s after the peak in BP (**figure 9**) (24). In humans, the maximum bradycardia due to the sympatho-inhibition is observed more than 2 s after the peak in BP (12; 86). In rats, the maximum bradycardia due to the sympathetic drive is observed more than 5 s after the peak in BP (24). Thus, the baroreflex parasympathetic reaction is much faster than the baroreflex sympathetic reaction (1.1s vs. 5s in rats). *In-vitro* studies on clusters of ventricular muscle cells showed that response of the heart to Ach is faster than to adrenaline (250ms vs. 3-6s, minimal latency) (54). This suggests that the difference in latency between parasympathetic and sympathetic actions on the heart is due to a delayed effect of neurotransmitters at the level of the cardiac ganglion. Thus, due to a delayed effect of the sympathetic limb of the cardiac baroreflex, a pulse interval at a certain moment is the result of: i). the previous pulse pressure that stimulate the parasympathetic system, and ii). of several previous pulse pressures that influence the sympathetic cardiac baroreflex (**figure 10**).

Thus, the **baroreflex loop** transforms a spontaneous variation in the BP in an opposite variation of HR. In turn this change in HR leads to an immediate change in BP given a relatively rigid *Windkessel* and a sluggish sympathetic response. This feedback regulation of the BP is mediated through both sympathetic and parasympathetic limbs. In a situation where the BP falls (**figure 11**) the baroreceptor afferent traffic to the NTS decreases. Increased cardiac and vascular sympathetic outflows are coordinated from here, together with a decreased parasympathetic activity. The decrease of the parasympathetic outflow results in a *passive* i) acceleration of the heart and ii) small increase of the

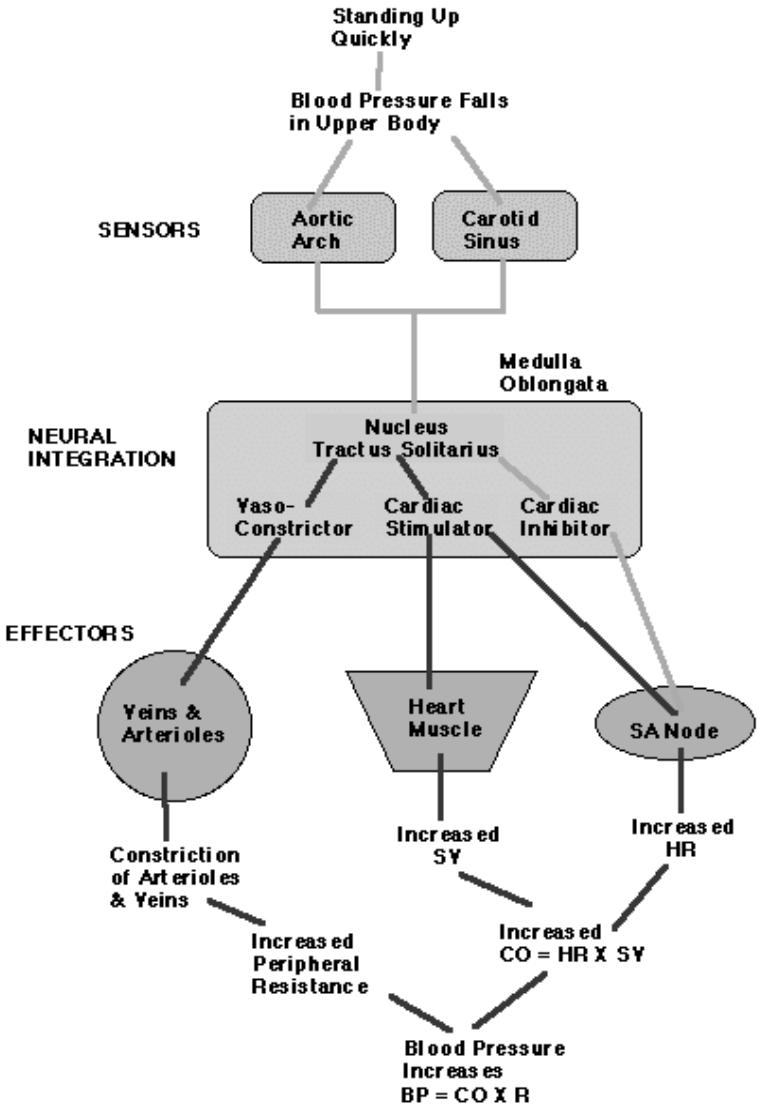


Figure 11. Baroreflex engagement upon pressure fall

When the BP falls in upper body, the baroreceptor output to the NTS decreases. From here, the parasympathetic system is inhibited and the sympathetic system stimulated. Thus, the specific effectors increase the HR and contractility, generating a greater cardiac output. In parallel, the sympathetic vasomotor limb of the baroreflex mechanism constricts the veins and arterioles, increasing the vasomotor tone. Increased cardiac output and peripheral resistance result in a rise in BP. In this way, the baroreceptor reflex is the rapid response system for dealing with changes in BP. Grey lines: inhibitory effects; black lines: excitatory effects. Figure adapted from http://members.aol.com/Bio50/LecNotes/LNPics/ln21g.gif.

heart contractility. The increase of the cardiac sympathetic outflow results in an *active* i) acceleration of the heart and ii) increase of the heart contractility. The increase of the HR and contractility leads to an increased cardiac output. The increase of the vascular sympathetic outflow results in an active vasoconstriction. Thus, the vasomotor tone is increased. The increase of the cardiac output on one hand and of the vasomotor tone on the other hand lead to an increase of the BP (**figure 11)**.

III. The von Bezold-Jarisch reflex

Activation of cardiopulmonary chemosensitive vagal afferent fibers originating in the heart and lungs by injection of substances such as veratrum alkaloids, serotonin, phenyl biguanide (PBG) and some prostaglandins produces profound and simultaneous reductions in arterial BP and HR and apnea (25; 78). This phenomenon was first described by von Bezold and Hirst in 1867 and characterized further by Jarisch and Richter in 1940. This reflex is known as the von Bezold-Jarisch reflex.

The coronary chemoreflex **afferents** are located mostly on the nerve endings within the left ventricular wall. Other chemoreceptors participating in the BJ reflex are situated in the right ventricle, atria and great vessels (great veins, pulmonary artery, and aorta), but these are less numerous. The afferent fibers of the chemosensitive receptors are unmyelinated C-fibers (**table 2**) that travel in the vagus nerve.

Table 2. Conduction velocities of different fibers

Type	Type	Conduction velocity
A	myelinated	~50 m/s
B	myelinated	Some m/s
C	unmyelinated	< 1 m/s

The **central** neurocircuitry that mediates the BJ reflex is not well known. The chemosensitive afferent vagal fibers project in the NTS (68). Some connection with the NA was also found (84). Electrolityc lesioning of these structures abolished the hypotensive and bradycardic components of the reflex (84). Neurons in the CVLM have also been found to have a role in the BJ reflex. These findings suggest that the central core circuitry of the cardiovagal and sympathetic components of the BJ reflex is similar to the baroreflex.

The same similarity is found also for the **efferent** side of the BJ reflex, namely the cardiac branch of the vagus.

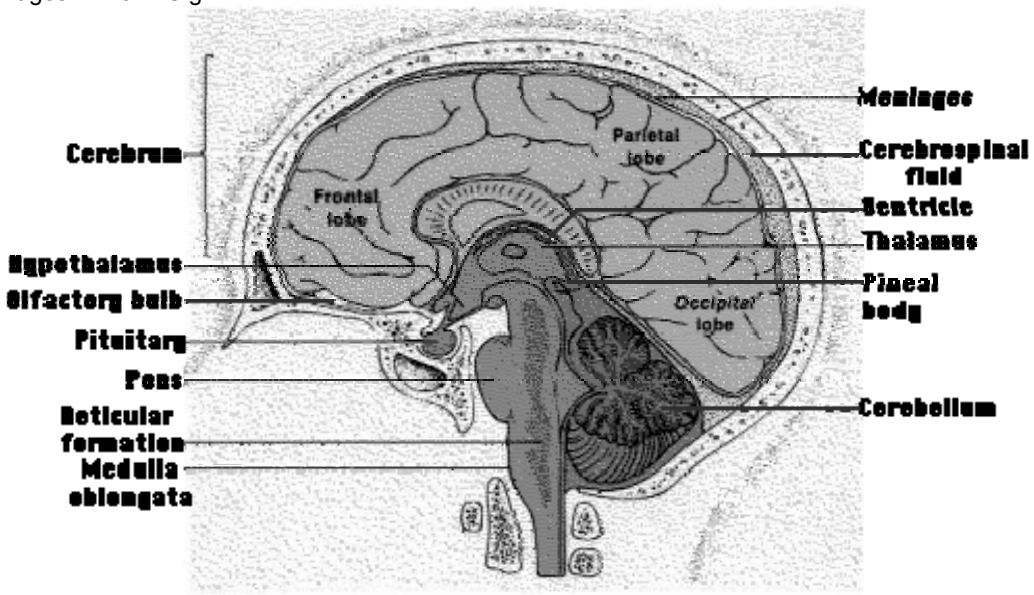
The physiological role of the BJ reflex is elusive (133). On one hand, BJ reflex is activated in specific situations during which there is ventricular overstretch, like in heart failure[3](93). On the other hand, coronary chemosensitive fibers are activated during myocardial ischemia and by certain agents that were used as radiocontrast media for coronary angiographic studies. A more recent hypothesis states that cardiopulmonary reflexes are defense mechanisms against chemical inhalants. Thus, cardiopulmonary reflexes serve initially to reduce the degree of inspired pollutant absorbed in the blood, then protect the vital organs from the potential toxicity of absorbed pollutant, and finally facilitate the elimination and deactivation of pollutant.

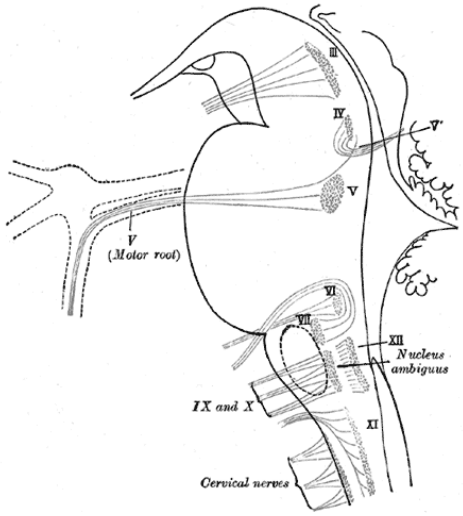
Elucidating the effects of the BJ reflex on the circulatory regulation would be useful for better understanding the physiology of various pathologies such as ischemic heart disease and congestive heart failure.

[3] Heart failure (CHF) is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump a sufficient amount of blood throughout the body.

Figure 12. Location of the *Medulla Oblongata* and Nucleus Ambiguus within the CNS, sagittal view in humans

A. The Medulla Oblongata, or *spinal bulb*, extends from the lower margin of the pons to a plane passing transversely below the pyramidal decussation and above the first pair of cervical nerves. At this level the medulla oblongata is continuous with the *medulla spinalis*. The anterior/ventral surface of the medulla oblongata is separated from the basilar part of the occipital bone and the upper part of the odontoid process by the dura mera of the brain and the occipito-axial ligaments. The posterior/dorsal surface of the medulla oblongata is received into the fossa between the hemispheres of the cerebellum. The upper portion of the medulla oblongata forms the lower part of the floor of the fourth ventricle. Figure adapted from http://users.rcn.com/jkimball.ma.ultranet /BiologyPages/B/BrainLS.gif.





B. The medulla oblongata is pyramidal in shape. The broad extremity of the medulla oblongata is directed upward toward the pons. Its narrow, lower end is continuous with the spinal cord. It measures about 3cm in length, about 2cm in breadth at its widest part, and about 1.25cm in thickness in humans. The central canal of the medulla spinalis is prolonged into its lower half, and then opens into the cavity of the fourth ventricle (junction of central canal and fourth ventricle: obex as opposed to calamus scriptorius). The medulla oblongata may therefore be divided into a lower *closed part* containing the central canal, and an upper *open part* corresponding with the lower portion of the fourth ventricle. Nuclei of origin of cranial motor nerves are schematically represented. The **NA** is a region of histologically disparate and scattered cells located just dorsal/posterior to the inferior olivary nucleus in the lateral portion of the rostral medulla. The NA gives rise to the efferent motor fibers of the vagus nerve (X), as well as to the efferent motor fibers of the glossopharyngeal nerve (IX). Figure adapted from http://commons.wiki media.org/wiki/Image:Gray697.png.

IV. The cardiac vagal motoneurons

The first evidence of induced bradycardia by stimulating the ANS effectors is from 1845 (quoted from (63)). Ernst and Eduard Weber discovered the inhibitory action of the vagus nerve upon the heart. Indeed, when stimulating the medulla oblongata and the vagi of a frog by means of a rotatory galvano-magnetic apparatus the heart could be brought to a standstill. The cardioinhibitory center lied in a region between the optic lobes and the calamus scriptorius (63).

IV.1. Location

Cell bodies of the CVMs are located in the *medulla oblongata* **(figure 12.B)**. The medulla oblongata is the lower portion of the brainstem **(figure 12.A)**. The medulla oblongata is located rostrally to the spinal cord and caudally to the pons, which is in turn ventral to the cerebellum. The medulla oblongata controls the autonomic functions of the body, e.g. respiration, circulation. The medulla oblongata integrates nerve signals and information between the forebrain and spinal cord.

The connection between the NTS and the CVM is monosynaptic at least for neurons controlling the atrioventricular conduction : neurons labelled anterogradely from the NTS are found in close apposition with neurons labelled retrogradely from the atrioventricular ganglion (dromotropic neurons) (10). The variability in diameter of the axons from NTS to the NAvl may explain the variability in the central delay in the cardiac baroreflex (quoted from (10)). This leaves open the question with respect to chronotropic CVM studied here.

There was some controversy whether the CVM are located in the NA or the dorsal motor nucleus (DMN) within the medulla oblongata. Initially, the sites were stimulated by using the prick of a needle as a method of excitation (63). Later, faradic stimulation was used. This proved to be inaccurate because i) the NTS, i.e. the sensory vagal nucleus, is very close to the DMN, and ii) the axons of the NA group curve back in hairpin loops towards the DMN before leaving the medulla (63). However, experiments done using local electrical stimulation (50) and local destruction of the DMN (11) suggested that NA is the main site of CVMs. Two new approaches clarified the organisation of the cardiac vagal motor control system.

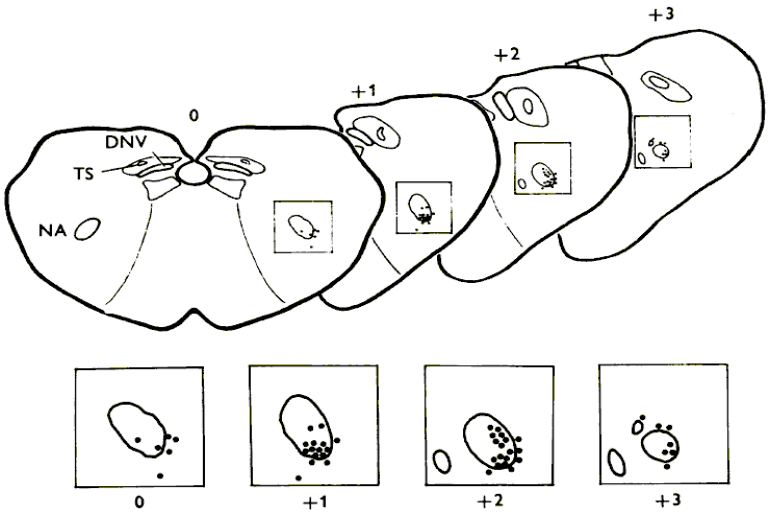
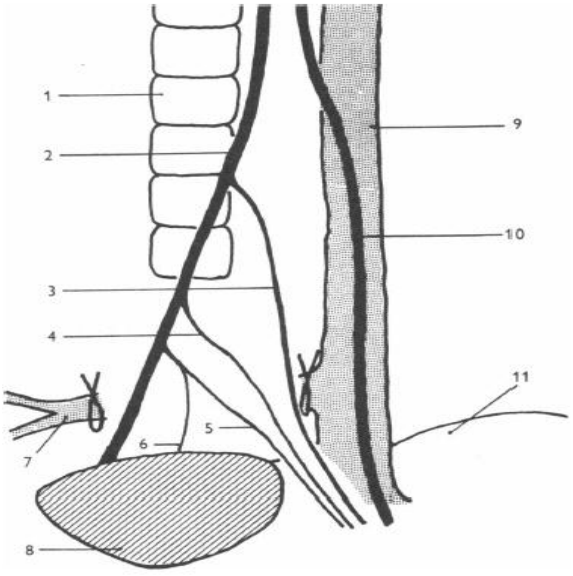


Figure 13. Location of CVM in cat

The positions of forty-six CVM are shown on four standard sections of the medulla taken at obex level, and at 1 mm intervals rostrally. The position of the confirmed CVM are marked by the expulsion of pontamine sky blue (PSB) dye. Inserts, 2 mm square, show details of their relation to the structure of the NA. Note the concentration of the units in a region ventro-lateral to the NA. Figure reproduced from McAllen (96).

TS, tractus solitarius; DNV, dorsal motor nucleus of the vagus; NA, nucleus ambiguus.

A



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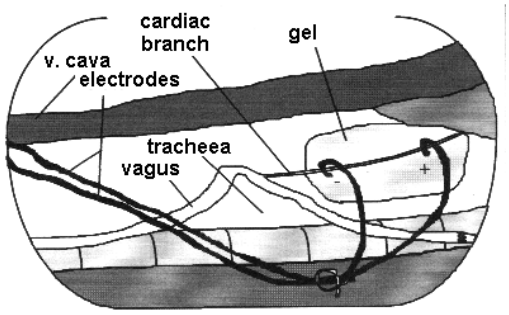

Figure 14. Schematic diagram of dissection of right cardiac vagal branches

A. In cat, on opening the chest, the fourth and fifth ribs are retracted to expose the upper lobe of the right lung, which is then ligated and excised to expose the azygos vein. This is then tied and cut to expose the thoracic vagus. The vagus is then searched for branches coursing towards the heart. There is always at least one branch at the level of azygos vein (caudovagal branch) and one several cm rostral to this (craniovagal branch) although there are sometimes two or three of either. In rat the anatomical disposition is similar. 1, trachea; 2, thoracic vagus; 3, craniovagal cardiac branch; 4, 5, caudovagal cardiac branches; 6, offshoot to lung; 7, divided azygos vein; 8, ligated root of upper lobe of lung; 9, superior vena cava; 10, phrenic nerve; 11, right atrium. Figure reproduced from McAllen (96).

B. In rat, the electrodes used for antidromical stimulation are carefully mounted on the cardiac branch. A pair of electrodes is placed under the cardiac branch, keeping it in mild tension. During the operation, the cardiac branch is kept wet. Drying might worsen the physiological condition of the nerve. The whole setup is secured in contact with silicon gel. Details of the operation are given in Appendix. Figure from Rentero.

Firstly, electrophysiological studies in cat established that most antidromically identified CVM originate in a region ventro-lateral to the NA (**figure 13**). A smaller percentage resides in the DMN (96-98). When stimulating the cardiac branch of the vagus units were antidromically activated (87). Some units could be found in an intermediary zone between the DMN and the NA (43). These units are closer functionally to the CVM of the DMN (76). The semicompact formation of the right NA can be located by stimulation of the cervical vagus (117). Low voltage stimulation (0.5-3V, 0.1ms pulses) of laryngeal motor axons that travel through the cervical vagus generate an antidromic field potential at the level of the NA (**figure 15.B**). Antidromically activated units were soon confirmed also in rat (107).

A second approach applied retrograde tracers, such as horseradish peroxidase (HRP), to the myocardium, the pericardium, the cardiac branches of the vagus and the fat pads around the heart (56; 60; 95). HRP tracings confirmed the locations discovered by electrophysiological studies. The majority of neurons labelled retrogradelly by cholera toxin-HRP injected into the right atrium were located in the NAvl. These cells were often ensheathed in 5HT immunoreactive axonal boutons (56; 60; 95; 106). More precisely, the majority of negative choronotropic CVM are found in the caudal NAvl; conversely the majority of negative dromotropic CVM are found in the rostral NAvl with less than 10% overlap (quoted from (10)). In rat, HRP tracings and medulla oblongata electrical stimulation done in same animals were in agreement (106). However, caution should be exercised when labelling neurons by introducing neuronal tracers onto the epicardium or into the pericardial cavity (49). Tracers have the tendency to diffuse through the mesothelial membrane of the pericardium and penetrate nearby organs. Thus, vicinity neurons (e.g. oesophageal units) might be erroneously labelled as CVM.

Finally whether CVM are indeed cholinergic is an unresolved issue as there is no positive antidromic identification of CVM coupled with precise anatomical tracing. Thus some neurons thought to be CVM projecting to the heart may in fact have been oesophageal motoneurons (D Hopkins, personal communication, 2001). Neurons retrogradely labelled from the sinoatrial ganglion and located in the external formation of the NA do not show immunoreactivity for choline acetyl transferase. By contrast neurons labelled in a similar manner during the same experiment and located in the dorsal motor nucleus of the vagus (DMV), the compact, loose, and semi-compact formations of the nucleus ambiguus do show immunoreactivity for choline acetyl transferase (128). The authors further discussed : "*Ruggiero et al (1990) reported that there were no cholinergic neurons in the ventral part of the NA. …the neurons projecting to the heart located ventrolateral to the [compact formation of the NA] were cholinergic parvicellular spindle-shaped perikarya. The neurons in the [external formation of the ambiguus] projecting to the heart are large polygonal cells according to the present and previous reports….some cardiac vagal nerve fibers released not only acetylcholine but also certain non-adrenergic, non-cholinergic substances such as CGRP and NO. These reports may support the presence of non cholinergic [cardiac vagal neurons] in the [external formation of the ambiguus]….the co-localization of CGRP with acetylcholine within the NA [suggests that] CGRP plays a role in modulating cholinergic neurotransmission*" (128). Much work remains to be done to delineate the anatomy and the physiology of CVM.

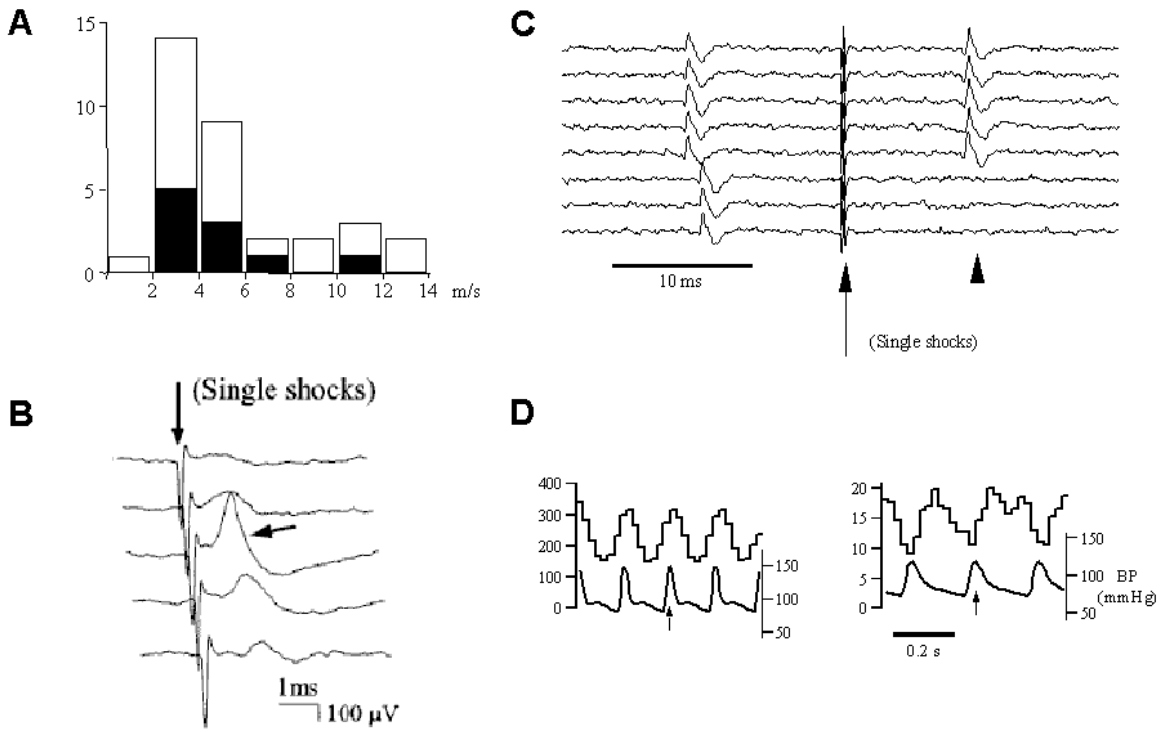

Figure 15. Identification of CVMs in the NA

A: Histogram of conduction velocities of 33 presumed CVM (open bars). 9 confirmed CVM are identified by collision test and are presented blackened. 2 other units, activated by BP rises, and, thus, presumed to be CVM, are presented blackened, too. The rest of 22 CVM showed little or no spontaneous activity, so the antidromic nature of their response could not be checked by the collision test. The axonal conduction velocities were calculated by dividing a constant conduction distance to the latency of antidromic activation. Note that the conduction velocity values of both presumed and positively identified CVM fall in the B-fiber range (1.6-13.8 m/s).

B: The semicompact formation of the NA is located by the maximum antidromic field potential to stimulating the cervical vagus. The antidromic field potential was recorded at 0.5mm step depths, in response to low-voltage stimulation (0.5-3V, single shock 0.1ms pulses) of the cervical vagus. Vertical arrow indicates stimulus artefact. Maximum potential is marked by slanted arrow.

C: Collision test for antidromic activation from cardiac branch. Successive traces show spontaneous (*left*) and antidromic (*right*) spikes of a single CVM. The stimulus (see artefact at vertical arrow) was triggered 11 ms (*top* 5 traces) or 10 ms (*bottom* 3 traces) after a spontaneous spike. Note that the CVM gave a constant-latency, antidromic spike (slanted arrow) when the delay was 11 ms but were cancelled by collision with the spontaneous (orthodromic) spike when the delay was 10 ms.

D: Pulse-triggered histograms of 2 CVM (*top* traces, data subjected to 3-point digital smoothing) and averaged arterial pressure. The CVM activity histograms shows regular peaks and troughs consistently linked to the averaged pulse wave. This pulse-locked character of the CVM is called cardiac rhythmicity or barosynchronicity. Note that there is a certain delay between the two waves. Arrows indicate the trigger time. Ordinates indicate spikes per 20-ms bin and BP. *Left*: 1,403 triggers; *Right*: 2,044 triggers. Figure adapted from Rentero (119).

IV.2. Projections

The axons of the CVMs project to the heart through the vagus nerve. Most of the fibers of the cardiac branch of the vagus (**figure 14**) contain “cardiac-type”, pulse-locked, efferent activity (79). Thus, most of the fibers of this branch are functionally cardiac-oriented, and not functionally projecting to the lungs or the oesophagus. Electrophysiological analysis in cat have shown that the CVM whose cell bodies originate in the NA are composed of B-fibers (96). These axons are myelinated, with conducting speeds of some m/s (**figure 15.A, table 2**). When stimulated antidromically, with electrodes mounted on the cardiac branch (**figure 14.B**), latencies of around 5-10 ms are obtained (**figure 15.C**). The CVM originating in the DMN are mainly slower C-fibers (107). These axons are unmyelinated. The conduction speed is under 1m/s. For these C-fibers, latencies of ~50ms are obtained when stimulating antidromically in the cardiac branch. Both populations project to similar clusters of ganglion cells on the atrial epicardium. This suggests functional overlap.

IV.3. Activity patterns

There is limited information on the activity patterns of CVM. This may be linked to the difficulty in obtaining *in-vivo* single unit extracellular recordings from CVM (64; 101). Indeed, the cell bodies of the relatively small population of CVM are located in the brain stem in a poorly defined and heterogeneous NA. Beside this, cells are scattered, in contrast with other areas (e.g. *locus coeruleus*, RVLM) where cells are tightly packed and thus, easier to find. The studies are also hampered by the deleterious effects of the surgical procedures (opening the chest, exposing the brain stem, anesthesia). These procedures are known to decrease the cardiac vagal activity. Recordings of the CVM bodies have been done in the cat (45; 96-98) and their efferent fibers (vagal nerve activity) in cat (79) and dog (61; 70; 118). A recent study over the activity patterns of CVM in rat used for the first time carbon-fiber microelectrodes for extracellular recording (119). This study confirmed the pulse-locked character of CVM (**figure 15.D**) and analysed the respiratory pattern. Another study introduced the multiple axonal recording (108). Simultaneous recording of B and C cardiac fibers of the vagus is done using a suction microelectrode method. Multiple spike-triggered averaging is used. Thus the central study itself is neglected. However, a larger number of cells is recorded.

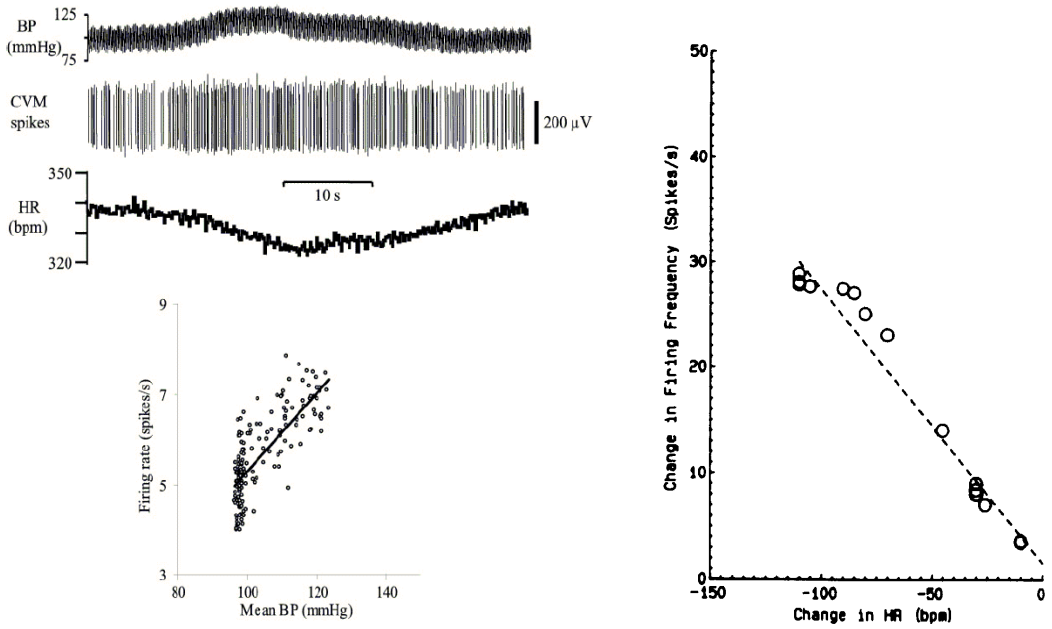

Figure 16. A. Demonstration of barosensitivity of CVM in rat

Arterial pressure is raised 20–50 mmHg by constricting the aorta with a pneumatic cuff. A: chart record of baroreceptor test. Top traces: BP, detected CVM spikes, and HR. B: scatter plot of 1-s values of mean firing rate vs. mean BP. The linear regression line for the relation is shown. The relation of firing rate to BP rise is highly significant ($p<0.001$). Bpm: beats/min. Figure reproduced from Rentero (119).

Figure 16. B. Relationship of firing rate of a CVM and HR

BP is raised by injecting 1-3µg phenylephrine boluses. Points represent number of spikes and values of HR in 14 sample periods taken at different levels of HR. Dashed line represents line of best fit ($r=0.9483$; $P<0.001$). Note the linearity of the response of the HR to the change in firing frequency of the CVM. However, these cells were not antidromically identified, and thus, positive identification is missing. Figure reproduced from Agarwal (2).

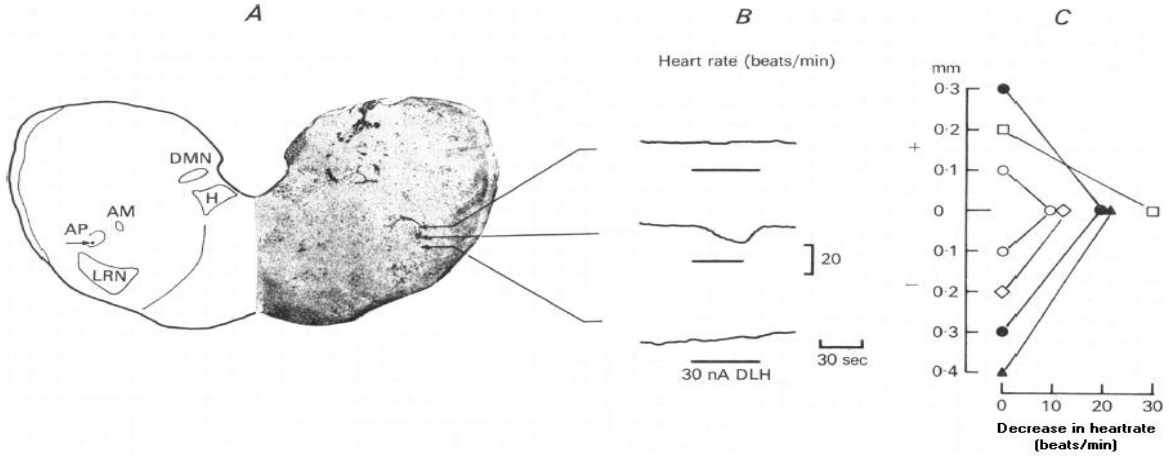

Figure 17. Effect of activating a single CVM on HR

A, photomicrograph showing transverse hemisection of cat medulla and diagrammatic hemisection on the left side. The location of CVM (marked by dye expulsion from the recording electrode) shown by the middle arrow on the right, and by the arrowed dot on the left. B. An excitatory amino acid, DL homocysteic acid (DLH), was delivered ionophoretically (30 nA), at the recording site shown by middle arrow to activate the cell body of the CVM, and at positions 300µm above and below the unit, marked by the other two arrows. HR only fell during DLH application close to the CVM (see middle trace). This demonstrates that there is little doubt that a single neuron can be activated and may generate the bradycardia. C. The effects on HR of activating five different CVM (each shown with a different symbol) using DLH (30-40 nA). The effects at the point of focal recording, and at points above, below or both are shown. Figure reproduced from McAllen (98).

AM, medial column of NA; AP, principal column of NA; H, hypoglossal nucleus; LRN, lateral reticular nucleus.

IV.3.1. On-going activity

Only a minority (5/23 in (97; 119) and 11/33 in (97; 119), so ~a quarter, in general) of CVM show spontaneous activity under experimental conditions (97; 119). When present, the activity is generally slow (<10 Hz) (119). This low percentage may be due to the effects of general anesthesia and surgical trauma (101; 119).

IV.3.2. Barosensitivity vs barosynchronicity

In response to a rise in BP, CVM activity is increased both by active cells firing faster and by recruitment of previously silent neurons (79; 119). When the BP is artificially raised by 20-50 mmHg, a related increase in the activity of single units CVM can be observed in rats (**figure 16.A)** (119). The relation between BP and CVM activity is linear. So far, no evidence of the effect on CVM of lowering the BP exists in rats.

When perievent histograms of CVM activity are triggered by the arterial pulse wave, clear peaks and throughs of cardiac periodicity are observed (**figure 15.D**). This characteristic of the CVM is called barosynchronicity (cardiac rhythmicity, pulse-locked character). CVM are excited by baroreceptors, whose rhythmic input links their firing to the pulse pressure.

Large and fast BP ramps (50-60 mmHg) that activate high-pressure ventricular baroreceptors are known to generate bradycardia. This suggests some neural connection to the CVM. However, such large increases in BP were purposely avoided in this study.

IV.3.3. Bradycardia effect upon stimulation of CVM

Bradycardia is easily obtained whenever the vagus nerve is stimulated. A more specific effect can be obtained upon stimulation of the cardiac branch of the vagus, since the cardiac branch is probably entirely composed of CVM axons (79). Stimulating a single CVM has an effect on the HR (98) (**figure 17**). Indeed, by applying an excitatory amino acid, DL homocysteic acid (DLH), next to the soma, a single CVM can be forced to fire. Thus, a bradycardia as large as 30 beats/min can be obtained in such a manner. Efferent C-fibres (unmyelinated) originating in the DMV contribute to a minor extent to the bradycardia (62).

The relation between the CVM activity and the HR is probably linear (**figure 16.B**). In one study, units increased their frequency to a maximum beyond which firing frequency was not increased even though the HR response increased (2). This suggests a saturation phenomenon. However, the lack of

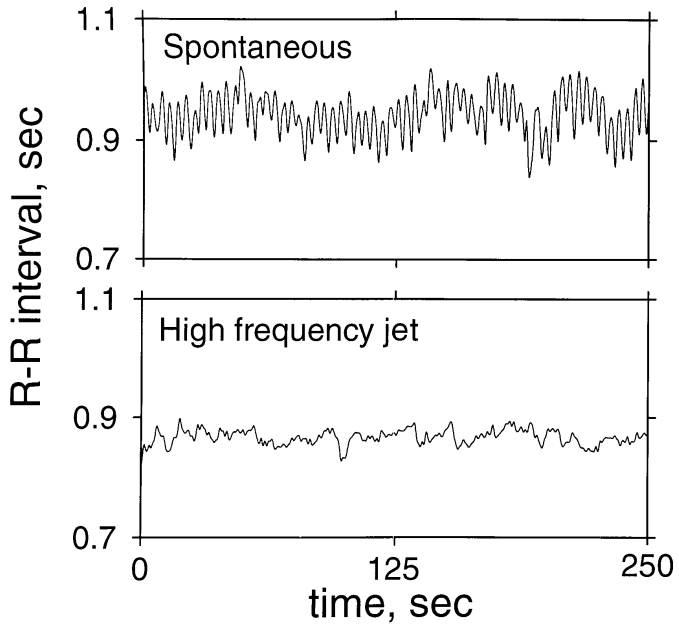

Figure 18. Recordings from an anesthetized healthy patient during spontaneous breathing and high frequency jet ventilation

The patients breathes 100% oxygen at a controlled frequency and uncontrolled tidal volume for 5 min. After anesthesia with fentanyl and induced muscle paralysis with intravenous vecuronium the patient is ventilated with high frequency jet ventilation. During high frequency jet ventilation, the ventilatory frequency is 5 Hz and the flow rate of 100% oxygen is 15 l/min. Note the absence of respiratory sinus arrhythmia in the case of high frequency jet respiration. Figure adapted from Koh (74).

antidromic identification in these experiments means that the cells under study cannot be positively identified as CVM. Indeed, the saturation phenomenon could be a consequence of misidentification of cells. If these were indeed CVM then there is no explanation for the observed extra-bradycardia. Therefore, this data needs to be confirmed by positive antidromic identification.

IV.3.4. Relationship with the respiratory drive

CVMs have two roles: i).optimise the gas exchange at the pulmonary level and ii). buffer the ventilatory-induced changes in BP. Thus some consideration of the respiratory drive to the CVM should be analysed.

The respiratory neurogenesis is defined at the CNS system level as the result of the inspiratory and expiratory neurons e.g. on the phrenic nerve. The *inspiration* and *expiration* are related to the activity of the phrenic nerve and the ventilatory cycle. The phrenic nerve has a cyclical activity, being composed of periods of high-frequency action potentials, during inspiration, and of periods of silence during expiration. Inspiration is an active phenomenon, while expiration is mostly passive in resting or anaesthetized animals.

During each respiratory cycle the heart beats more rapidly in inspiration and slows during expiration. This phenomenon is referred to as *respiratory sinus arrhythmia* (RSA) (6). Rapid changes in HR activity help to buffer the circulation during rapid perturbations such as the respiratory fluctuation in filling pressure (20) and help to improve ventilation/perfusion homogeneity. Indeed, at the onset of the inspiration: i). the volume of the lungs increases and ii). HR increases : thus more blood flows to the alveoli during the filling of alveoli with air. In this way the ventilation/perfusion ratio is improved in a rhythmic manner (139).

However, the link between sinus arrhythmia[4] and respiratory sinus arrhythmia has not been unequivocally delineated: indeed, the suppression of this RSA was observed only in anesthetized healthy patients (74) using a temporary and reversible suppression of ventilation (high frequency jet ventilation) (**figure 18**). This has not been demonstrated in healthy volunteers, without the influence of anesthesia or sedation.

[4] refers to the irregularity of the sinus node activity due to the variability in the ANS control of the heart

I.

2 sec

II.

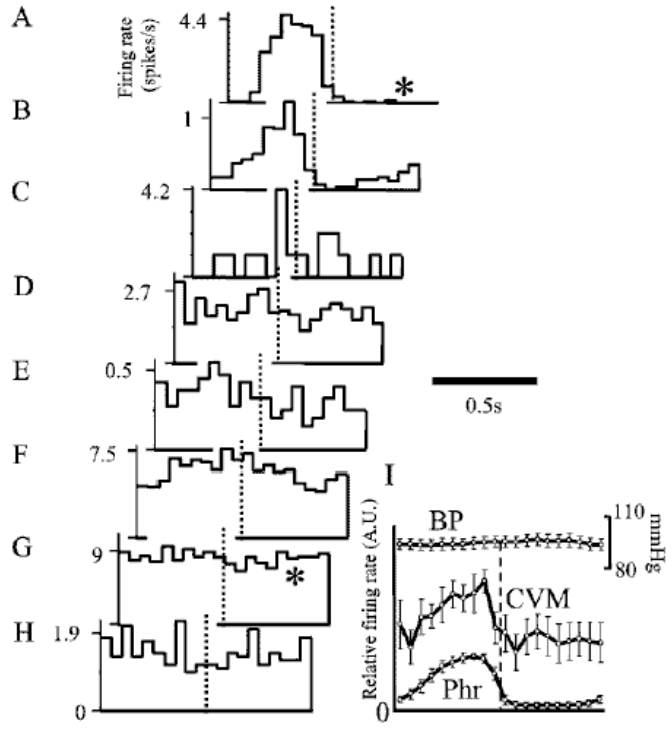

Figure 19. Modification of CVM activity by respiratory drive

I. CVM activity was recording in the medulla of the *cat* by means of double-barrelled micropipettes (one used for recording, and the other for DLH iontophoresis). The inactive neurone only fired in the presence of 40nA DLH. Note the post-inspiratory firing pattern of this unit. Traces from top downwards: unit activity, rectified and smoothed phrenic activity (inspiration: up), femoral arterial BP (calibration bar 0-200 mmHg). Anesthesia : alpha-chloralose. Figure reproduced from McAllen (98).

II.. A-H. Respiratory cycle-triggered histograms of 8 CVM, in the medulla of the *rat*. Histograms were triggered (at vertical dotted line) by the rapid decline in phrenic (Phr; *B-F*, *H*) or central respiratory activity (*A*, *G*, *). Histograms cover 0.5 s before and after the trigger, in 50-ms time bins. I. Grouped data, showing (from above) mean BP (*n* = 7), normalized mean bin counts of CVM taken from histograms *A-H* (CVM, *n* = 8), mean normalized phrenic or central respiratory activity (Phr, *n* = 8), both plotted in arbitrary units (A.U.) but with the baseline set at zero activity. The CVM activity is strongly related to the phrenic respiratory pattern. Note that in contrast with the post-inspiratory activation observed in the cat, the rat CVM activity has an inspiratory activation. In all cases, the dotted line indicates the trigger time, error bars give SE, and zero levels are set at the baseline. Numbers of triggers: *A*, 680; *B*, 807; *C*, 19; *D*, 294; *E*, 875; *F*, 401; *G*, 221; and *H*, 213. Anesthesia: urethane. Figure reproduced from Rentero (119).

The anatomical contiguity of the CVM in the NA with the respiratory neurons might generate interactions between the two sets of neurons. Indeed, by analysing the single unit activity (SUA) of most CVM, a correlation between the phrenic activity and the CVM activity pattern can be easily inferred (**figure 19**). In large mammals such as cats, dogs, and humans, RSA is often prominent and is entirely due to fluctuations in CVM activity. Experiments done in cat, dog, and (by inference) human demonstrated that CVM activity is greatest during early expiration called post-inspiration (**figure 19.I**) (36; 61; 98).

The electrophysiological basis for “respiratory gating” of CVM was deciphered through experiments done in anaesthetized cats (45). Neurones in the NA receive baroreceptor inputs which modulate their membrane potentials. Such baroreceptor influences vary according to other ongoing respiratory membrane potential fluctuations. The ability of CVM to fire in response to excitatory amino acids fluctuates in parallel with these respiratory fluctuations of CVM membrane potentials. Also, a locally applied muscarinic antagonist atropine appears to remove the inspiratory inhibition of the CVM discharge. Thus, the dominant feature that sets the respiratory firing pattern of CVM appears to be synaptic inhibition. Therefore, a one-way effect of respiratory drive over the circulatory neurons is demonstrated. In line with this idea, low doses of systemic atropine enhance cardiac vagal efferent activity in dogs (69).

In rat CVM activity is greatest during inspiration (**figure 19.II**) (119). This is linked to the “reversed” respiratory pattern of sympathetic nerve activity to skeletal muscle, which is inspiratory in cats but expiratory in rats (52). In fact, the HR in rats shows very little fluctuation with the respiratory cycle. This reflects the high resting HR and short respiratory cycle. The neuroeffector delay between the arrival of CVM action potentials and their effect on HR (~200–400 ms in cats; (17)) also occupies a greater proportion of the respiratory cycle in rats than in larger animals. An inspiratory maximum in CVM firing rate could thus easily have its main bradycardic action in the expiratory period, effectively “normalizing” the respiratory pattern of HR variation. Any resultant HR effects would be very small, however, and unlikely either to give a clear guide to the underlying pattern of rat CVM activity or to have great consequences for cardiovascular function. However, a later study (13) demonstrates that HR normally increases during inspiration in conscious, freely moving rats, similar to the RSA pattern that occurs in other species and that this pattern is disrupted in the presence of general anesthetics, including inversion in the case of urethane. Therefore, previous results (119) might be a consequence of the use of urethane anesthesia.

IV.3.5. CVM and the sympathetic system

Electrophysiological and anatomical data describing the medullary pathways mediating the baroreceptor reflex supported the idea of independent control of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) at the level of the *medulla*. Recent evidence, mostly indirect, has suggested that baroreceptor control may also include interactions between sites in the medulla which were thought to be independently involved in controlling the PNS or SNS. Electrical stimulation of the central end of the cut cervical vagus inhibits the activity of RVLM neurons (127). However, stimulating the central end of the vagus is very non-specific, since it activates dozens of different afferent fibre types, and is known to cause parasympathetic bradycardia with sympathetic vasomotor inhibition. Microinjection of the local anesthetic lidocaine in the NA elicits increases in arterial pressure (AP) without changes in HR. Electrolytic lesions of the NA facilitate the establishment of hypertension (HBP) produced by sinoaortic deafferentation (88; 89). These facts provide indirect evidence that NA neurons influence the activity of RVLM or CVLM neurons, which control sympathetic tone. However, a lesion of the NA will also destroy the CVLM and this will surely cause HBP. There may be no change in HR if there was little vagal tone before the lesion. Microinjection of the excitatory amino acid glutamate into the NA influences the activity of cardiovascular neurons in the RVLM (99). In this study, NA provides an inhibitory input to 2/3 of identified cardiovascular neurons in the RVLM. A reciprocal pathway between the NA and CVLM exists (100). Numerous tyrosine-hydroxylase+ terminals are found in the NAvl, in close apposition with negative inotropic preganglionic neurons identified by retrograde tracing from the cranial medial ventricular ganglion (94). These neurons are not cholinergic but lie directly ventral to cholinergic neurons in the dorsal column of the NA (94). In addition, tyrosine-hydroxylase+ labelled neurons are located in close proximity to cells retrogradely labelled from the sinoatrial ganglion, using cholera-toxin (128). The anatomical possibility exists for a direct relationship between presympathetic neurons and CVM. This adds to the physiological evidences (99; 100). However, i). CVMs in these studies were not antidromically identified; ii). stimulating the NA will also stimulate the CVLM. Therefore, the conclusions of these studies are unsecure : central sympathetic-parasympathetic interactions remain to be thoroughly worked out.

V. Summary

The autonomous nervous system regulates the BP through its cardiovascular sympathetic and parasympathetic limbs. Sympathetic limb adapts the BP to emergency situations (33). Sympathetic response has a delay of some seconds in unanesthetized conscious freely moving rats (24). Parasympathetic division is responsible for ultra-short-term BP regulation, as its action is fast (24). The cardiac vagal activity buffers small beat-by-beat BP variations by fine-tuning the HR mainly (**figure 5** legend). Changes in BP are sensed by the baroreceptors. The information is sent to the NTS, in the medulla oblongata. Special emergency cardiovascular situations are also signalled to the NTS by the ventricular chemoreceptors. In turn, the NTS sends information to the CVM, which originate mainly in the NA (96). Physiological studies (96-98), together with retrograde tracing (56; 60; 95; 106) confirmed the NA as the main location of the CVM. The CVM axons are mostly, but not all, B-fibers (conducting speed of some m/s, (96)). CVM project through the vagus nerve to the cardiac ganglion. CVM are believed to be tonically active, but most of the recorded cell units are silent, due to the experimental conditions (101; 119). As expected, spontaneous BP rises generated by aortic occlusion increase the CVM activity (119). The relation between the BP and the CVM activity during the pressure increase is probably linear. Increases in CVM activity generate bradycardia. In contrast, no evidence on what happens to the CVM when BP is lowered exists. Stimulating the cardiac branch generates a proportional bradycardia. Stimulating a single CVM has an important bradycardic effect (98). CVM activity is clearly modulated by the arterial pulse wave. This phenomenon is called barosynchronicity (97). By analysing the CVM activity patterns, a modulation by the respiratory drive could be observed in most units (98; 119). This is closely related to the RSA, a mechanism that is believed to adjust the pulmonary blood flow to the ventilatory cycle. Indeed, the respiratory neurons, in anatomical contiguity with the CVM, exert an inhibitory action on the CVM activity during inspiration in *cats* (45). The inhibitory effect of the CVM on the sympathetic RVLM cells demonstrates a pathway between the central sympathetic and parasympathetic sites of the baroreflex (99; 100). However, since the neurones in the study were not antidromically identified, this hypothesis awaits further confirmation.

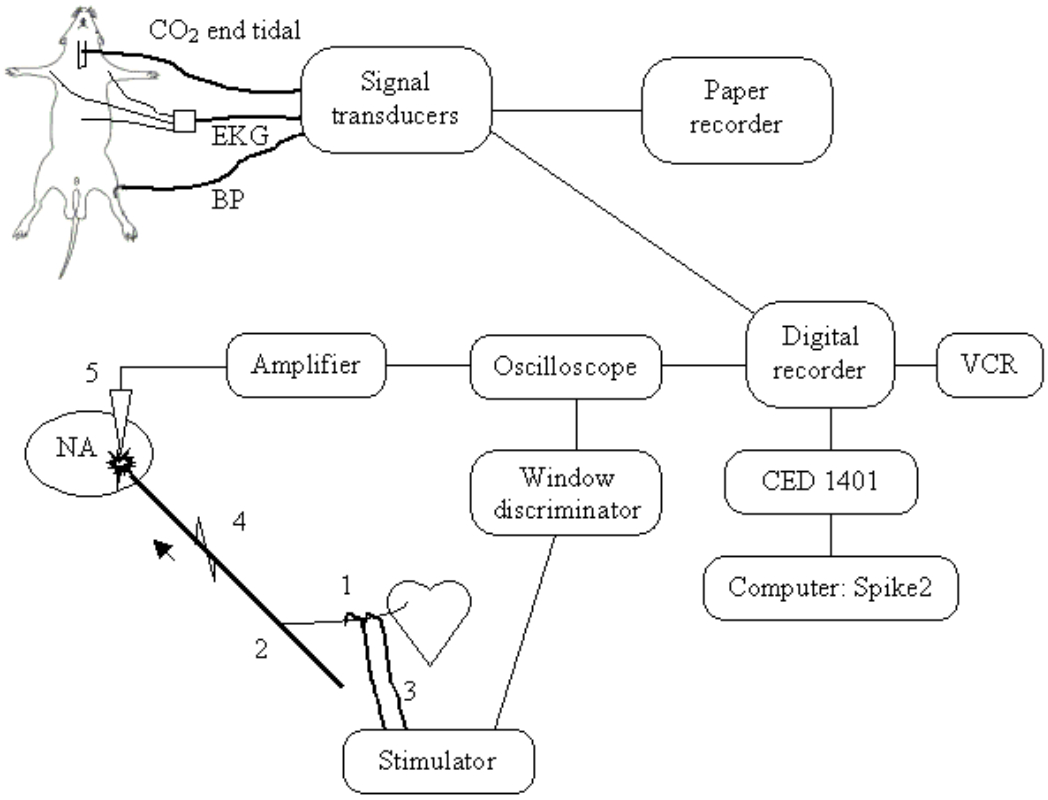

Figure 20. Experimental set-up for CVM signle unit activity extracellular recording in urethane-anesthetized rats

The cardiac branch is placed on a pair of electrodes made from teflon-coated silver wire, bared at the tips, keeping it under mild tension. Thus, only vagal efferences projecting on the sino-atrial node are stimulated. Antidromic action potentials are detected in the NA of the medulla oblongata with a carbon-fiber microelectrode. The signal is amplified and visualised on an oscilloscope. CVM are identified by fixed latency, collision test, and pulsed-locked activity criteria. A window discriminator is used for spike detection. Unitary activity of identified CVM is recorded, together with BP, EKG and CO_2 end-tidal signals, by using a digital recorder and a VCR for later off-line processing. 1: cardiac branch; 2: vagus; 3: stimulating electrodes; 4: antidromic action potential; 5: carbon-fiber recording electrode.

NA: nucleus ambiguus; EKG: electrocardiogram.

OBJECTIVES

Both the baroreceptor-HR and the BJ reflexes have a short-term action on BP. A normal cardiac baroreflex reduces BP fluctuations, i.e. BP lability. Upon stimulation of chemoreceptors located in the ventricles (25), the BJ reflex has an unloading effect and thus, reduces the ventricular stress. Both cardiac baroreflex and BJ reflex share a common path: the cardiac vagal efferent supply to the heart. In case of a depressed reflex, it appears that cardiac vagal activation is beneficial.

Recent studies confirmed the possibility of analysing the CVM activity in the rat now most favoured for invasive studies (119). Methods presented in this study seem to suit at best the needs of our study. Indeed, *in-vivo* extracellular-recording of CVM single unit activity (**figure 20**) in urethane-anesthetised rats, by using carbon-fiber microelectrodes, seem to be the best way to quantify the cardiac vagal activity. This method, although highly invasive, appear to be closest to normal physiological conditions. CVM are fully identified by physiological criteria: i). constant-latency response to antidromic stimulation from the cardiac branch of the vagus; ii). absolute refractory period; iii). collision testing; iv). pulse-locked character; v). barosensitivity.

α-2 agonists, such as **clonidine**, are known to improve circulatory stability. In the same line, the brain natriuretic peptide **(BNP)** is believed to be a cardioprotective neurohormone (130). One of the actions of the BNP is to improve the effects of the BJ reflex. However, their action on the cardiac vagal activity was assessed by indirect measures such as HR, HR variability and BP variability, cardiac baroreflex sensitivity. By indirect evidence, the BNP was demonstrated to enhance the parasympathetic activity in conscious sheep (130). Thus, an analysis of the action of these two drugs on the cardiac vagal activity will clarify the mechanism of action.

RESULTS

A - Cardioinhibitory actions of clonidine assessed by cardiac vagal motoneuron recordings

I. Introduction

In mammals, sympathetic and parasympathetic divisions of the ANS work together to adjust the blood flow to the needs of the organism (chap. I). HR is controlled by the cardiac sinoatrial node. In an isolated heart, the sinoatrial node generates a constant rhythm. Both sympathetic and parasympathetic divisions of the ANS modify the cardiac rate by a direct action on the sinoatrial node. Parasympathetic division exerts a fast action by adjusting the HR to buffer the variations of BP. The sympathetic system leads also to a slower modification of the HR. This action of the sympathetic system is performed through the intra-synaptic release of catecholamines. Besides, the sympathetic system modifies the vasomotor tone. Sympathetic system regulates mainly the BP set-point through its slower action. Autonomic dysfunction of the SNS is correlated with HBP. Sympathetic overactivity is associated with essential HBP (67).

Thus, two entirely different mechanisms are involved in beat-by-beat BP regulation: i) the mechanism buffering the BP fluctuations (seconds to tens of seconds) and ii) the mechanism maintaining the BP set-point on a long-term basis (minutes to days). These two mechanisms seem to be independent. However, given the difficulty of obtaining direct measures of cardiac nerve traffic, most studies that delineate the contribution of the subdivisions of the ANS to the regulation of BP and HR are indirect. Perhaps the most widely applied method is the use of selective pharmacological blockade of the vagal and sympathetic innervations of the heart (8; 66). Indeed, the change in the functional state or response of an organ after selective blockade of an autonomic branch, for example, provides an index of the contribution of that branch to the autonomic control of the organ. The change in RR interval after withdrawal of cardiac vagal activity with atropine has been used often as an index of vagal control of the heart under baseline condition. Moreover, the remaining cardiac control after vagal blockade as opposed to combined sympathetic and vagal blockade serves as an index of residual sympathetic control. This approach using subtractive logic has been most useful in elucidating the autonomic origins of various frequency components of HR variability (HRV) (75; 111) and produces results that are in agreement with other measures of sympathetic and parasympathetic control. Thus, the parasympathetic control of the heart is responsible for the beat-by-beat sinus arrhythmia.

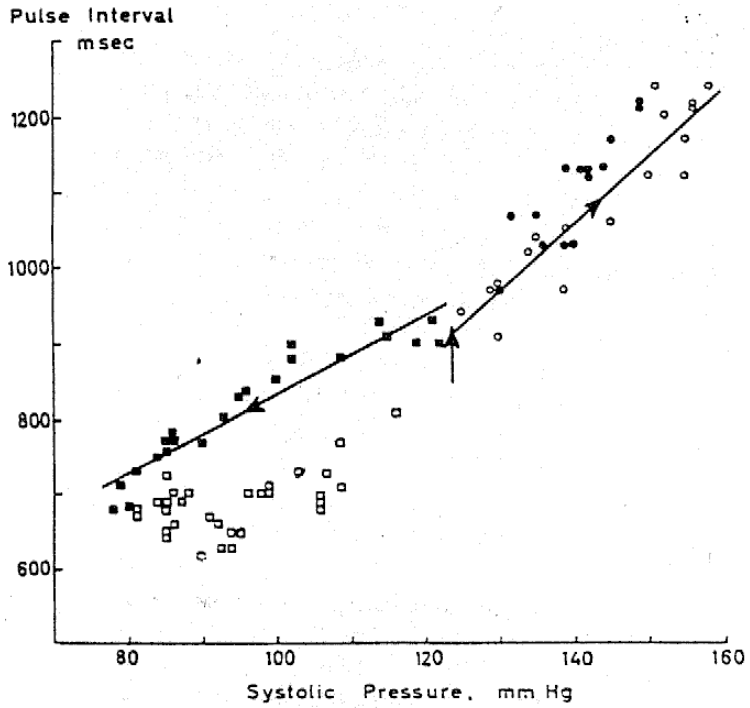

Figure 21. Comparison of the response of pulse interval to rising and falling arterial pressure after one injection of phenylephrine and one inhalation of amyl nitrite in man

The vertical arrow shows resting level of systolic pressure and pulse interval. Open circles = pressure falling to original level; closed circles = pressure falling to original level; closed squares = pressure falling after amyl nitrite; open squares = pressure rising to original level. Note the asymmetry of the response. The solid lines are the regression lines relating pressure and pulse interval. Figure reproduced from Pickering (116).

Hypertension (HBP) is a pathology where the BP is chronically elevated. Persistent HBP is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure. HBP can be classified as either *essential* or *secondary*. Essential HBP is the term used when no specific cause can be found to explain a patient's condition. Secondary HBP means that the high BP is a result of another specific disease, as renal disorder, endocrine disorders (e.g. phaechromocytoma, hyperaldosteronism), tumours, drugs, obstructive sleep apneea. Generally, in humans, the limit for the value of BP which is defined as arterial HBP is 140 mmHg for the systolic BP, and of 90 mmHg for the diastolic BP (5). There are many classes of medications for treating HBP, called *antihypertensives*, which act by lowering BP. Evidence suggests that reduction of the BP by 5-6 mmHg can decrease the risk of stroke by 40%, of coronary heart disease by 20-25%, and reduces the likelihood of dementia, heart failure, and mortality from vascular disease (26).

Clonidine is a centrally-acting α-2 agonist prescribed historically as an anti-hypertensive agent. Clonidine selectively stimulates α-2 receptors in the brain, decreases local concentrations of catecholamine in specific nuclei and thus lowers plasma catecholamine concentrations. Clonidine has two main effects:

- *long-term effect*, by decreasing SBP and HR. This is mostly an effect of the inhibitory action of clonidine on the sympathetic system.

- *short-term effect*, by increasing HR variability, and decreasing BP lability. The fact that HR variability increases with clonidine suggests an increased cardiac vagal activity. This awaits direct confirmation. Decreased BP lability might be correlated with the increased HR variability (39; 112). However, decreased BP lability is a consequence of a lowered cardiac vagal activity and/or decreased sympathetic tone.

Sodium nitroprusside is a potent peripheral vasodilator that affects both arterioles and venules. It reduces both vasomotor tone as well as venous return, thus decreasing both preload and afterload. Sodium nitroprusside is often administered intravenously to patients who are experiencing an acute hypertension or in severe cardiogenic heart failure. In situations where cardiac output is normal, the effect is to reduce BP. Despite its toxicity, nitroprusside is still used because it remains an effective drug in certain clinical circumstances such as malignant HBP or for rapid control of BP during vascular surgery and neurosurgery.

Baroreflex assessment method. Baroreflex SBP-RR interval relationship was calculated historically by plotting RR interval (or HR) changes vs. a pressure ramp (124) (**figure 21**). This pressure ramp is artificially generated (valsalva *manœuvre*, head-up tilt, neck chamber *technique*, drug-induced modifications with phenylephrine, angiotensin, sodium-nitroprusside etc). Newer methods of analysis

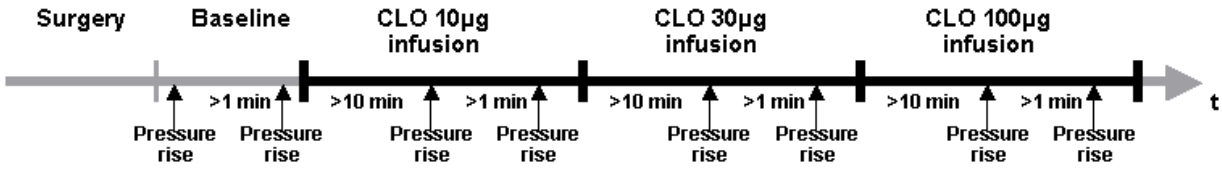

Figure 22. Protocol used for CVM activity recording under clonidine infusion in urethane-anesthetized rats

Clonidine was infused in 10, 30, 100 µg/kg cumulative doses via the femoral vein, over 5-10 min to minimize pressure changes. 2-3 pressure rises (amplitude inferior or equal to 40 mmHg) were generated by inflation of the balloon of the PTCA catheter for each dose. At least 2 minute intervals were allowed between inflations for full recovery of BP and HR to baseline values. CVM and HR responses were measured. Clonidine was prepared in 5 or 50 µg/ml solutions. CLO 10µg: Clonidine 10µg/kg. Total duration of recording: 1-3h. Total duration of experiment: 9-15h.

appeared since the development of computational techniques (32; 137). These are able to analyse short sequences of *spontaneous* fluctuations of pressure. Thus, presumably, a value closer to the real cardiac baroreflex gain can be obtained because: i). real-life, i.e. not evoked, artificial, sequences are analysed; ii). sequences contain smaller pressure excursions, in contrast to induced ones that rarely occur in real life; iii). large artificially-induced pressure excursion might also stimulate other reflexes (triggered by intraventricular high pressure mechanoreceptors) that might overestimate the calculated cardiac baroreflex gain; iv). a great number of sequences are generated, and an average can be quickly calculated (thus discarding the errors due to the minute-to-minute variability of the baroreflex); v). the technique may be non-invasive. However, such techniques could not be applied when analysing the baroreflex at the central level, i.e. when calculating the SBP-CVM relationship, because: i). one single CVM represents poorly the baseline activity of the whole population of CVM; ii). CVM in rat are often silent, or present low activity in baseline conditions. Thus, similar spontaneous correlations between the SBP and CVM activity, like the one between SBP and RR interval, could not be made. Therefore, assessment of the cardiac baroreflex at the central level has to be done using a pressure ramp generating method. This would: i). generate large pressure ramps that can be easily correlated with corresponding CVM increased activity and ii). bring some baseline inactive CVMs to some degree of activity during the pressure rises, so that a SBP-CVM relationship could be calculated.

Pressure-ramp generating method. In order to generate the pressure ramp three methods could be used: i). the phenylephrine-nitroprusside bolus injections. This method appeared unsuitable to the present protocol where three cumulative doses of clonidine are tested (**figure 22**). In order to have better results and to manage at best the effects of the variability of the baroreflex, 2-4 pressure ramps were generated per condition. Given the non negligible volume of phenylephrine and nitroprusside per pressure ramp challenge and the numerous times this should have been done for a complete dataset in one rat, it was concluded that this could affect the volemia of the rat; ii). arterial blood flow blocking through inflation of a balloon placed next to the upper abdominal aorta (77). This method requires a highly invasive surgery. Beside this, during pressure ramps the aorta is occluded and thus, the BP cannot be monitored at the femoral level. A cannulation of the carotid is possible, but this would imply further invasive surgery; iii). inflatable balloon placed inside the aorta (21). A percutaneous transluminal coronary angioplasty catheter (PTCA) with an inflatable balloon at its tip is advanced to the thoracic aorta through a femoral catheterisation. This is a similar method to the previous one, but has the advantages that is less invasive and that BP could be monitored during the pressure ramps. Through the complete blocking of the aorta, it is possible to generate either fast or slow rising BP ramps. It is also possible to rapidly release the blockade of the vessel. However, care has to be taken in order to generate pressure rises of less than 40-50mmHg, in order not to stimulate ventricular high-pressure mechanoreceptors (38). This method seems to be the most adapted to the needs of this study.

Objective. The question is how does clonidine contribute to smaller beat-by-beat variations of BP, controlled mainly by the sympathetic and parasympathetic systems? While its common effect of inhibiting the sympathetic outflow is known, the action of clonidine on the parasympathetic system has been analysed previously by indirect methods mainly. Thus, the present study analyses the cardiac vagal activity directly at the central level by using a combination of recently developed methods: *in-vivo* CVM SUA recording in the rat using carbon-fiber electrodes (119) and generation of pressure ramps by means of a PTCA catheter (21).

Central-acting anti-hypertensive agent clonidine will be compared with peripheral vasodilator nitroprusside in terms of action on the CVM, in an attempt to qualify their effect on the central parasympathetic core.

II. MANUSCRIPT n°1

Cardioinhibitory actions of clonidine assessed by cardiac vagal motoneuron recordings

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CARDIOINHIBITORY ACTIONS OF CLONIDINE ASSESSED BY CARDIAC VAGAL MOTONEURON RECORDINGS

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Abstract

Background : Cardiac vagal activity is now considered as an important therapeutic target. However, there is a lack of direct data on how cardiac vagal motoneurons (CVM) respond to parasympathomimetic agents.

Methods : Rats were anesthetized with urethane and mechanically ventilated. Single unit activity was recorded in the nucleus ambiguus from CVM, identified by antidromic activation from the cardiac vagal branch and their barosensitivity.

Results : Nitroprusside lowered systolic blood pressure (SBP), increased heart rate (HR) and inhibited CVM activity (n=5 cells in 5 rats). Clonidine 1-100 µg.kg-1 i.v., however, lowered SBP, but increased CVM activity (n=8 cells in 8 rats). It also enhanced their barosensitivity. An unsuspected further finding was that clonidine significantly increased the occurrence of CVM firing spikes separated by short (< 30 ms) interspike intervals (“doublet”).

Conclusion : Such grouped patterns are known to enhance neurotransmitter release. Therefore, these data provide a new mechanism by which clonidine can further potentiate parasympathetic actions on the heart.

Key words : nitroprusside, alpha-2 agonist, clonidine, cardiac vagal motoneuron, cardiac vagal preganglionic neuron, single unit activity, firing pattern, baroreceptor-heart rate reflex, baroreflex, gain.

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INTRODUCTION

An inverse relationship exists between beat-by-beat blood pressure (BP) variability (“pressure lability”) on the one hand, heart rate (HR) variability (sinus arrhythmia) and baroreceptor-heart rate reflex (“cardiac baroreflex”) sensitivity [1] on the other hand. Thus cardiac vagal activity may play a key role to buffering pressure lability. Following major surgery, increased pressure lability and reduced HR variability appeared linked (figure 3B&D in [2]). Furthermore, cardiac vagal de-activation is also an hallmark of hypertension (HBP) [3] and congestive heart failure (CHF) [4]. Following myocardial infarction, the lowered sensitivity of the cardiac baroreflex is associated with an increased incidence of sudden death [5]. Although there is no cause-effect relationship between cardiac vagal activation and successful therapy, cardiac vagal activation is considered as an index of successful treatment of cardiovascular disease [6].

While indirect measures of cardiac vagal activity such as HR or HR variability have been heavily exploited [7], *direct* information on the central regulation of cardiac vagal activity is scarce. Cardiac vagal motoneurons (CVM, cardiac vagal preganglionic neurons; cardiac vagal inhibitory preganglionic neurons) are located in the external formation of the nucleus ambiguus (NA) of cats and rats [8-9], where they may be recorded *in vivo* [10-11]. The sensitivity of the cardiac baroreflex analyzed at HR level is increased by alpha-2 agonists [12], beta-blockers, M1 antagonists (pirenzepine), converting enzyme inhibitors or angiotensin antagonists. Surprisingly, little information pertaining to the effects of drugs on cardiac vagal activity analyzed at CVM level or on vagal fibers is available [13] : this may be linked to the difficulty of recording CVM *in vivo* [14]. Hypotensive agents such as clonidine reduce pressure lability [2-15], simultaneously generate a large sinus arrhythmia and increase the sensitivity of the cardiac baroreflex in hypertensive patients recovering from major surgery [2].

Given the a) clinical utility of alpha-2 agonists in recruiting cardiac vagal activity and reducing pressure lability [16] b) renewed interest in the use of centrally acting agents [17-18] c) increased cardiac vagal activity observed experimentally following clonidine [19-24], and because the opposite effects of a peripherally acting vasodilator, nitroprusside (SNP) and clonidine on baroreflex function, CVM neurons were hypothesized to be activated by clonidine and inhibited by SNP. Unexpectedly, upon data analysis, the firing pattern of CVM appeared altered following clonidine and thus was analyzed.

MATERIALS AND METHODS

Anesthesia : Experiments approved by the Rhône-Alpes Committee for the care of Animals were performed on Sprague-Dawley male rats (Harlan, Gannat, France, 325-400g). Methods were as described [11]. Isoflurane anesthesia was switched over to urethane 1.4 g.kg-1 i.v. over 15-30 min before recording. During recordings, the animal was paralyzed (metocurine iodide 0.2mg i.v., Metubine®, Lilly, Indianapolis, IN). This was done once anesthesia had been established to abolish withdrawal reflex. Paralysis was allowed to wear off between doses to ensure adequate anesthesia. If necessary, urethane (10–20% of original dose) was given i.v. before re-establishing paralysis. After tracheotomy, rats were mechanically ventilated (f~72/min; ~40 % O_2/air; end-tidal CO_2~25-30 and ~30-35 mmHg during surgery and recording respectively; Engström Elisa Duo, Gambro Engström, Bromma, Sweden). The rectal temperature was kept at ~37.5°C (Harvard, Edenbridge, Kentucky). The bladder was canulated and drained. The right femoral vein was catheterized. A percutaneous transluminal coronary angioplasty catheter with an inflatable balloon at its tip (PTCA catheter 2-3mm, Boston Scientific, Galway, Ireland) was introduced through the right femoral artery up to the thoracic level, to monitor BP and generate pressure rises [25] (“balloon inflation”). A slow infusion (<3ml/h) through the arterial lumen of the PTCA catheter prevented hypovolemia [26] and clotting. A 3 lead electrocardiogram (EKG) was set up.

Surgery : The right thoracic vagus was exposed through a thoracotomy (2nd right intercostal space). The cranio-vagal cardiac branch (“branch”) was identified by its ability to cause bradycardia when stimulated electrically (20-50Hz, 2-7 V, 0.05ms) [11]. A small sheet of polyethylene was inserted beneath the branch to insulate it. A pair of electrodes (teflon-coated silver wire bared at the tips, 125 µm) was placed under the branch and secured in contact with silicon gel (Wacker, Munich, Germany). The wires were secured to avoid pulling on the nerve. The viability of the branch was then re-checked by stimulating through the implanted electrodes. The experiment was discontinued if this failed to cause bradycardia. The rat was fixed in a stereotaxic frame, with the head ventroflexed sufficiently to bring the surface of the medulla horizontal. The tail was pulled to stabilize the medulla. The medulla was exposed by removing part of the occipital bone with rongeurs. The dura and the atlanto-occipital membrane were reflected.

Single unit recording : Carbon fiber electrodes were made [27] with the fiber to protrude 10–15 µm beyond the micropipette. Electrical contact was made by a chlorided silver wire with 2M NaCl in the pipette shaft. Electrodes were lowered vertically through the dorsal surface of the medulla (1.5–2.2 mm to the right of the calamus scriptorius; depth=1.5-2 mm corresponding to the external formation of the NA). Unit activity was recorded differentially between the carbon fiber electrode and a reference silver wire placed on the medullary surface using a preamplifier (Grass P16, Quincy, MA). The signal was amplified (x10,000) and band-pass filtered (300-3000Hz) before display on an oscilloscope (Tektronix 5111A, Beaverton, OR). The unit signal was digitized at 18.5 kHz (Instrutech, New York, NY) then recorded (JVC, Friedberg, Germany) along with BP, EKG, CO2 (digitized at 4.5 kHz), stimulus, vocal messages and event markers. Signals were also recorded with a computer-based system (“Micro 1401”; Spike2 software, CED, Cambridge, U.K), for which the CVM signal was digitized at 15 kHz. On-line spike discrimination was performed with a time amplitude window discriminator (FHC, Brunswick, ME). CVM were sought by their fixed-latency response to stimulation of the branch (0.5–5 mA, 0.05 ms, ~1 Hz [11]). Signal averaging software (Spike2 v3.21, C.E.D., Cambridge, U.K) helped to localize CVM. Once a unit recording had been isolated (figure 1A), it was subjected to time-controlled collision testing (figure 1B) [11-28]. Unit discrimi-

isolated (figure 1A), it was subjected to time-controlled collision testing (figure 1B) [11-28]. Unit discrimination and collision tests were re-checked and edited off-line from the recorded signals.

Nitroprusside : bolus doses of nitroprusside (2 µg, sodium nitroprusside dihydrate in saline, 25 µg/ml, Fluka, Buchs, Switzerland) were administered i.v. In some experiments, phenylephrine (2 µg, 25 µg/ml, Boehringer, Ingelheim, Germany) was then injected at the pressure nadir to cause a rising pressure ramp (figure 3).

Clonidine : clonidine (5 or 50 µg/ml in saline, Sigma, St Louis, MO) was infused in 1, 3, 10, 30, 100 µg/kg i.v. cumulative doses, over ~10 min. Two or three BP rises of ≤ 30-50 mmHg [29] were generated by balloon inflation after each clonidine dose. At least 2-minute intervals were allowed between inflations for full recovery of BP and HR to baseline values. CVM and HR responses were measured over 1s windows. Once the pharmacological experiment was completed, the animal was killed with an overdose of chloral hydrate (200 mg i.v.).

Firing Pattern : Barosynchronicity (cardiac rhythmicity, pulse-locked character) of neurons was tested online, by generating pulse-triggered correlation histograms of single unit activity in 5 ms bins, using Spike2 (figure 1C). The stored BP and EKG signals were converted back from Instrutech to analog signals, then redigitized at 1 and 4 kHz respectively (Keithley KPCMCIA 16AIAO, Cleveland, OH). Systolic BP (SBP)-RR interval and SBP-CVM relationships were generated with custom-made software (RECAN©, Alpha-2 Ltd, Lyon, France). Discriminated spikes were imported in RECAN from Spike2 files and converted to firing rate integrated over 1s window (e.g. figure 2A). The data were analyzed in 2 ways (see Results): a) counting every spike as a single event; b) counting double spikes occurring within 30 ms as a single event. Interspike interval histograms of single unit activity were also generated, using 10ms bins. The 100s periods used for interspike-interval analysis were divided in 5s-window intervals. Spikes and double spikes were averaged over 5s windows. Double spikes (“doublet”) were counted on these intervals and plotted against the mean firing rate obtained on same periods. Triple spikes (“triplet”) were considered as 2 double spikes. Slopes were averaged over baseline and clonidine 100µg.kg^{-1} periods respectively.

Nitroprusside : total CVM spike counts were measured for 50s before (baseline) and 50s immediately after the injection (response; figure 2B). To analyze the baroreflex during nitroprusside-phenylephrine sequences, CVM firing rate was averaged over 6 respiratory cycles and plotted against SBP. Linear regression lines were calculated for selected segments of the BP trace, handling separately the sections where pressures were below and above baseline levels.

Cardiac baroreflex measured at the CVM : In order to study the SBP-CVM relationship, SBP and CVM activity were averaged over 3 respiratory cycles (~2.5 s), to avoid confounding effects of respiratory modulation [11]. Data were taken from the full pressure sequence due to balloon inflation, including the transient hypotension following deflation, ending when BP returned to baseline. Linear regression lines were calculated separately for sections above and below resting BP. The appropriate reflex delay to allow for was calculated as the number of heart beats, which maximized the correlation coefficient (r) between the two signals (averaged SBP and CVM firing rate). Cases where r was <0.7 were discarded. Duplicate measurements were averaged.

Cardiac baroreflex analyzed at the heart: SBP and R-R interval signals were filtered at 0.8Hz and 0.3Hz respectively using a 201-coefficients low-pass finite impulse response filter. Only the first 5s of the balloon-induced pressure rise were used to calculate the slope of the SBP-RR relationship (cardiac baroreflex analyzed at the heart), to measure selectively the parasympathetic component of the reflex : indeed the slower, sympathetic, mechanism contributes only after 5s [30]. In this case, the appropriate reflex delay to apply was computed by measuring the number of heart beats between the mid-heights of the SBP and R-R interval rises. Linear regression was then applied to calculate the SBP-RR interval slope.

Statistical analysis : Data are expressed as mean±SD in text and ±SEM in figures. SBP, RR, SBP-CVM slope and SBP-RR interval slope data were analyzed using one-way repeated measures ANOVA (STATISTICA 5.1, Statsoft, Tulsa, OK), after verifying visually the normal distribution using log-normal plots. If ANOVA suggested significance, the LSD test post-hoc was used. The firing rate, the normalized firing rate, and the SD of SBP and RR intervals did not present normal distributions. Therefore non-parametric Friedman analysis, followed by Wilcoxon paired test were used, if appropriate. $p<0.05$ was chosen as significant.

RESULTS

Sixteen units which met the criteria for CVM units [10-11] were recorded in 39 rats (table 1 and figure 1). However, given the complexity of the experiments, all parameters (HR, BP, unit activity) could not be collected in all instances (details in table 1). Thus, 13 units were selected for detailed study: 5 were tested with nitroprusside and 8 were used for clonidine studies.

A peripheral vasodilator, nitroprusside, led to brief hypotension (SBP: 128.4±4.0 to 81.6±13.7 mmHg), increase in HR (387.5±7.8 to 392.3±11.7 bpm, n=5) and inhibited all 5 CVM units (figure 2). Vehicle did not modify pressure, HR or CVM activity (n=5). When nitroprusside was followed by phenylephrine administered immediately after the nadir in pressure, a clear relationship linked CVM unit activity to the level of pressure (figure 3A). However, the slope of the CVM-SBP relation was greater at pressures above resting levels than below (figure 3B), indicating non-linearity.

A centrally acting vasodilator, clonidine, led to decreased pressure (SBP : 145.5±16.6 to 122.6±15.6 mmHg, $p<0.05$), HR (371.8±25.7 to 350.7±26.2 bpm, ns, n=8 rats) and increased CVM activity (n=8 cells, $p<0.05$, figure 4A). Vehicle did not modify pressure, HR or CVM activity (n=8 cells). When one silent CVM was encountered, clonidine activated it (figure 4B). Clonidine also led to a change in CVM activity pattern (figure 5A): the single CVM action potentials under baseline conditions were often replaced by high frequency double or triple spikes following clonidine (figures 5, 6) in 5 out of 8 cells, presenting with the highest baseline firing rate, especially when the incidence of doublets is normalized for pressure (figure 5C). When CVM interspike intervals were grouped into four ranges (divisions: 30, 600, 1300 ms, figure 5B), clonidine clearly increased the numbers of short intervals, especially those <30 ms, at the expense of long intervals (n=5 cells; figures 5B-D). Even if double spikes were counted as single events, the firing rate remained significantly elevated after clonidine 100 µg.kg-1 (8.62 Hz vs. baseline : 5.28 Hz when all spikes were counted; 7.19 Hz vs. baseline : 5.08 Hz when double spikes were counted as single events; $p=0.04$ in both cases). Finally, double spikes firing pattern was observed as more prominent during pressure rises than during resting conditions (figures 5E-F) following clonidine 100 µg.kg-1.

Cumulative doses of clonidine (1-100 µg.kg-1 i.v.) decreased SBP and HR (see above), while increasing sinus arrhythmia (figure 6) and CVM firing rate in a dose-dependent manner (figures 8A-E; n=8 cells for unit activity, SBP and RR interval). Vehicle and the lowest clonidine doses (1 and 3 µg.kg-1) caused no significant effect (data not shown). The slope of the SBP-CVM activity relationship (cardiac baroreflex analyzed at CVM level, n=8 cells, figures 7C-D and 8F) was significantly enhanced at clonidine 100 µg.kg-1 compared with baseline ($p=0.043$), but the SBP-RR interval relationship not significantly so (cardiac baroreflex analyzed at heart level, n=8 rats, figure 8G, $p=0.66$).

DISCUSSION

Peripherally- and centrally-acting vasodilators exerted contrasting effects on CVM activity. The centrally acting agent, clonidine, increased CVM activity and modified CVM firing pattern from single spike firing to double or triple spikes volleys of action potentials. Lastly, the slope of the cardiac baroreflex analyzed at CVM level was increased.

METHODOLOGY

Criteria for identification of CVM units [10-11] were met. A pressure-unit relationship (cardiac baroreflex relationship analyzed at CVM level) allowed to relate this pressure-unit relationship to the pressure-RR relationship [31] (cardiac baroreflex analyzed at the heart level). Care was taken to increase pressure by <30-50 mm Hg to avoid stimulating ventricular mechanoreceptors [29] and loosing the unit. The present analysis follows an analysis performed with respect to the sympathetic vasomotor baroreflex : sympathetic premotoneurons and lumbar sympathetic discharge exhibit an identical relationship to BP (figure 9-3 in [32]). These [32] and present data demonstrate tight coupling between brain stem cardiovascular cells and their parasympathetic and sympathetic effectors, cardiac or vascular, for *both* divisions of the autonomic nervous system.

Urethane suppresses the bradycardia evoked by clonidine [33] and is associated with a high parasympathetic activity [34]. Thus low dose clonidine (1-3 µg.kg-1) elicited little increase in CVM activity, against an already existing high baseline activity. Accordingly, low dose clonidine failed to increase aortic depressor nerve discharge in chloralose-urethane anesthetized rabbits [22]. Despite this drawback, urethane was used, as isoflurane silences CVM activity (Cividjian, unpublished observations).

Lastly, CVM are under important respiratory influences detailed elsewhere [10-11]. Given the complexity of the experiments, the phrenic nerve was not recorded. Thus no analysis on how clonidine may alter the respiratory input to CVM was attempted.

FINDINGS

No attempt has been made so far to record CVM during decreases in pressure. When the baroreflex is challenged with nitroprusside, hypotension and increase in HR are accompanied by an inhibition of the CVM (figure 2). One interesting finding is the fact that hypotension does not always entirely suppresses CVM firing (figure 2C) and raise the question of a possible baro-independent component of CVM activity. This cardiac vagal de-activation (figure 2) appears reciprocal to the sympathetic activation observed during nitroprusside-induced hypotension [35]. The pressure-unit relationship slopes do not appear linear when responses to nitroprusside and phenylephrine are considered (figure 3B), as in humans [36].

By contrast to nitroprusside, clonidine induced hypotension and bradycardia *and* an activation of CVM. Thus, these drugs present *opposite* effects on the sympathetic and parasympathetic efferent limbs of the baroreflex. Second, the effect of clonidine on the sympathetic baroreflex is ascribed to at least a dual site of action : rostral ventrolateral medulla [35] and intermediolateral cell column [37]. By contrast, the effect of clonidine on the cardiac

baroreflex arc is less delineated. The activation of CVM appears in line with the parasympathetic activation described with centrally acting agents : a) the bradycardia evoked by clonidine is observed even after beta-blockade [23]. This bradycardia is suppressed by vagotomy [23] b) clonidine enhances the bradycardia evoked by pressure rise following pre-treatment with guanethidine, reserpine and beta-blockers [19] [20] c) clonidine increases the discharge of the primary afferent baroreceptor neurons (first-order neurons) [21]. Thus, clonidine may act at two or more levels within the cardiac baroreflex arc : primary afferent baroreceptors neurons [21-22] and centrally, at or before CVM (present data). A site of action in the nucleus tractus solitarius appears unlikely [21], however. Third, clonidine may activate previously silent units (n=1, figure 4B). Thus, one mechanism leading to increased sensitivity of the cardiac baroreflex may be recruitment of the pool of active CVM : this has to be documented. Fourth, the most unexpected finding was the change in CVM firing pattern : single spikes were seen at baseline, in contrast to high frequency volleys of action potentials after clonidine (figures 5 and 6). This effect of clonidine is even more prominent during pressure rises, as opposed to resting pressure (figure 5E). This last observation is in line with a previous hypothesis on baroreflex assessment [38] : the “sequence” technique observes spontaneous pressure changes around resting pressure and reveals a lower slope of the cardiac baroreflex as opposed to the slope measured after phenylephrine challenge. Here, balloon inflation leads to large pressure changes (figure 5E and 7), even if care is taken to avoid stimulation of high pressure intraventricular mechanoceptors. The present data suggest that the difference in slope measured by the sequence technique opposed to the drug-induced technique [38] may be a consequence of recruitment of CVM or changed firing pattern or both. Fifth, the shift in CVM firing patterns to include frequent doublets, especially in cells with high resting activity, prompts the following questions: a) are frequent doublet spikes the natural accompaniment of high activity in CVM; or b) does the higher prevalence of doublets after clonidine indicate a direct effect of clonidine on CVM ? The analysis illustrated in figure 5 c,d found that the relation between the incidence of doublets and mean firing rate is altered by clonidine, strongly supporting the latter view. Sixth, could these volleys of action potential increase acetylcholine (Ach) release at the level of the sinus node ? This may indeed be the case [39-40]. It is established that grouped, high frequency stimulation patterns are more effective than tonic patterns at releasing neurotransmitters [41-42]. Furthermore, “larger concentrations of [Ach] seem to produce a more than proportional inhibitory lengthening” of the RR interval [43]. These factors could all enhance the effectiveness of cardiac slowing following clonidine. The recruitment of CVM and their doublet or triplet firing following clonidine may also underlie the increase in beat-by-beat sinus arrhythmia under resting conditions (figure 6). Finally, the observation of a changed pattern of firing strongly supports the study of CVM at single unit level as *the* tool to delineate the intrinsic relationship between cardiac vagal activity and sinus arrhythmia, during pharmacological or physiological manipulation, as opposed to observation of plain HR or of vagal recording.

However, the demonstration of a definite link between changed CVM firing pattern and improved pressure lability would be difficult : pressure lability is almost non-existent in anesthetized rats. Could this be applied to conscious hypertensive humans ? After clonidine, as each increase in pulse pressure is followed by a volley of action potential, and possibly larger acetylcholine release, any minor increase in vasomotor sympathetic activity and corresponding surge in pressure is followed within the same beat [44] by a larger reduction in instantaneous HR, as opposed to preceding instantaneous HR. This is compatible with the observation that BP lability is almost totally suppressed while HR variability is largely enhanced in hypertensive humans recovering from major surgery and treated with clonidine (compare respective BP and HR traces in figures 3B vs. 3D in [2]).

Finally, the recruitment of silent unit (figure 4) and the changes in firing patterns (figure 5) may be brought together with an increased slope of the SBP-CVM activity relationship (figure 8F) : activity increases *while* pressure decreases, suggestive of a resetting and of increased sensitivity of the central limb of the cardiac baroreflex. Such an increased sensitivity of the integrative mechanism fits with the increase in SBP-RR relationship, previously repeatedly demonstrated [12-22-24].

Can this pharmacological increase in cardiac baroreflex sensitivity be related to similar phenomenon under physiological condition [45] ? An excitatory amino-acid, DL homocysteic acid, micro-ejected in the ventrolateral periaqueductal grey matter (PAGvl), enhances the bradycardia evoked by stimulation of the aortic depressor nerve [46]. Thus, the pharmacological stimulus, clonidine, may mimic the physiological condition, PAGvl stimulation, in sensitizing the central limb of the cardiac baroreflex [46].

PERSPECTIVES

Firstly, the cardiac vagal activity is viewed as a key target to manipulate the cardiovascular system [6]. If an increased routine for CVM recording leads to higher success rate, the present model will allow pharmacological investigation. For example, B-natriuretic peptide increases CVM activity during *von Bezold-Jarisch* reflex [47]. Also, some drugs increase the slope of the cardiac baroreflex (see introduction). Because of the therapeutic importance of the cardiac vagal activity [6], these drugs would benefit from an analysis of the cardiac baroreflex at CVM level : this model should bring molecules to activate the cardiac vagal activity without side effects. Secondly, the change in firing pattern and increased slope observed here may be related to reduced pressure lability [2] (see above). Outcome is linked to beat-by-beat pressure lability, independently from the *mean* level of pressure [48-51] : would reduced lability following alpha-2 agonist be linked to improved outcome in hypertensive patients ? Thirdly, low HR variability and low baroreflex sensitivity are also associated with poor outcome following myocardial infarction [5]. Some CVM present dromotropic [52] and possibly bathmotropic properties. However, they have not yet been electrophysiologically identified. Given the therapeutic importance of cardiac vagal activity in relationship to arrhythmia, this matter should be deciphered. Lastly, the present findings may be of interest as cardiac vagal activity is defective in CHF [4] and as clonidine improves symptoms of CHF [17]. This matter is of importance as clonidine is no longer widely used in the cardiology setting for the treatment of HBP and CHF, at variance with its use in the anesthesia/critical care setting. Thus examining the effects of newer centrally acting agents (rilmelidine, monoxidine) on CVM and pressure lability may strengthen the rationale for impacting centrally the cardiovascular system in HBP and CHF.

Peripherally- and centrally-acting hypotensive agents respectively inhibit and activate cardiac vagal motoneurons. Clonidine changes the firing pattern of cardiac vagal motoneurons toward volleys of action potentials. Finally, the sensitivity of the central limb of the cardiac baroreflex is increased. Given the sympatholytic and parasympathomimetic effect of alpha-2 agonists, these properties may explain the reduced pressure lability observed in HBP following centrally-acting agents.

1. Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertinieri G et al. Blood pressure variability in man : its relation to high blood pressure, age and baroreflex sensitivity. Clin.Sci. 1980; 59: 401s-404s.

2. Parlow J, Begou G, Sagnard P, Cottet-Emard JM, Levron JC, Annat G et al. Cardiac baroreflex during the postoperative period in patients with hypertension : effect of clonidine. Anesthesiology 1999; 90: 681-692.

3. Julius S, Pascual AV, London R. Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension. Circulation 1971; 44: 413-418.

4. Eckberg DL, Drabinsky M, Braunwald E. Defective cardiac parasympathetic control in patients with heart disease. N.Engl.J.Med. 1971; 285: 877-883.

5. La Rovere MT, Bigger JT, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998; 351: 478-484.

6. Townend JN Littler WA. Cardiac vagal activity : a target for intervention in heart disease. Lancet 1995; 1: 937-938.

7. Bigger JT & Schwartz PJ. Markers of vagal activity and the prediction of cardiac death after myocardial infarction. In: Levy MN, Schwartz PJ (editors). Vagal control of the heart : experimental basis and clinical implications. Armonk: Futura; 1994. pp. 481-508.

8. Hopkins DA, Bieger D, deVente J, Steinbusch WM. Vagal efferent projections : viscerotopy, neurochemistry and effect of vagotomy. Progress in Brain Research 1996; 107: 79-96.

9. Nosaka S, Yamamoto T, Yasunaga K. Localization of vagal cardioinhibitory preganglionic neurons within rat brain stem. J Comp Neur 1979; 186: 79-92.

10. McAllen RM Spyer KM. The baroreceptor input to cardiac vagal motoneurones. J.Physiol.(London) 1978; 282: 365-374.

11. Rentero N, Cividjian A, Trevaks D, Pequignot JM, Quintin L, McAllen RM. Activity patterns of cardiac vagal motoneurons in rat nucleus ambiguus. Am.J.Physiol. 2002; 283: R1327-R1334.

12. Sleight P, West MJ, Korner PI, Oliver JR, Chalmers JP, Robinson JL. The action of clonidine on the baroreflex control of heart rate in conscious animals and man and on single aortic baroreceptor discharge in the rabbit. Archives of International Pharmacodynamy and Therapeutics 1975; 214: 4-11.

13. Gilbey MP, Jordan D, Richter DW, Spyer KM. Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. J.Physiol.(London) 1984; 356: 65-78.

14. Mendelowitz D. Advances in parasympathetic control of heart rate and cardiac function. NIPS 1999; 14: 155-161.

15. Girard A, Fevrier B, Elghozi J-L. Cardiovascular variability after rilmelidine challenge : assessment of acute dosing effects by mean of spectral analysis. Fundamental and Clinical Pharmacology 1995; 9: 366-371.

16. Parati G, Di Rienzo M, Ulian L, Santucciu C, Girard A, Elghozi J-L et al. Clinical relevance of blood pressure variability. J.Hypertension 1998; 16: S25-S33.

17. Manolis AJ, Olympios C, Sifaki M, Handanis S, Bresnahan M, Gavras I et al. Suppressing sympathetic activation in congestive heart failure : a new therapeutic strategy. Hypertension 1995; 26: 719-724.

18. Tank J, Diedrich A, Szczech E, Luft FC, Jordan J. Alpha-2 adrnergic transmission and human baroreflex regulation. 43 2004; 1035-1041.

19. Kobinger W Walland A. Evidence for a central activation of a vagal cardiodepressor reflex by clonidine. Eur.J.Pharmacol. 1972; 19: 203-209.

20. Kobinger W Walland A. Modulating effect of central adrenergic neurons on a vagally mediated cardioinhibitory reflex. Eur.J.Pharmacol. 1973; 22: 344-350.

21. Laubie M, Schmitt H, Drouillat M. Action of clonidine on the baroreceptor pathway and medullary sites mediating vagal bradycardia. Eur.J.Pharmacol. 1976; 38: 293-303.

22. Korner PI, Oliver JR, Sleight P, Chalmers JP, Robinson JS. Effect of clonidine on the baroreceptor-heart rate reflex and on single aortic baroreceptor fibre discharge. Eur.J.Pharmacol. 1974; 28: 189-198.

23. Robson RD Kaplan HR. An involvement of ST155 [2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, Catapress R) in cholinergic mechanisms. Eur.J.Pharmacol. 1969; 5: 328-337.

24. Tank J, Jordan J, Diedrich A, Obst M, Plehm R, Luft FC et al. Clonidine improves spontaneous baroreflex sensitivity in conscious mice through parasymapthetic activation. Hypertension 2004; 43: 1042-1047.

25. Cividjian A, Rentero N, McAllen RM, Quintin L. A simple method for generating a blood pressure-unit activity relationship for central cardiovascular neurons in the rat. Experimental Physiology 2002; 87: 535-538.

26. Quintin L, Gillon JY, Saunier CF, Chouvet G, Ghignone M. Continuous volume infusion improves circulatory stability in anesthetized rats. J.Neurosci.Methods. 1989; 30: 77-83.

27. Kuras A Gutmaniene N. Preparation of carbon-fibre microelectrode for extracellular recording of synaptic potentials. J.Neurosci.Methods. 1995; 62: 207-212.

28. Lipski J. Antidromic activation of neurones as an analytical tool in the study of the central nervous system. J.Neurosci.Methods. 1981; 4: 1-32.

29. Faris I, Iannos J, Jamieson G, Ludbrook J. Comparison of methods for eliciting the baroreceptor-heart rate reflex in conscious rabbits. Clin Exp Pharmacol Physiol 1980; 7: 281-291.

30. Coleman TG. Arterial baroreflex control of heart rate in the conscious rat. Am.J.Physiol. 1980; 238: H515-H520.

31. Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. Circ.Res. 1969; 24: 109-121.

32. Guyenet P. Role of the ventral medulla oblongata in blood pressure regulation. In: Loewy AD, Spyer KM (editors). Central Regulation of Autonomic Functions. Oxford: Oxford University Press; 1990. pp. 145-167.

33. Armstrong JM, Lefevre-Borg F, Scatton B, Cavero I. Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. J Pharmacol Exp Ther 1982; 223: 524-535.

34. Katona PG, Lipson D, Dauchot PJ. Opposing central and peripheral effects of atropine on parasympathetic cardiac control. Am.J.Physiol. 1977; 232: H146-H151.

35. Sun MK Guyenet P. Effect of clonidine and gamma-aminobutyric acid on the discharges of medullo-spinal sympathoexcitatory neurons in the rat. Brain Res. 1986; 368: 1-17.

36. Pickering TG, Gribbin B, Sleight P. Comparison of the reflex heart rate response to rising and falling arterial pressure in man. Cardiovasc.Res. 1972; 6: 277-283.

37. Guyenet P Cabot JB. Inhibition of sympathetic preganglionnic neurons by catecholamines and clonidine : mediation by an alpha-adrenergic receptor. J.Neurosci. 1981; 1: 908-917.

38. Parlow J, Viale JP, Annat G, Hughson RL, Quintin L. Spontaneous cardiac baroreflex in humans : comparison with drug-induced responses. Hypertension 1995; 25: 1058-1068.

39. Levy MN, Iano T, Zieske H. Effects of repetitive bursts of vagal activity on heart rate. Circ.Res. 1972; 30: 186-195.

40. Dexter F, Yang T, Levy MN. Analysis of vagally induced sinus arrhythmia. J Theor Biol 1992; 159: 507-511.

41. Gonon F. Non-linear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 1988; 24: 19-28.

42. Pernow J, Schwieler J, Kahan T, Hjemdahl P, Oberle J, Wallin BG et al. Influence of sympathetic discharge pattern on norepinephrine and neuropeptide Y release. Am.J.Physiol. 1989; 257: H866-H872.

43. Brown GL Eccles JC. The action of a single vagal volley on the rhythm of the heart beat. J.Physiol.(London) 1934; 82: 211-241.

44. DeBoer RW, Karemaker JM, Strackee J. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. Am.J.Physiol. 1987; 253: H680-H689.

45. Hilton SM Spyer KM. Participation of the anterior hypothalamus in the baroreceptor reflex. J.Physiol.(London) 1971; 218: 271-293.

46. Inui K, Murase S, Nosaka S. Facilitation of the arterial baroreflex by the ventrolateral part of the midbrain periaqueductal grey matter in rats. J.Physiol.(Lond) 1994; 477: 89-101.

47. Toader E, McAllen RM, Cividjian A, Woods RL, Quintin L. Effect of systemic B-type natriuretic peptide on cardiac vagal motoneuron activity. Am.J.Physiol. 2007; 293: H3465-H3470.

48. Parati G, Pomidossi G, Albini F, Malaspina D, Mancia G. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. J.Hypertension 1987; 5: 93-98.

49. Kikuya M, Hozawa A, Ohokubo T, Tsuji I, Michimata M, Matsubara M et al. Prognostic significance of blood pressure and heart rate variabilities : the Ohasama study. Hypertension 2000; 36: 901-906.

50. Sander D, Kukla C, Klingelhofer J, Winbeck K, Conrad B. Relationship between circadian blood pressure patterns and progression of early carotid artherosclerosis : a 3 year follow up study. Circulation 2000; 102: 1536-1541.

51. Aronson S, Fontes ML, Miao Y, Mangano DT. Risk index for perioperative renal dysfunction/failure : critical dependence on pulse pressure hypertension. Circulation 2007; 115: 733-742.

52. Massari VJ, Johnson TA, Gatti PJ. Cardiotopic organization of the nucleus ambiguus ? an anatomical and physiological analysis of neurons regulating atrioventricular conduction. Brain Res 1995; 679: 227-240.

Neurone	Protocol	Data inclusion	Latency (ms)	Refractory period (ms)	Collision time (ms)	Firing rate (spikes/s : Hz)	Conduction velocity (m/s)
N1	CLO	****	6.78	3.2	7.36	1.83	10.32
N2	CLO	*	5.26	2.6	6.54	3	13.31
N3	CLO	***					
N4	CLO	**	7.56	5.8	8.42	7.5	9.26
N5	CLO	**	10.91	2	12.35	9.7	6.42
N6	CLO	*	5.03	3.94	5.85	6.5	13.92
N7	CLO	***	10.02	3	10.36	7.5	6.99
N8	CLO	**	5.51	5	6.7	0	12.7
N9	CLO	***					
N10	CLO	****	6.71	1.5	7.5	1	10.43
N11	NPS	**	5.66	1.54	6.43	0.01	12.37
N12	NPS	**	7.52	1.76	8.71	1	9.31
N13	NPS	**	8.63	3	9.7	4.5	8.11
N14	NPS	**	8.55	4.4	12.2	0.15	8.19
N15	NPS	**	7.66	3.17	9.78	1	9.14
N16	CLO	****	8.18	3	10.8	0.4	8.56
N17	CLO	**	12.21	4	14	1.3	5.73
N18	CLO	**	5.51	1.9	6.22	0.1	12.70
Mean			**7.7**	**3.1**	**9.0**	**2.9**	**9.8**
SD			**2.2**	**1.3**	**2.5**	**3.3**	**2.6**

Table1: CVM characteristics. Data were obtained in different animals. Latency is measured between the stimulation artefact and the generated action potential. * indicates CVM not included in presentation (see results); ** : CVM, HR and SBP data included in analysis; *** : HR and SBP data included in analysis; **** : CVM and SBP data included in analysis.

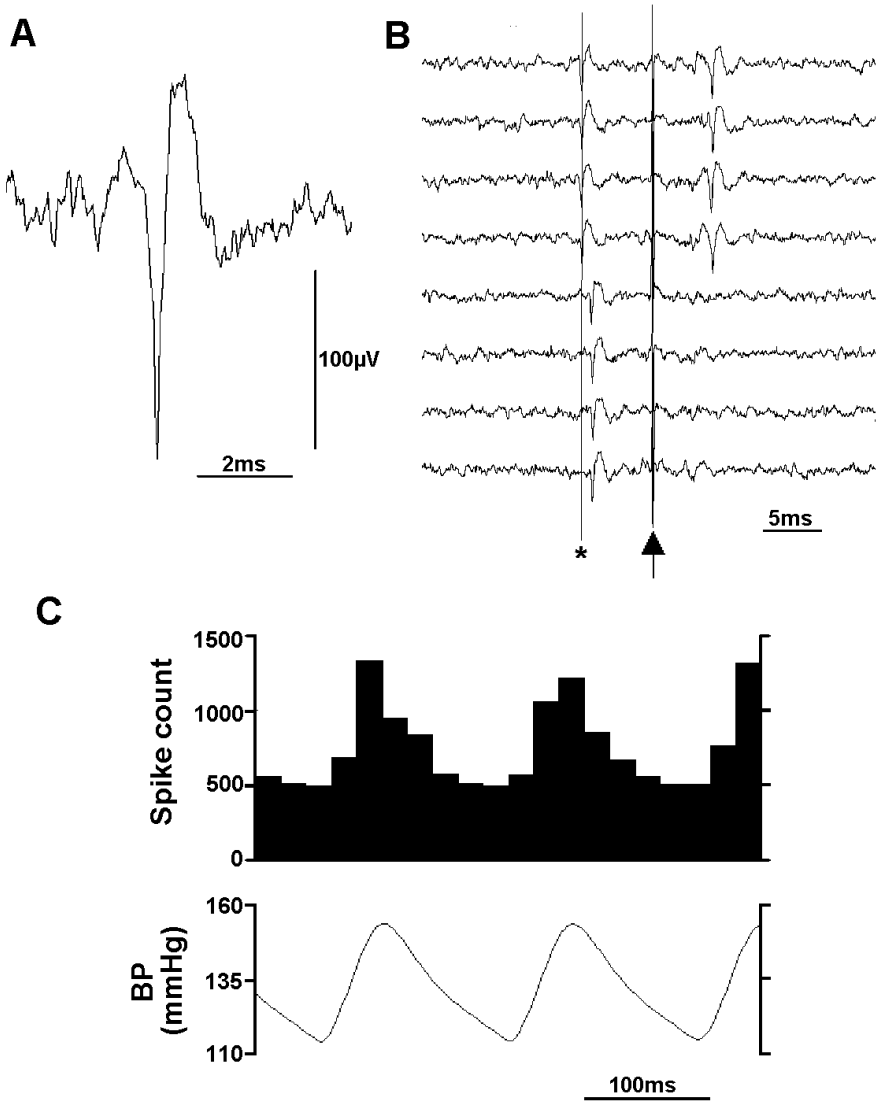

Figure 1 : Identification of a cardiac vagal motoneuron (CVM). A: Spontaneous discharge of a CVM. B: Collision test with a stimulus (artefact at vertical arrow) triggered 6 ms (*top* 4 traces) and 5 ms (*bottom* 4 traces) after a spontaneous spike (*left* side). The CVM gives a constant-latency, antidromic spike for a 6 ms delay but the depolarization is cancelled by the orthodromic spike when the delay is 5 ms. Vertical line marked by star is traced at 6ms. C: Pulse-locked character of the unit (barosynchronicity): upper trace shows histogram of same CVM spontaneous activity (6703 cycles, 20ms bins), locked to arterial pulse pressure (lower trace).

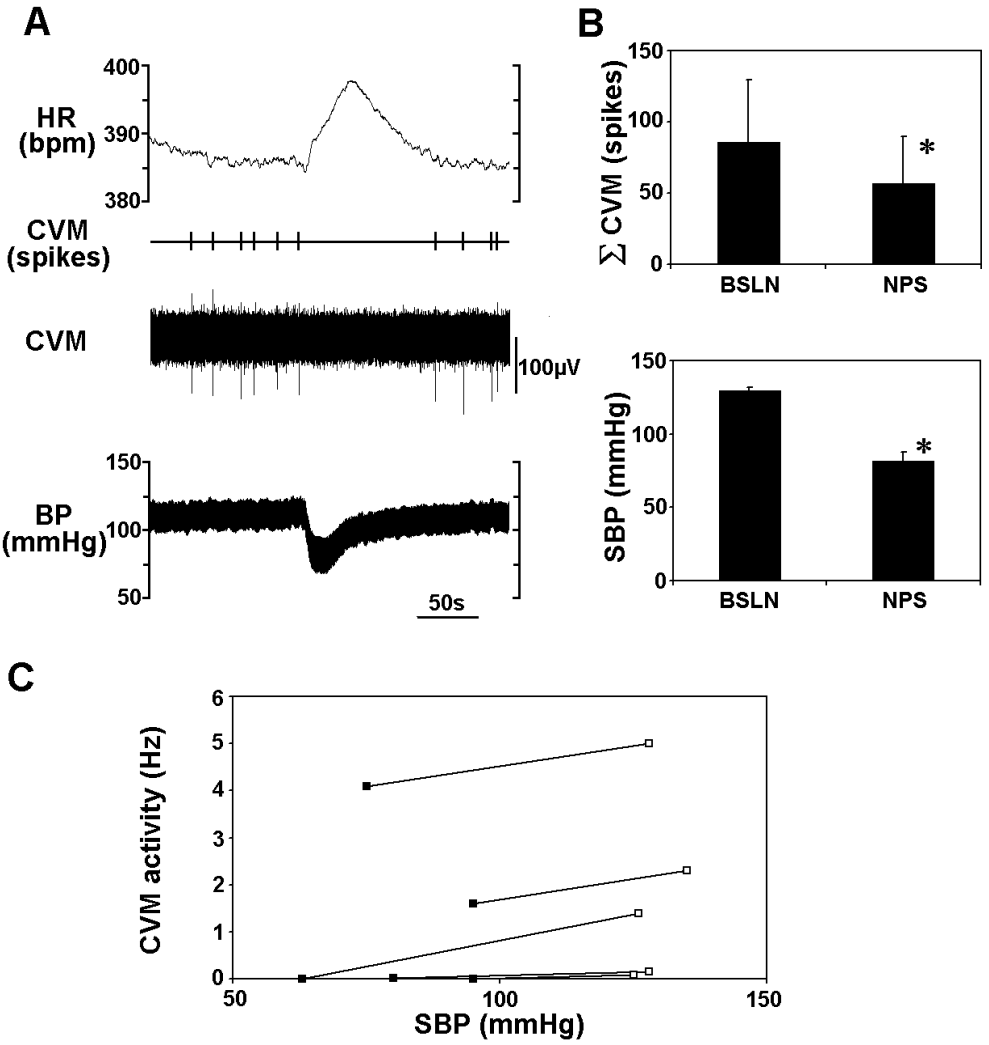

Figure 2 : A. CVM activity decreases during pressure fall evoked by nitroprusside. From bottom to top : BP, raw CVM signal, CVM detected spikes, HR (from EKG). B. CVM activity (*top*) decreases when nitroprusside is administered in 5 out of 5 rats. *-$p<0.05$ vs. Baseline for the considered variable. (n=5 cells in 5 rats). The total CVM spike counts was measured for 50s before [baseline] and 50s immediately after injection [response]. C. Plot shows that nitroprusside decreases CVM activity and SBP in 5 out of 5 rats. Baseline: open squares; nitroprusside : closed squares.

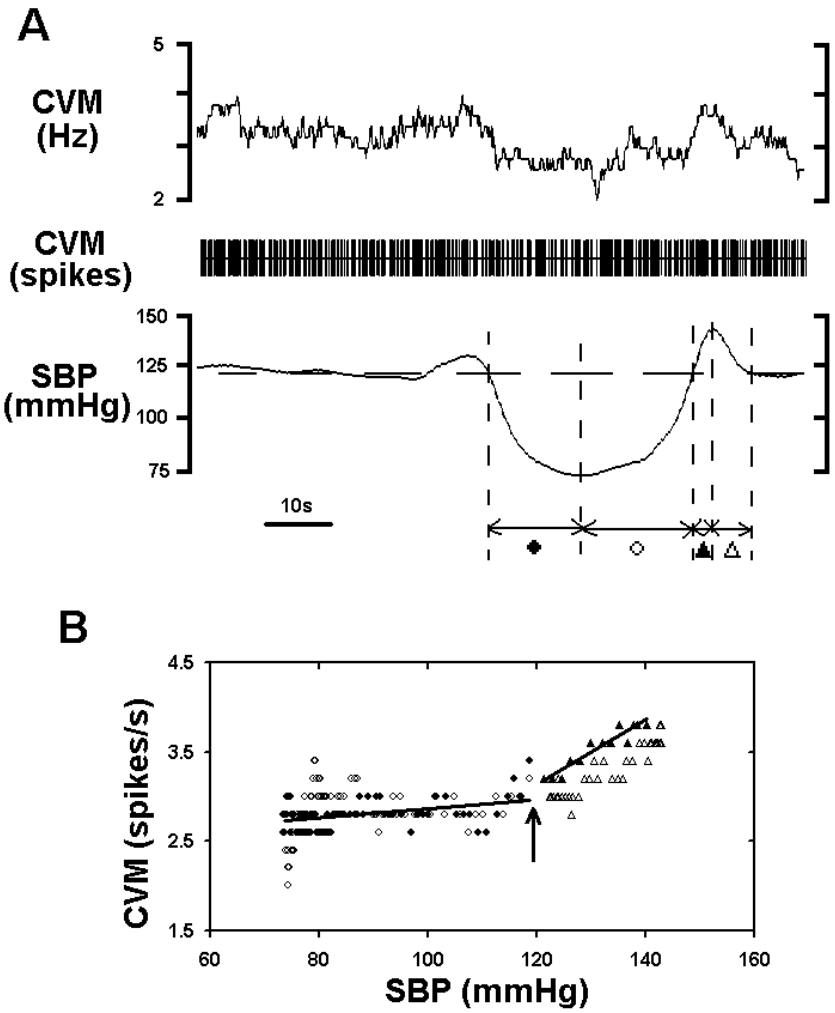

Figure 3 : A. Typical trace of CVM activity following nitroprusside-phenylephrine challenge. Phenylephrine was injected immediately following the nadir in pressure. Traces from bottom to top : SBP, CVM detected spikes, CVM (Hz). CVM activity and SBP averaged over 6 ventilatory cycles. Horizontal line corresponds to baseline SBP level. Vertical lines show pressure inflection points with corresponding symbols in figure B. B. Relation between the CVM activity and SBP for the sequence in A. Vertical arrow : baseline SBP and CVM activity. Closed circles = pressure fall after nitroprusside; open circles = pressure rising to baseline level. Closed triangles = pressure rise after phenylephrine; open triangles = pressure falling back to baseline. The solid lines are the regression lines relating the systolic pressure and the CVM activity during fall and rise in pressure (closed circles and triangles respectively; R^2=0.1663, R^2=0.9069; n=1 cell). Data may also fit a sigmoid (R^2=0.63, not shown).

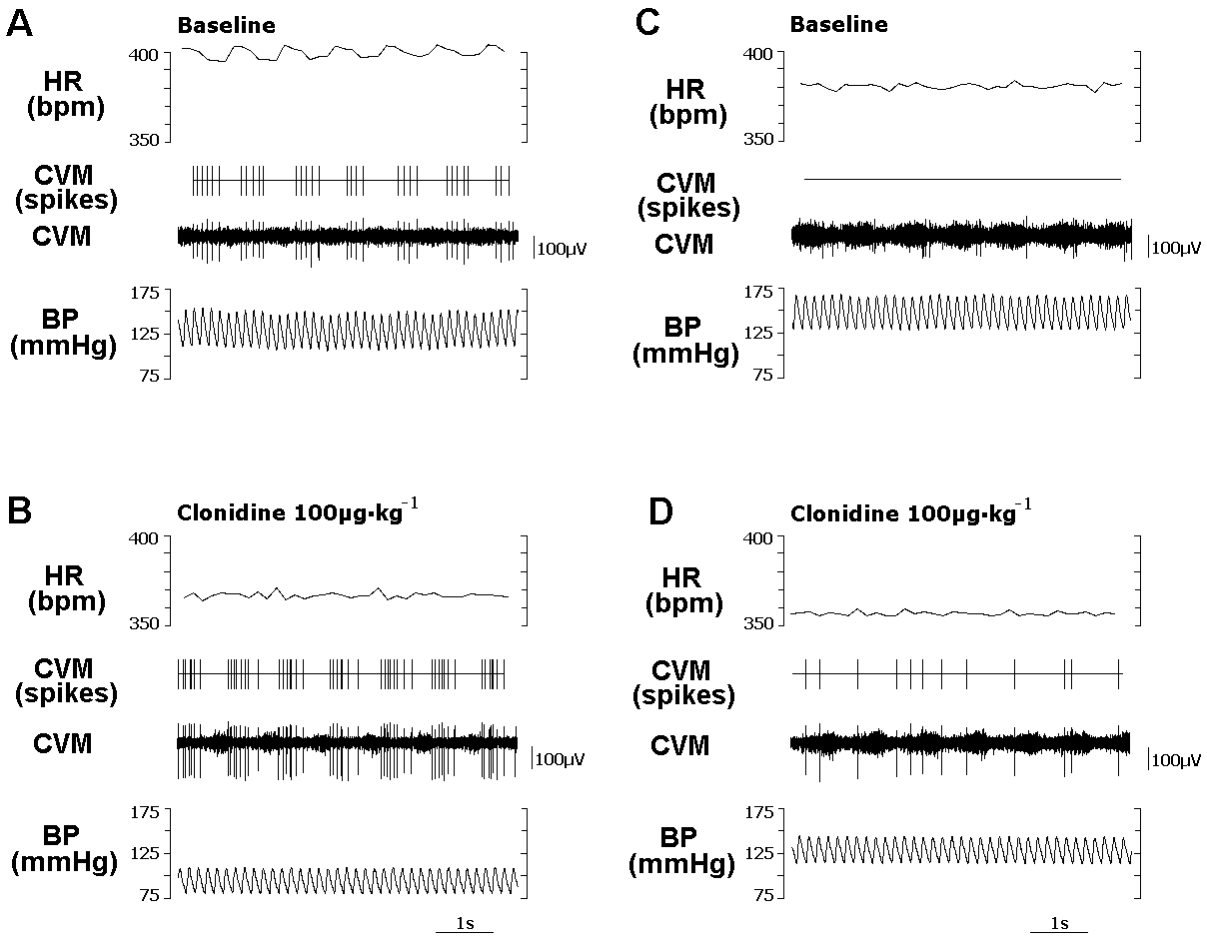

Figure 4 : Activation of CVM unit following clonidine 100 µg.kg^{-1} i.v. A,B : typical trace for one active unit at baseline. C,D : typical trace for one inactive unit at baseline. Traces from bottom to top : pulse pressure (BP, mm Hg), CVM activity (raw signal), CVM detected spikes, Heart rate (HR from EKG).

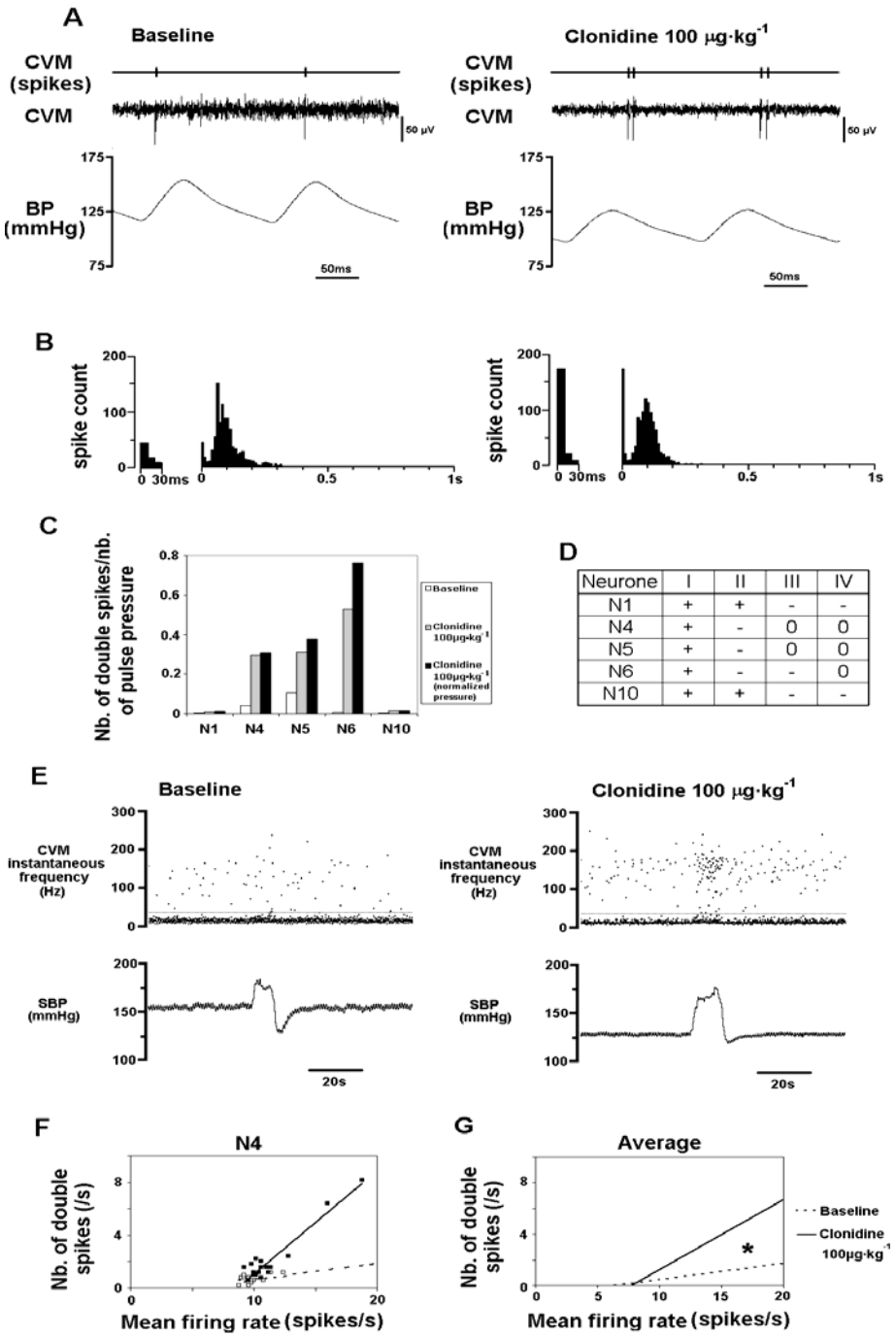

Figure 5 : Interspike intervals decrease following clonidine 100µg.kg^{-1} i.v (n=5 cells). A: Single spike (left) and double spikes ("doublet"; right : spikes with interspike interval<30 ms). Note the increase in double spikes after clonidine 100µg.kg^{-1} under resting conditions. Traces (bottom to top) : pulse pressure (BP), raw CVM activity, detected CVM spikes. B: Interspike interval histogram of the same CVM in baseline (left) and clonidine 100 µg.kg^{-1} i.v. (right) conditions. Each histogram has at left a time-axis zoom (3x) of the 0-30ms interval. Note the large increase of firing in the 0-30 ms interval Numbers of triggers (*baseline, clonidine 100 µg.kg^{-1}*): 988, 1119; C: The number of double spikes per pulse pressure increases in 5 cells, especially when number of double spikes are normalized to baseline pressure. D: Changes in interspike interval (expressed as percentage of interspike interval : + for increasing, - for decreasing and 0 for unchanged) after clonidine 100µg.kg^{-1} . Interspike intervals are divided in four ranges : I : 0-30ms (double spikes); II : 30-600 ms; III : 600-1300 ms, IV, >1300 ms. Firing increases in the double spike interval range. By contrast, firing decreases in longer interspike interval ranges, indicative of tighter coupling between BP and CVM activity. E: The increase in double spike is even more prominent during pressure rise (balloon inflation) following clonidine 100 µg.kg^{-1} i.v. Left and Right : Typical traces at baseline and following clonidine 100 µg.kg^{-1} respectively. Traces (bottom to top) : CVM instantaneous firing frequency (Hz), pulse pressure (BP, mm Hg). Horizontal line shows frequency corresponding to 30ms interspike interval. Dots above horizontal line correspond to double spikes. F : number of double spikes vs. mean firing rate for 1 cell before (open symbols and dotted regression line) and after clonidine 100µg.kg^{-1} i.v. (closed symbols and solid regression line). G : aggregated data for 5 cells in 5 different rats *:p=0.043 for slopes.

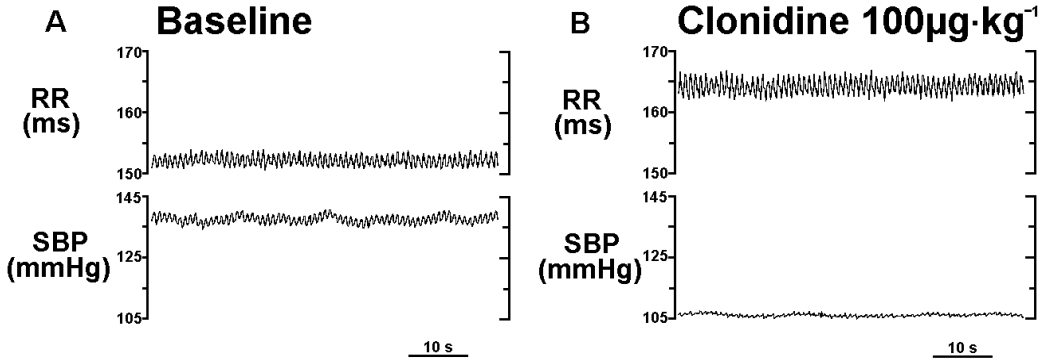

Figure 6. Raw traces of RR interval (*top*) and systolic blood pressure (SBP, *bottom*) in one rat during baseline (A) and clonidine 100 µg.kg-1 i.v. (B)

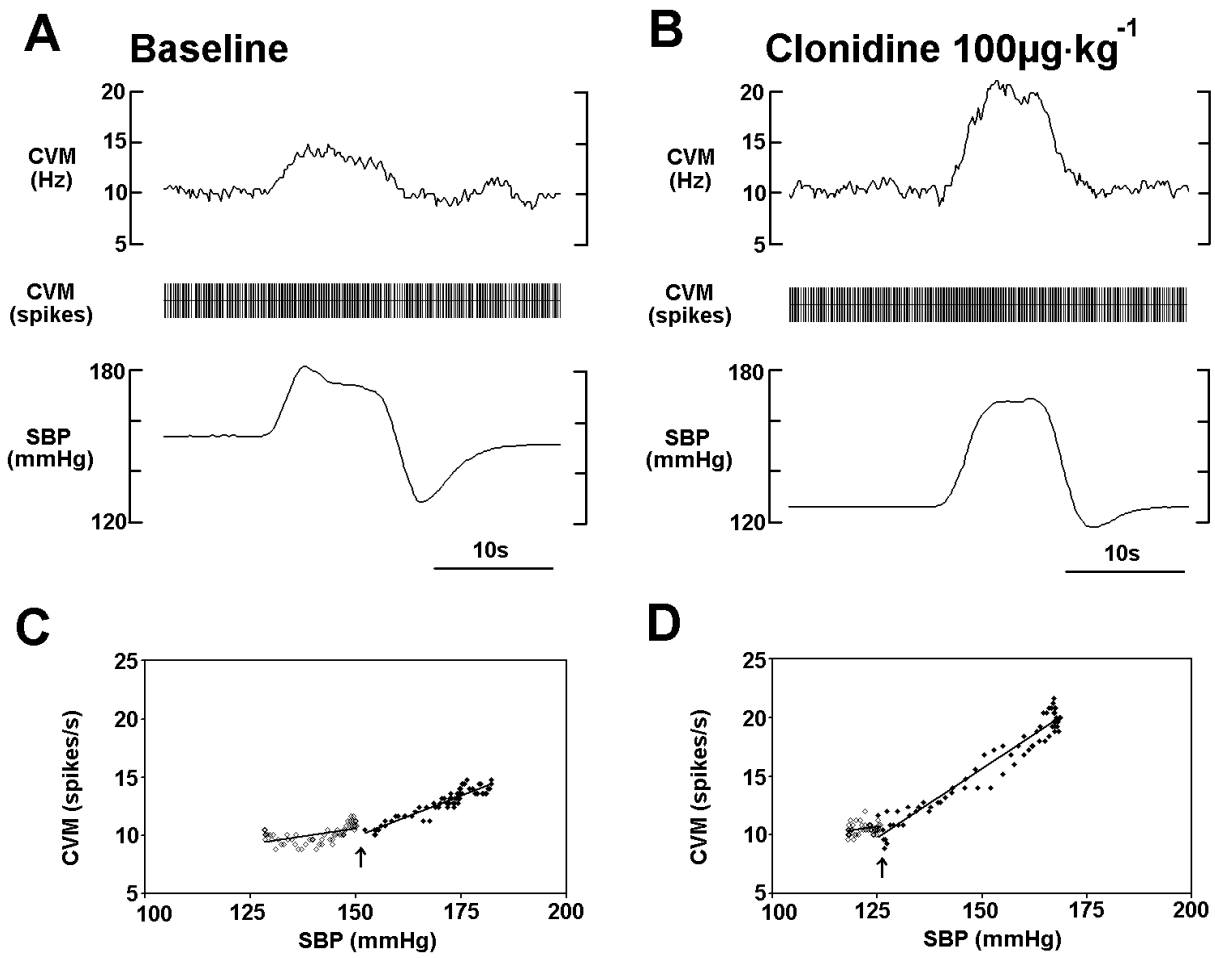

Figure 7 : Typical trace of CVM activity following balloon inflation (*top*) in baseline (A) and clonidine 100 µg.kg^{-1} i.v. (B) conditions. Traces from bottom to top : SBP, CVM detected spikes, CVM (Hz). CVM activity and SBP averaged over 3 ventilatory cycles. Scatter plot of mean firing rate vs. mean systolic blood pressure for a CVM during balloon inflation in baseline (C) and clonidine 100 µg.kg^{-1} i.v. (D) conditions. The linear regression lines for the relations are shown (R^2=0.33 and 0.07 for reduction in pressure for baseline and clonidine 100.µg.kg-1 respectively; R^2=0.87 and 0.95 for rises in pressure for baseline and clonidine 100 µg.kg^{-1} respectively). arrow : resting SBP and CVM activity.

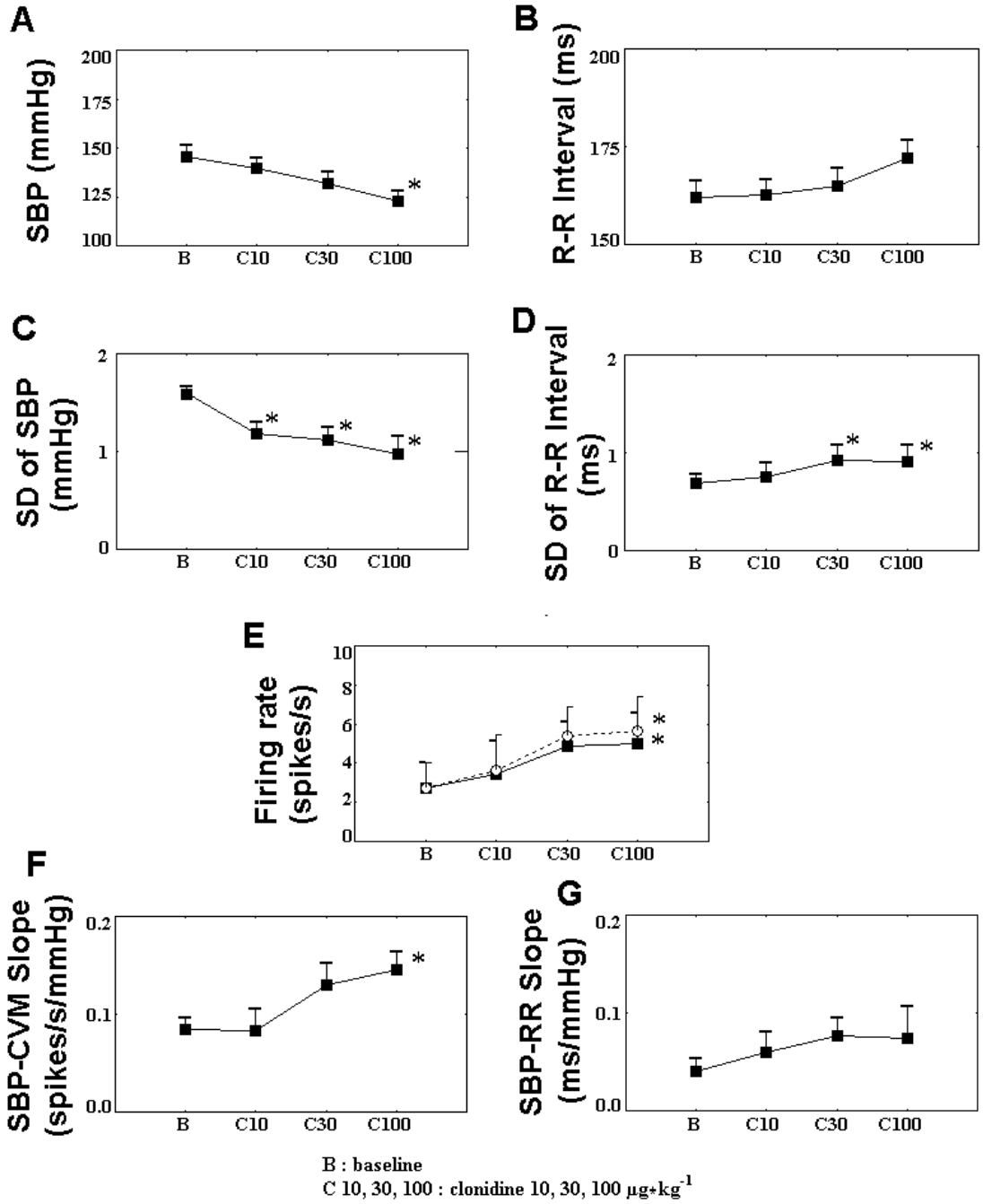

Figure 8 : Effect of cumulative doses of i.v. clonidine on circulatory variables, unit activity and cardiac baroreflex analysed at CVM and heart levels (n=8 cells in 8 different rats). A: Systolic blood pressure (SBP). B: R-R interval. C: Standard deviation of SBP. D: Standard deviation of R-R interval. E: Closed squares: mean firing rate. Open circles: normalized firing rate : mean firing rate multiplied by the ratio between SBP at baseline and current SBP. F: slope of SBP-CVM activity (cardiac baroreflex analysed at CVM level) during balloon inflation. Note the increased sensitivity. G: slope of SBP-R-R interval (cardiac baroreflex analyzed at heart level) during balloon inflation. B : Baseline; C10 : clonidine 10 µg.kg^{-1} i.v; C30 : clonidine 30 µg.kg^{-1} i.v., C100 : clonidine 100 µg.kg^{-1} i.v. * : $p<0.05$ vs. baseline for the considered variable.

III. Discussion

Methods

In this study, CVMs were recorded in the NA of the rat. Rigorous identification was used (96-98; 119). Following antidromic stimulation from the cardiac branch of the vagus, constant latency units were confirmed by collision-testing and barosensitivity (**ms1 table 1** and **ms1 figure 1**).

The originality in the methods used in this work is the SBP-CVM relationship (**ms1 figure 3** and **7**). This is similar to the SBP-RR relationship (124), but constitutes an analysis at the central level of the cardiac baroreflex which is opened. Here, this was called cardiac baroreflex analysed at CVM level. This relationship is similar to the one presented in a study of the sympathetic neurones located in the RVLM (**figure 8**) (51).

Hypotension and tachycardia appear after nitroprusside administration (**ms1 figure 2,3**). Indeed, nitroprusside is a peripheral vasodilator, and thus the BP decreases. CVM activity decreases too, and HR generally increases, in the first seconds after the bolus is administered. In fact, the diminished CVM activity together with the correlated increased HR are the effect of the cardiac baroreflex.

Hypotension and bradycardia appear after clonidine administration (**ms1 figure 4** and **8 A,B**). Hypotension is a consequence of sympathetic system inhibition. The decrease in HR was short of significance, perhaps due to the small sample or to the use of urethane.

The CVM activity increases following clonidine administration, as expected. In contrast, the CVM firing rate decreases when nitroprusside bolus injection is administered. This clearly confirms the different actions of these two drugs at central level. One increases the cardiac vagal activity while the other one silences it, presumably as a secondary effect of the induced hypotension. In another study, clonidine decreased RVLM sympathetic neurons activity, while nitroprusside increased their firing rate (126). Thus, these drugs have an opposite effect on the parasympathetic and sympathetic limbs of the cardiac baroreflex.

The firing pattern of CVM is changed by clonidine. When interspike intervals are generated for the CVM activity, a shift towards the lower intervals is obvious after clonidine administration (**ms1 figure 5 B,C,D**). This could be partly explained by the fact that clonidine increases the CVM activity. However, a more complex phenomenon where spontaneous, fast firing patterns are improved by clonidine might be involved (**ms1 figure 5 A**). In an attempt to perform an interspike interval analysis, four domains corresponding to different physiological states of the CVM were used (**ms1 figure 5** legend). A

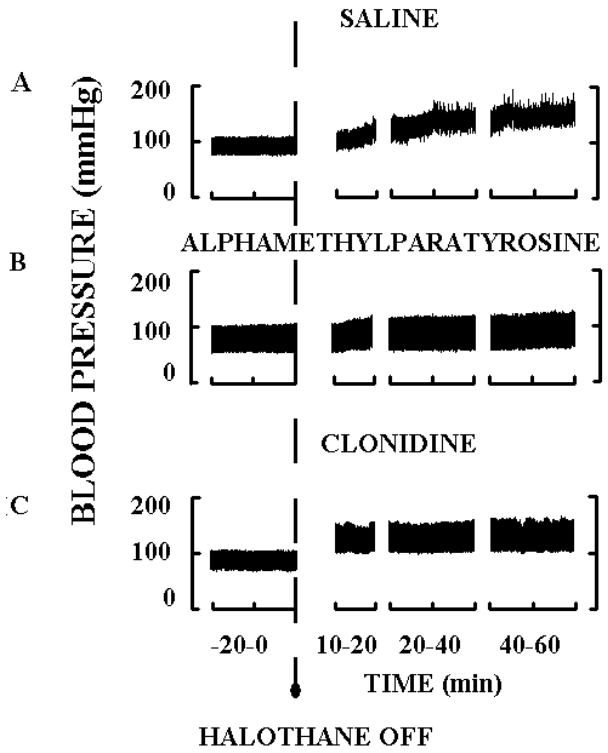

Figure 23. Typical pressure trace in a paralyzed rat upon halothane discontinuation

BP lability increases after halothane discontinuation. Movements (vibrissae, lower jaw, upper limbs, then lower limbs) are associated with a slow breakthrough on which several rapid breakthroughs are superimposed. Note that the slow and rapid breakthroughs are largely reduced when αmethylparatyrosine (B) or clonidine (C) is administered as compared to the control (A). (Cividjian, unpublished data)

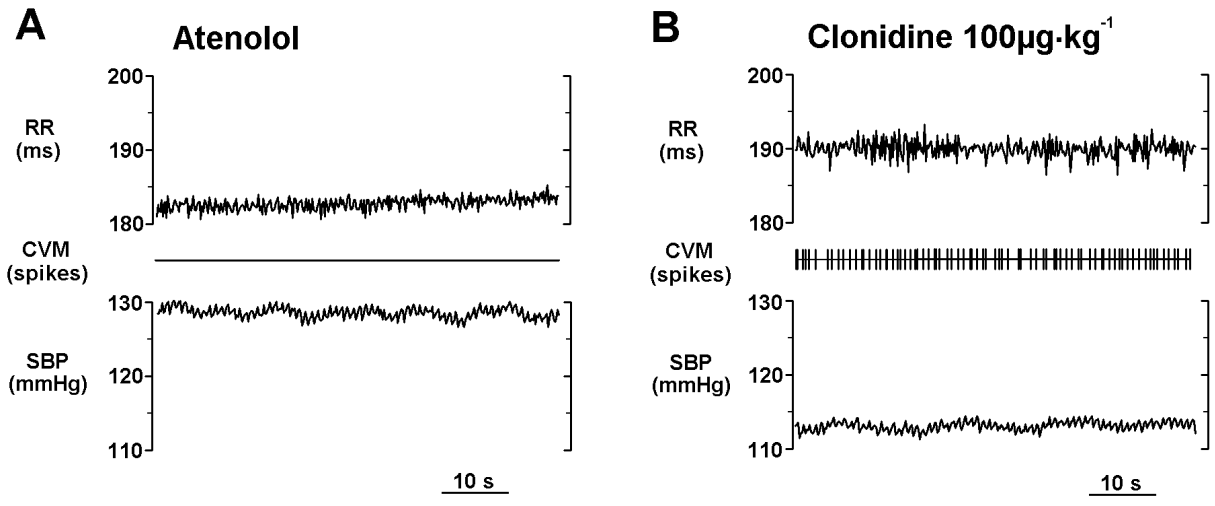

Figure 24. BP lability, sinus arrhythmia and CVM activity following cardiac vagal activation with clonidine 100 µg.kg^{-1} i.v.

Typical traces of RR interval (top), CVM activity (middle) and systolic blood pressure (SBP, bottom) in one animal following cardiac sympathetic blockade with a β-blocker, atenolol 2 mg.kg-1 (A) and clonidine 100 µg.kg^{-1} i.v. (B). Atenolol, which does not cross the blood brain barrier, does not modify the slope of the cardiac baroreflex. Note the reduction in pressure lability, increase in CVM activity and increase in sinus arrhythmia after clonidine. (Toader, submitted)

significant increase in percentage is observed in the lowest interspike domain (0-30ms), corresponding to the fast-firing state of the CVM. This was an unexpected finding. Actually, for the same mean firing frequency, different firing patterns have different effects on the output (47). Thus, a phasic discharge pattern might be more potent to generate bradycardia than a regular, tonic activity. This could be achieved by an increased Ach release at the level of the sinus node (31; 85). Thus, "larger concentrations of Ach seem to produce a more than proportional inhibitory lengthening" of the RR interval (17), in line with a possible non-linear functioning of the system. During pressure ramps the frequency of the double spikes (*doublets*) increased, together with the firing rate (**ms1 figure 5E**). This suggests that the increase in mean firing rate with clonidine might be a possible mechanism for the increase in percentage of doublets. However, the number of doublets divided by the mean firing rate is significantly increased with clonidine (**ms1 figure 5F,G**). This implies that at the same mean firing rate, the percentage of doublets is higher with clonidine. Therefore, clonidine increases the reactivity of the CVM, possibly by a direct action on alpha-2 receptors located on or close to CVM cell bodies themselves.

The BP lability and HR variability present, most often, an inverse variation. This is in line with previous studies (39). Both BP and RR interval variabilities significantly decrease and increase, respectively, with clonidine (**ms1 figure 6** and **8 C,D**). This is in agreement with previous findings and shows that the experimental set-up used in this study could be the model for analysing, at central level, the inverse relationship between HR variability and BP lability found in humans (**figure 2**) (91; 113).

In mammals, normal BP variability coexists with normal HR variability and normal cardiac baroreflex gain. By contrast, in sinoaortic baroreceptor-denervated animals the BP variability is increased and the cardiac baroreflex gain and HR variability are depressed (9; 28). Accordingly, several studies reported a coexistence of increased BP variability, decreased HR variability and depressed baroreflex gain (90; 113). Generally, the situation of normal BP variability, HR variability and baroreflex sensitivity in normal mammals, on the one hand, contrasts with the situation of increased BP variability, depressed HR variability and depressed baroreflex sensitivity in mammals showing a dysfunction of both the cardiac and vascular sympathetic and cardiac parasympathetic systems, on the other hand. In this study (ms1) an increased RR interval variability following clonidine administration suggests an increase of the parasympathetic outflow. This is highly correlated with the corresponding measured increase in CVM activity (**ms1 figure 8E**).

The sympatholytic effect of the clonidine might also be responsible for the reduction in BP lability. Previous studies done in rats show that a drug which blocks catecholamine synthesis, alphamethylparatyrosine, totally suppresses BP lability while cardiac vagal activity and baroreflex slope are lowered (**figure 23**) (23). In this model, sympathetic inhibition stands alone to explain the absence of pressure lability under environmental stress. However, the sympatholytic effect of clonidine is not responsible alone for the reduction in BP lability. In fact, in experiments done under β-blockade, using atenolol, clonidine reduced BP lability (**figure 24**). RR interval variability increased. The reduced

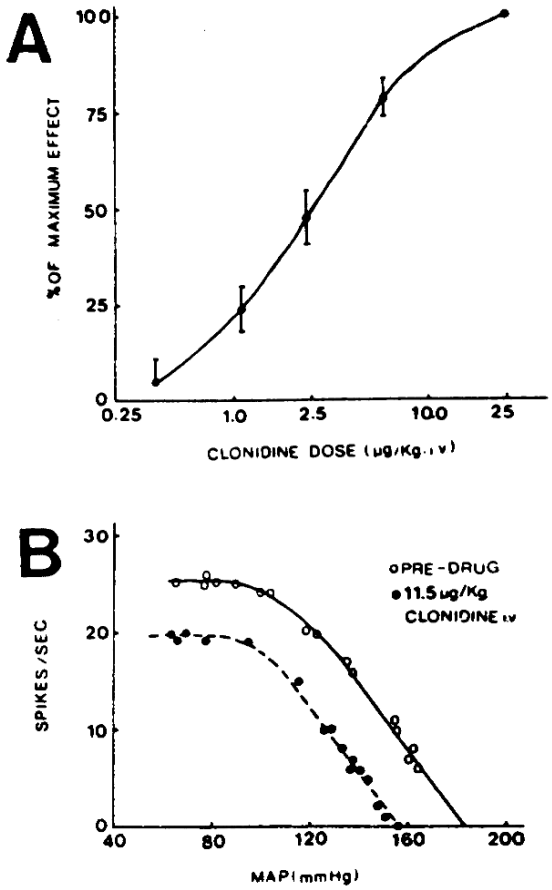

Figure 25. Effects of clonidine administration on the activity of sympathetic cardiovascular neurons in halothane-anesthetized rats

RVLM is critical in the maintenance of the sympathetic tone and in the operation of sympathetic limb of cardiac baroreflex. (A) Cumulative dose-response curve representing the hypotensive effect of clonidine (mean ± SEM, n=4; maximum effect 32mmHg). (B) Effect of clonidine on pressure-activity curve of a slow-conducting RVLM cardiovascular neuron with spinal projection presumably adrenergic. The parallel downward shift of response curve illustrates that despite a lowering of baseline sympathetic activity, responsiveness (reactivity) of sympathetic activity was preserved after clonidine administration. This illustrates that clonidine lowers pressure set-point but leaves intact reactivity of sympathetic efferent limb. The BP set-point is decreased due to a decrease in the output of the RVLM barosensitive bulbospinal neurons, which leads to a decreased SNS activity. Note that by contrast, clonidine increases both CVM set-point and reactivity, i.e. the SBP-CVM relationship (see **ms1 figure 7** and **8**). Figure reproduced from Sun (126).

BP lability can not be attributed in this case to the sympatholytic effect of clonidine, since the cardiac sympathetic system is already inhibited by atenolol. Therefore, BP lability decrease with clonidine (**figure 2** and **ms1 figure 6**) is the likely result of *two distinct but combined* phenomena : firstly, vascular sympathetic inhibition, and then, secondly, cardiac vagal activation that further fine tunes pressure on a beat-by-beat basis on top of a low background vascular sympathetic activity.

The cardiac baroreflex gain at central level (SBP-CVM relationship, **ms1 figure 7**) is clearly increased by clonidine. However, this was not the case for the cardiac baroreflex gain at peripheral level (SBP-RR relationship). Significant increase of the SBP-CVM slope (**ms1 figure 8F**) could be explained by:

a. Non-linearity of the CVM activity. The relation between BP and CVM is supposed to be linear. However, there is a reduced number of studies in this respect and so the hypothesis of linearity could be mistaken. Thus, for two different values of resting CVM activity, the reactivity of the CVM to changes in pressure might be different. This could be a mechanism of increasing the central cardiac baroreflex by improving the CVM reactivity.

b. A change in the firing pattern. If clonidine acts directly on the CVM, then it might be possible that for a same input to the CVM a doublet to be generated during clonidine instead of a single spike. This explanation implies an improved CVM reactivity, too.

c. Clonidine could also improve the neural input to the CVM. Indeed, an increased activity of the first and/or second order neurons in the reflex arc would increase the CVM activity at rest, but also the response of the CVM to a pressure rise.

Thus, clonidine increases cardiac vagal activity set point and increases the SBP-CVM slope. In a previous study that analysed the sympathetic command of the heart, clonidine decreased the set-point of RVLM neurons activity, but left unchanged the slope of the sympathetic limb of the cardiac baroreflex (**figure 25**) (126). This suggests an opposite mechanism of action (decreased set point, unchanged slope) for the sympathetic baroreflex as opposed to the parasympathetic baroreflex (increased set point and slope).

The SBP-RR interval slope is increased, but not significantly. This is in line with some previous studies done in rats (135) and humans (104). However, this was unexpected, since the SBP-CVM relationship was significantly increased by clonidine. Possible reasons could be:

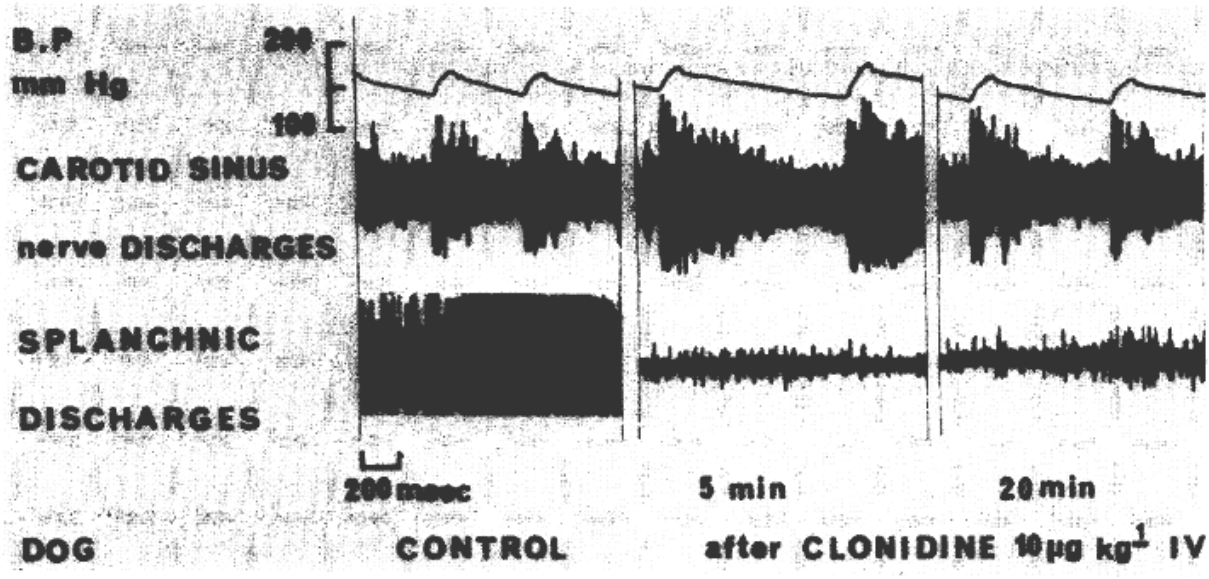

Figure 26. Action of clonidine (10 µg/kg i.v.) on the carotid sinus nerve and splanchnic discharges

In anaesthetized dogs, clonidine 10 pg/kg i.v. induced a transient hypertension, linked to blocking of peripheral extrasynaptic α2 receptors, followed by a long lasting hypotension. During the hypertensive phase, the sinocarotid nerve potentials were strongly increased (middle and right panels). When BP returned to its control value, the spontaneous firing of the carotid sinus nerve was still markedly increased. During the hypotensive phase, the discharges were no different from the discharges seen during the control phase or were decreased depending on the blood pressure. The fact that there was high neural activity in spite of a decrease in blood pressure is in support of an altered relationship between blood pressure and carotid sinus nerve discharges. In all these experiments, splanchnic discharges were reduced after i.v. injection of clonidine. Figure reproduced from Laubie (83).

a. The presence of stimulating electrodes on the cardiac branch. Indeed, the action potential could be blocked at the level of the electrodes, either by an electrophysiological phenomenon, or by a physiological damage of the nerve;

b. SBP-RR interval relationship is influenced by both sympathetic and parasympathetic systems. Normally, the sympathetic nervous activity is inhibited by clonidine at high-dose (126). Besides, care was taken when selecting the pressure ramp sequences. The sympathetic delay of action is greater than 5s, i.e. the period taken into consideration. Therefore, effects of sympathetic inhibition are unlikely;

c. A saturation phenomenon at the level of the heart. This hypothesis is unlikely, since stimulating the cardiac branch can even completely stop the heart. The bradycardia obtained during pressure rises is not the maximal bradycardia that heart can withstand;

d. RR interval vs. HR analysis. Some studies suggest that an unsignificant change in RR interval analysis might be significant when the analysis is transformed in HR values;

e. The analysis was not performed in the same rats for SBP-CVM slope and for SBP-RR interval relationship. Not all the data was collected in all rats, due to experimental conditions. Thus, the averages of SBP-CVM slope and SBP-RR interval slope might be poorly correlated, given the small population sample.

The site of action of clonidine is supposed to be at the level of the CVM or upstream within the cardiac baroreflex arc, given the increase in CVM activity. This is in line with previous studies in dogs, where clonidine actions at the level of the first order neurons (**figure 26**)(83). However, the changed pattern of the CVM activity with clonidine suggests a direct action on the CVM itself. This is in disagreement with pervious findings (83), but could be explained by the differences in clonidine doses.

Overall, the central and peripheral vasodilators clonidine and nitroprusside have different actions on the CVM. Besides its anti-hypertensive effect, clonidine increases CVM activity, together with baroreflex gain at CVM level. Thus, clonidine has two actions: i). it lowers the BP and HR set-points, through its inhibitory action on the vascular and cardiac sympathetic system; ii). it reduces the BP lability, presumably by lowering SNS activity, and improving the vagal control of the heart. In this way, clonidine shifts the sympathovagal balance towards the cardioprotective side, by i).inhibiting the sympathetic activity (51) (**figure 25**) and ii). increasing the parasympathetic output (ms1). As previously stated, clonidine reduces BP lability in the ambulatory and post-operative setting (22). On the other hand, nitroprusside has a different mechanism of action. It decreases the BP set-point, and meanwhile, the CVM activity is *passively* silenced. This is a normal response of the cardiac baroreflex to the decrease in BP.

B - Effect of systemic B-type natriuretic peptide on cardiac vagal motoneuron activity

I. Introduction

Natriuretic peptides are neurohormones released by the heart, blood vessels and kidney in response to ventricular volume expansion, pressure overload, and increased wall tension. They have a cardioprotective role in patients presenting with congestive heart failure (CHF). Natriuretic peptides family include mainly:

a. Atrial natriuretic peptide (ANP), which is released primarily from the atrium of the heart. ANP binds to natriuretic peptide receptor A. ANP is a better marker of acute overload and/or rapid cardiovascular hemodynamic changes than other NP.

b. Brain natriuretic peptide (BNP), also known as B-Type Natriuretic Peptide, was initially discovered in the porcine brain, hence its name. The BNP is a 32 amino acid polypeptide secreted mainly in the left ventricle. BNP binds to and activates natriuretic peptide receptor A. The half-life of the BNP in plasma is 20 minutes. BNP is marketed as Neseritide or Natrecor for the treatment of CHF.

c. C-type natriuretic peptide, predominantly secreted by noncardiac tissues (e.g. endothelium).

BNP is believed to have a more potent action on the ANS, since it increases vagal bradycardia in response to a rapid vasopressor more than ANP or CNP (129; 130). BNP does not potentiate the arterial baroreflex response.

BNP main effects can be divided in three types (**figure 3**):

a. Short-term action on the sympathovagal balance. BNP influences the autonomic control of the heart itself, by inhibiting the cardiac sympathetic nerve activity (18). It is also supposed to have an enhancing effect on the parasympathetic cardiac reflexes (129; 130). However, there is no direct evidence regarding the cardiac vagal activity.

b. Medium-term actions on circulation and hormonal status: i). renal natriuresis and diuresis; ii). inhibition of other pressor systems, i.e. renin-angiotensin-aldosterone system, vasopressin, catecholamines; iii). vasodilatation (at high doses or in CHF); iv). Plasma sequestration from the vascular compartment.

c. Long-term action on the cardiac hypertrophy.

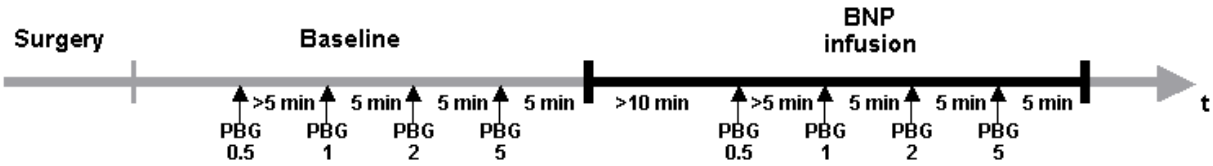

Figure 27. Protocol used for CVM activity recording under BNP infusion in urethane-anesthetized rats

Upon completion of surgery and once a CVM unit is identified, the jugular catheter is filled with PBG. Baseline recordings are performed during administration of bolus doses of 0.5µg, 1µg, 2µg, 5µg of PBG, using a Hamilton syringe. A minimum of 5 minutes recovery time was allowed between PBG doses. Two to three repeat injections were made of each PBG dose. Then B-type natriuretic peptide (BNP) is infused. Identical PBG administration is repeated after 10-15 minutes, to allow BP and HR to reach a steady state. PBG was dissolved freshly in saline at a concentration of 50µg/ml. BNP was infused at a rate of 25pmol/kg/min via the femoral vein.

Thus, BNP generates a proportional physiological response to the left ventricular dysfunction. BNP is released in cardiovascular conditions where the ventricle is overstretched. Elevated levels of BNP in the blood are used as diagnostic and prognostic tools in CHF. Plasmatic BNP percentage increases with age and is slightly more concentrated for women. Finally, the BNP concentration can be increased in valvular diseases, pulmonary primitive arterial HBP, cirrhosis, hyperthyroid, Cushing disease, renal dysfunction, hypoxia and cerebral tumours.

One of these presumed cardioprotective actions is to enhance the BJ (cardiopulmonary) chemoreflex. This action has been shown in several species. It may be produced by all three natriuretic peptides, although BNP is the most potent (53; 130). However, despite the fact that BNP is believed to improve the cardiac vagal activity, there is no evidence on the central mechanism of action.

An easy way to trigger the BJ reflex is to inject small doses of 5HT3 receptor agonist phenylbiguanide (PBG) close to the right atrium. By measuring the CVM activity, together with the HR, to equivalent doses of PBG before and after BNP infusion (**figure 27**), an analysis of the effect of BNP on the central arm of the BJ reflex will be performed.

II. MANUSCRIPT n°2

Effect of systemic B-type natriuretic peptide on cardiac vagal motoneuron activity

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Effect of systemic B-type natriuretic peptide (BNP) on cardiac vagal motoneuron activity

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ABSTRACT

Intravenous B-type natriuretic peptide (BNP) enhances the bradycardia of reflexes from the heart, including the *von Bezold-Jarisch* reflex, but its site of action is unknown. The peptide is unlikely to penetrate the blood-brain-barrier, but could act on afferent or efferent reflex pathways. To investigate the latter, two types of experiment were performed on urethane-anesthetized (1.4 g/kg, i.v.) rats. First, activity was recorded extracellularly from single cardiac vagal motoneurons (CVM) in the nucleus ambiguus. CVM were identified by antidromic activation from the cardiac vagal branch and by their barosensitivity. Phenylbiguanide (PBG), injected via the right atrium in bolus doses of 1-5 micrograms to evoke the von Bezold-Jarisch reflex, caused a dose-related increase in CVM activity and bradycardia. BNP infusion (25 pmol/kg/min, i.v.) significantly enhanced both the CVM response to PBG (n=5 rats) and the reflex bradycardia, but the log-linear relation between those two responses over a range of PBG doses was unchanged by BNP. The reflex bradycardia was not enhanced in 5 matched time-control rats receiving only vehicle infusions. In 5 further rats the cervical vagi were cut and the peripheral right vagus was stimulated supramaximally at frequencies from 1-20 Hz. The bradycardic responses to these stimuli were unchanged before, during and after BNP infusion. We conclude that systemic BNP in a moderate dose enhances the *von Bezold-Jarisch* reflex activation of CVM, in parallel with the enhanced reflex bradycardia. That enhancement is due entirely to an action before the vagal efferent arm of the reflex pathway.

Key words: cardiopulmonary, von Bezold-Jarisch, B Natriuretic Peptide, phenylbiguanide, bradycardia, heart, chemoreflex, parasympathetic, vagus, cardiac vagal motoneurons, single unit activity.

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INTRODUCTION

Increased sympathetic drive and reduced vagal activity are prognostic of a poor outcome in cardiovascular disease (12;14;29). Many studies have shown the benefits of reducing sympathetic activity in cardiovascular disease (10) but actions on the vagal arm of autonomic nervous system have been under-exploited. It is likely that enhancing vagal drive to the heart will be beneficial because efferent vagal stimulation can improve outcome after myocardial infarction in dogs (29). Mechanisms that may enhance vagal tone are therefore of interest for their therapeutic potential in cardiovascular disease.

The cardiac natriuretic peptides - atrial natriuretic peptide (ANP), b- and c-type natriuretic peptides (BNP and CNP) - have actions which may be interpreted as cardioprotective (31). Indeed, BNP (Nesiritide®) has proved clinically useful in the treatment of congestive heart failure (5;16). One of these presumed cardioprotective actions is to enhance bradycardic reflexes such as the *von Bezold-Jarisch* (cardiopulmonary) chemoreflex and the 'ramp' (cardiac) high pressure baroreflex. This action has been shown in several species, and may be produced by all three natriuretic peptides, although BNP is the most potent (23;27). This action is mediated by particulate guanylate cyclase (pGC) receptors (25), but their location is as yet uncertain. On the one hand, experiments on rats and sheep have found that BNP, in a dose which enhanced the von Bezold-Jarisch and 'ramp' (cardiac) baroreflex, did not alter the bradycardic response to arterial baroreceptor loading (as assessed by the 'steady state' baroreceptor reflex (26;27). These findings have been interpreted as showing modulation specifically of reflexes from the heart, with cardiac afferents being the most likely site of hormone action, echoing early studies on ANP (1;28). On the other hand, Paterson and colleagues demonstrated that higher concentrations of BNP (and CNP) enhanced vagal efferent neurotransmission to the guinea pig heart *in vitro*, by an action mediated via cyclic GMP (8). While the latter workers found ANP ineffective, Atchison and Ackermann had previously demonstrated that more moderate doses of ANP *in vivo* enhanced the bradycardic effect of vagal stimulation in rats (2). Corresponding data on BNP are lacking.

The present study was undertaken firstly to test directly whether BNP, in systemic doses which enhance cardiac reflexes, has any effect on the activity of cardiac vagal motoneurons. This approach effectively opens the vagal cardio-cardiac reflex loop, allowing us for the first time to distinguish actions before and after its efferent arm. Secondly, we sought to test whether BNP has any demonstrable action on the parasympathetic efferent arm of the von Bezold-Jarisch reflex pathway, between the cardiac vagal motoneurons and the heart.

MATERIALS AND METHODS

Preparation

Experiments were performed on 32 Sprague-Dawley male rats (Harlan, Gannat, France, 350-400g), and were approved by the Rhône-Alpes Committee for the care of Animals. Rats were anesthetized with 5% isoflurane in oxygen and given a tracheotomy, after which they were mechanically ventilated with 2% isoflurane in oxygen during surgery. End tidal CO_2 was adjusted to 25-28mmHg during surgery. Rectal temperature was maintained at ~ 37.5°C with a servo-controlled heating blanket (Harvard, Edenbridge, Kentucky). The bladder was cannulated and allowed to drain spontaneously. The right femoral vein and artery were catheterized for injection of drugs, and monitoring of arterial blood pressure, respectively. Normal saline was infused at ~3ml/h through the arterial catheter to keep it patent and to avoid hypovolemia (19). A third catheter was advanced through the right jugular vein until its tip rested close to the right atrium. Subcutaneous silver wire electrodes were placed across the thorax to record the EKG.

CVM recording and time control experiments

The right thoracic vagus was exposed through a thoracotomy at the second intercostal space. The craniovagal cardiac branch was identified anatomically then tested for its ability to slow the heart (0.05ms pulses of 2-7 V, delivered at 20Hz via a paired silver wire electrode) (20). A small polyethylene sheet was inserted beneath the branch to insulate it from the underlying tissue. A pair of electrodes made from teflon-coated 0.005inch silver wire (A-M Systems, Carlsborg, WA), bared at the tips, was placed under the cardiac branch and fixed in contact with the nerve by embedding the area in silicon gel (Wacker, Munich, Germany). The leads were also sutured to the thorax to avoid pulling on the nerve. The thoracotomy was then closed and the viability of the nerve rechecked by stimulating through the implanted electrodes: if this failed to give a bradycardia, the experiment was discontinued.

The rat was fixed prone in a stereotaxic frame, with the head ventroflexed. The tail was clamped and kept under mild tension. The dorsal surface of the medulla (now approximately horizontal) was exposed via a dorsal approach, and part of the occipital bone was removed with rongeurs. The atlanto-occipital membrane and dura were incised and reflected.

When surgery was complete, anesthesia was switched from isoflurane to urethane (1.4g/kg iv, administered over ~30 min). End tidal pCO_2 was adjusted to 30-35mmHg. When a stable plane of anesthesia had been established, sufficient to abolish withdrawal reflexes, the animal was paralyzed with metocurine iodide (0.2 mg, i.v., Metubine®, Lilly, Indianapolis, IN). Paralysis was allowed to wear off between doses so that the adequacy of anesthesia could be checked before repeating the paralysis. If necessary, additional urethane (10–20% of original dose) was given to deepen anesthesia. At the end of the experiments, rats were killed with an overdose of chloral hydrate (0.2g, i.v.).

Carbon fiber electrodes were made as described by Kuras and Gutmaniene (13), with the fiber protruding 10–15 µm beyond the end of the glass micropipette. Electrical contact was made via 2M NaCl in the pipette shaft. Unit activity was recorded differentially with respect to a reference silver wire on the medullary surface, using a Grass P16 preamplifier (Grass, Quincy, MA). The signal was amplified (x10,000) and filtered (100–3000 Hz) before be-

ing displayed on an oscilloscope and stored, along with blood pressure, EKG, stimulus and event markers on magnetic tape (JVC, Friedberg, Germany) and computer (CED Micro 1401 interface and Spike2 analysis program; CED, Cambridge, UK).

Electrodes were inserted vertically through the dorsal surface of the medulla, aiming for a region 1.5–2.0 mm to the right of the calamus scriptorius, and at a depth corresponding to the external formation of the nucleus ambiguus (NA) (9). CVM were sought by their antidromic response to stimulation of the craniovagal cardiac branch, using a search stimulus of 0.5–1 mA, 0.05 ms width, delivered at ~1 Hz. A signal averaging program in Spike2 was used to help locate CVM. Once a unit recording of sufficient amplitude and stability had been isolated, and showed spontaneous activity, it was subjected to time-controlled collision testing (15;20). Spike discrimination was performed on-line with a time-amplitude window discriminator (FHC, Brunswick, ME) and off-line from the analog signal (digitized at 15-20 kHz), using the Spike2 program. The accuracy of discrimination was carefully checked off-line and edited to eliminate mistakes.

Vagal stimulation experiments

The right and left cervical vagi were exposed through a large ventral incision in the neck, and cut. The caudal end of the right vagus was placed over a pair of silver wire hook electrodes under a pool of silicon gel (Wacker, Munich, Germany). The maximal stimulus voltage to activate cardiac vagal motor axons was measured by delivering 20 Hz stimuli of 0.05 ms width. Test stimuli were thereafter delivered at 1.5 times the maximal voltage. The effects of vagal stimulation were observed on a chart recorder and the heart rate measured from a digital meter, triggered by the R-wave of the EKG and smoothed with a 1 s time constant (Gould Recorder 2600S, Cleveland, Ohio). The EKG waveform was displayed on the computer screen at the time, and also checked off-line from the recorded signal. Supramaximal stimulus trains were delivered in a set sequence: 1, 2, 5, 10, 20, 40, 20, 10, 5, 2 and 1Hz. Each stimulus train (except at 40 Hz) was continued until a plateau heart rate response was achieved. The next stimulus was given once stable control conditions were re-established. In each case the difference in heart rate between the plateau and the pre-stimulus control was measured. Junctional rhythms often appeared during 40 Hz stimulation, and these data were discarded.

Protocols

In CVM recording experiments, phenylbiguanide (PBG, Lilly, Indianapolis, IN) was dissolved freshly in saline at a concentration of 50 µg.ml-1. After filling the jugular catheter, bolus doses of 1µg, 2µg (both in 5 rats) and either 4 µg (2 rats) or 5 µg (1 rat) of PBG were administered, using a Hamilton syringe. A minimum of 10 minutes of recovery time was allowed between PBG doses to avoid tachyphylaxis. Two to three repeat injections were made of each PBG dose before, and during, BNP infusion. The CVM response to each PBG injection was defined as the extra spikes occurring within the 30s following the stimulus. The fall in heart rate was measured with respect to the pre-stimulus control value.

After the control series of tests in both types of experiment, B-type natriuretic peptide (rat BNP-45, Bachem, Bubendorf, Switzerland) was infused at a rate of 25 pmol/kg/min via the femoral vein. After allowing 10-15 minutes to reach a steady state, test PBG stimuli were repeated, using the same PBG doses as before (2-3 repeats

of each dose) in each rat. In the case of vagal stimulation experiments, the same sequence of supramaximal stimulus trains was repeated before, during and 15-30 minutes after the BNP infusion was stopped.

Time control experiments were performed on 5 rats, which were each individually matched to a successful CVM recording experiment. The surgical preparation was the same, except no microelectrodes were inserted. Each control rat received PBG doses on the same time schedule as in the counterpart CVM recording experiment, but received vehicle infusion in place of BNP. The falls in heart rate with PBG were measured as detailed above.

Analysis

Repeat responses to the same dose of PBG in the same condition (e.g. before BNP) were averaged. To examine the effect of BNP, each animal's mean bradycardic and CVM responses to PBG (all doses combined) before BNP were compared with its responses to the same PBG doses during BNP infusion. The significance of the BNP effect on all animals was assessed by paired t-test. In time control experiments, the effect of vehicle infusion on the bradycardic response to PBG injections was assessed by paired t-test in the same way. Baseline blood pressure, heart rate and CVM spike rate were also measured in each rat during control conditions and 15 minutes after the onset of BNP infusion: the within-animal effect of BNP was compared for the group, using paired t-tests. The same procedure was used to assess the effect of vehicle infusion on blood pressure and heart rate. To compare vagal stimulus-response relations, the bradycardia in beats/min was plotted against the log of the stimulus frequency or the log of the CVM response (see above). Least-squares regression lines were constructed for these data before BNP and during BNP: the slopes and intercepts of those regression lines before and during BNP were compared for the group by paired t-test. Values are stated as mean $\pm$ SEM and changes are expressed as mean $\pm$ SED (standard error of the difference). Differences were considered significant at $P<0.05$.

Results

Extracellular single unit recordings were made from the region of the right nucleus ambiguus of 22 rats. Eight CVM were identified by antidromic activation from the cardiac branch of the right vagus as described previously (20) (Figure 1). Five spontaneously active CVM in five rats were studied with the full protocol. Their properties matched those previously described (17;20). Their conduction velocities were in the B-fiber range (10.0 ± 1.2 m/s). Their spontaneous activity (mean rate 0.14 ± 0.10 spikes/s) was strongly modulated by the arterial pulse, as shown by cardiac cycle-triggered histograms (Figure 1B). They were also activated by rises and inhibited by falls in arterial blood pressure, as reported previously (20) (not shown here). This combination of antidromic activation from the cardiac vagus and barosensitivity was considered sufficient to identify these neurons as CVM (17;20).

When PBG was injected close to the right atrium, CVM activity abruptly increased in parallel with the classic von Bezold-Jarisch reflex bradycardia and hypotension. A representative recording is illustrated in Figure 2A. CVM activity peaked within 1 second of its onset and then declined to a plateau level, which usually persisted for 30 seconds or more (Figure 2A). The CVM response and the bradycardia were dose-related (Figure 3A and B).

When BNP was infused intravenously at 25 pmol/kg/h, there was a modest fall in resting arterial pressure (from 119.0±4.7 to 105.8±4.5 mm Hg), but no change in mean heart rate or CVM activity (381±14.4 to 382.8±16.2 bpm, and 0.14±0.10 spikes/s unchanged, respectively). In response to PBG injections in the presence of BNP, there was a modest enhancement in both the mean CVM response and the mean bradycardia (figs 3A, 3B; $p < 0.05$ in each case).

It was found empirically that the change in heart rate in each response to PBG was closely related to the logarithm of the CVM response (expressed as the extra spikes within the first 30 seconds). Figure 4A illustrates for one CVM how that relation was unchanged in the presence of BNP: the enhanced CVM responses and the enhanced bradycardias shifted upwards along the same line of relation. For none of the 5 CVM tested did the slope or the intercept of the regression line of data obtained before BNP differ significantly from that of data during BNP infusion. Figure 4B illustrates the corresponding regression lines for each CVM, constructed from all responses to PBG before and during BNP infusion: r^2 values were between 0.83 and 0.98. It thus appears that there was a strong, fixed relationship between CVM activity and bradycardia, which was unaffected by BNP.

In 5 time control rats, resting blood pressure was 126.2±10.0 mmHg before and 122±10.1 mm Hg during vehicle infusion. The corresponding values for resting heart rate were 391.6±6.1 and 394.6±7.9 bpm. The mean reflex bradycardia in response to PBG (all doses) was 64.5±17 bpm before and 60.2±16 bpm during vehicle infusion (-7%). None of these changes was significant ($p > 0.05$, $n = 5$).

In order to test whether BNP had any direct action on the reflex pathway distal to the CVM, the cervical vagi were cut in 5 other rats, and the cardiac end of the right vagus was directly stimulated. The bradycardic response to a range of increasing stimulation frequencies up to 20 Hz showed an approximately linear-log relation (Figure 5). The responses obtained before, during and after infusion of BNP (at the same rate as in CVM recording experiments) were superimposable (Figure 5), and their regression lines were not significantly different before and during BNP ($p = 0.90$ for slope, $p = 0.28$ for intercept).

Discussion

The present study demonstrates that cardiac vagal motoneurons (CVM) in the rat, with cell bodies in the nucleus ambiguus and B-fiber axons in the right cardiac branch, are activated by right atrial phenylbiguanide (PBG). This confirms recent findings in the cat (30). We found, moreover, that PBG activated CVM dose-dependently, and that this was closely paralleled by a dose-related bradycardia. Both the bradycardia and the CVM activation were significantly enhanced in the presence of the cardiac natriuretic peptide BNP, but the relation between the CVM activation and reflex bradycardia was unchanged. Control experiments established that the reflex enhancement was due to BNP rather than to time or vehicle. Moreover, the bradycardic response to stimulation of the efferent vagus at a range of frequencies was also unaltered by BNP. Together, these results indicate that BNP acts to enhance the von Bezold-Jarisch reflex bradycardia at a site upstream to the vagal efferent arm of the baroreflex arc.

The action of BNP, as well as ANP and to a lesser extent, CNP to enhance the von Bezold-Jarisch reflex bradycardia is well established (22-25) : this action is mediated by particulate GC-receptors, as it is blocked by the pGC-receptor antagonist HS-142-1 (25). The site of this action of BNP and the other natriuretic peptides has been inferred to be on cardiac afferents. The reasons for this are that BNP enhances *von Bezold-Jarisch* (23) and high pressure, ramp cardiac mechanoreceptor reflexes (27) but, at the same doses, is without effect on steady state (principally arterial) baroreflexes (26). Also in keeping with a cardiac rather than arterial origin of this effect is the observation that the ability of ANP to enhance ramp mechanoreceptor and von Bezold-Jarisch reflexes was still evident in rats after sino-aortic denervation (22). It is therefore likely that BNP enhances the PBG-induced activity in unmyelinated cardiac ventricular afferents (21). This may involve an interaction between 5HT3 and natriuretic peptide receptors on or near cardiac afferent terminals (6;25). Chemo- and mechanosensitive cardiac afferents terminate centrally in the caudal part of the nucleus tractus solitarii (4) where they activate neural pathways to CVM in the nucleus ambiguus ((11), this study).

On the efferent side, ANP has been proposed to have a ganglionic site of action (on the sympathetic side) by Floras (7), and a presynaptic vagal efferent action by Atchison & Ackermann (2). Paterson and colleagues also showed that high doses of CNP, and to a lesser extent BNP, enhanced vagal efferent transmission in guinea pig hearts *in vitro,* but saw no such effect with ANP (8). The reasons for such discrepancies are uncertain, but may involve differences between natriuretic peptides, species and dose. The present study used modest doses of BNP *in vivo*, which effectively enhanced bradycardic reflex responses, yet there was no evidence for any action of the peptide on the vagal efferent pathway. Indeed, CVM responses and reflex bradycardias remained robustly linked. The possibility that BNP acts at a central nervous site to enhance cardiac reflexes has not yet been eliminated, however. Although the peptide is unlikely to cross the blood-brain barrier, it could conceivably act on neurons of the sensory circumventricular organs – the area postrema, subfornical organ or the organum vasculosum laminae terminalis (18). This awaits further study.

The baseline effects of BNP in this study were modest. Arterial pressure fell by ~13mmHg, yet there was no compensatory tachycardia. Nor was there any change in CVM activity in response to the fall in baseline arterial pressure. In this respect also, the quantitative relationship between CVM activity and heart rate remained robustly linked. Although anesthesia may blunt arterial baroreflex responses, this lack of compensation to the hypotensive effects of BNP, is observed also in the conscious state in dogs, sheep and humans (3;23;32).

The present data add to the understanding of the enhancement of cardiac vagal activity induced by BNP, which is evidently by an action upstream to the cardiac vagal efferent pathway. This action may contribute to the therapeutic benefits of BNP (Nesiritide®) in congestive heart failure (5;16).

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1. Ackermann, U., T. G. Irizawa, S. Milojevic, and H. Sonnenberg. Cardiovascular effects of atrial extracts in anesthetized rats. *Can.J.Physiol.Pharmacol.* 62: 819-826, 1984.
2. Atchison, D. J. and U. Ackermann. Influence of atrial natriuretic factor on autonomic control of heart rate. *Am.J.Physiol.* 258: R718-R723, 1990.
3. Brunner-La Rocca, H. P., D. M. Kaye, R. L. Woods, J. Hastings, and M. Esler. Effects of intravenous brain natriuretic peptide on regional sympathetic activity in patients with chronic heart failure as compared with healthy subjects. *J.Am.Coll.Cardiol.* 37: 1221-1227, 2001.
4. Butcher, J. W. and J. F. Paton. K+ channel blockade in the NTS alters efficacy of two cardiorespiratory reflexes in vivo. *Am.J.Physiol.* 274: R677-R685, 1998.
5. Colucci, W. S. Neseritide for the treatment of decompensated heart failure. *Journal of Cardiac Heart Failure* 7: 92-100, 2001.
6. Deliva, R. D. and U. Ackermann. Atrial natriuretic peptide and mechanisms of cardiovascular control. Role of serotoninergic receptors. *Am.J.Physiol.* 274: R711-R717, 1998.
7. Floras, J. S. Inhibitory effect of atrial natriuretic factor on sympathetic ganglionic neurotransmission in humans. *Am.J.Physiol.* 269: R406-R412, 1995.
8. Herring, N., J. A. B. Zaman, and D. J. Paterson. Natriuretic peptides like NO facilitate cardiac vagal transmission and bradycardia via a cGMP pathway. *Am.J.Physiol.* 281: H2318-H2327, 2001.
9. Hopkins DA, Bieger D, deVente J, and Steinbusch WM. Vagal efferent projections : viscerotopy, neurochemistry and effect of vagotomy. *Progress in Brain Research* 107: 79-96, 1996.
10. Hunt, S. A., D. W. Baker, M. H. Chin, M. P. Cinquegrani, A. M. Feldmannd, G. S. Francis, T. G. Ganiats, S. Goldstein, G. Gregoratos, M. L. Jessup, J. Noble, M. Packer, M. A. Silver, and L. W. Stevenson. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult : executive summary. *Circulation* 104: 2996-3007, 2001.
11. Jones JFX, Wang Y, and D. Jordan. Heart rate responses to selective stimulation of cardiac vagal C fibres in anesthetized cats, rats and rabbits. *J.Physiol.(London)* 489: 203-214, 1995.
12. Kaye, D. M., J. Lefkovits, G. L. Jennings, P. Bergin, A. Broughton, and M. Esler. Adverse consequences of high sympathetic nervous activity in the human failing heart. *J.Am.Coll.Cardiol.* 26: 1257-1263, 1995.
13. Kuras, A. and N. Gutmaniene. Preparation of carbon-fibre microelectrode for extracellular recording of synaptic potentials. *J.Neurosci.Methods.* 62: 207-212, 1995.
14. La Rovere, M. T., J. T. Bigger, F. I. Marcus, A. Mortara, and P. J. Schwartz. Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 351: 478-484, 1998.
15. Lipski, J. Antidromic activation of neurones as an analytical tool in the study of the central nervous system. *J.Neurosci.Methods.* 4: 1-32, 1981.
16. Maisel, A. S. Neseritide : a new therapy for the treatment of heart failure. *Cardiovascular Toxicology* 3: 37-42, 2003.
17. McAllen, R. M. and K. M. Spyer. The baroreceptor input to cardiac vagal motoneurones. *J.Physiol.(London)* 282: 365-374, 1978.
18. McKinley, M. J., R. M. McAllen, P. Davern, M. E. Giles, J. Penschow, N. Sunn, A. Uschakov, and B. J. Oldfield. The sensory circumventricular organs of the mamalian brain. *Advances in Anatomy Embryology and Cell Biology* 172: 1-122, 2003.
19. Quintin, L., J. Y. Gillon, C. F. Saunier, G. Chouvet, and M. Ghignone. Continuous volume infusion improves circulatory stability in anesthetized rats. *J.Neurosci.Methods.* 30: 77-83, 1989.
20. Rentero, N., A. Cividjian, D. Trevaks, J. M. Pequignot, L. Quintin, and R. M. McAllen. Activity patterns of cardiac vagal motoneurons in rat nucleus ambiguus. *Am.J.Physiol.* 283: R1327-R1334, 2002.
21. Schultz, H. D., D. G. Gardner, C. F. Deschepper, H. M. Coleridge, and J. C. Coleridge. Vagal C-fiber blockade abolishes sympathetic inhibition by atrial natriuretic factor. *Am.J.Physiol.* 255: R6-R13, 1988.
22. Thomas, C. J., A. M. Allen, R. M. McAllen, and R. L. Woods. ANP potentiates nonarterial baroreflex bradycardia : evidence from sinoaortic denervation in rats. *Autonomic Neuroscience : Basic and Clinical* 97: 89-98, 2002.
23. Thomas, C. J., C. N. May, A. D. Sharma, and R. L. Woods. ANP,BNP,CNP enhance bradycardic responses to cardiopulmonary chemoreceptor activation in conscious sheep. *Am.J.Physiol.* 280: R282-R288, 2001.
24. Thomas, C. J., A. J. Rankin, G. A. Head, and R. L. Woods. ANP enhances braycardic responses in normotensive but not spontaneously hypertensive rats. *Hypertension* 29: 1126-1132, 1997.
25. Thomas, C. J. and R. L. Woods. Guanylyl-cyclase receptors mediate cardiopulmonary vagal reflex actions of ANP. *Hypertension* 41: 279-285, 2003.
26. Thomas, C. J. and R. L. Woods. Do natriuretic peptides modify arterial baroreflexes in sheep ? *Experimental Physiology* 89: 709-715, 2004.

27. Thomas, C. T., G. A. Head, and R. L. Woods. Cardiac reflex actions of atrial, B-type and C-type natriuretic peptides in conscious rats. *J.Hypertension* 17: 801-806, 1999.

28. Thoren, P., A. L. Mark, D. A. Morgan, T. P. O'Neal, P. Needleman, and M. J. Brody. Activation of vagal depressor reflexes by atriopeptins inhibits renal sympathetic activity. *Am.J.Physiol.* 251: H1252-H1259, 1986.

29. Vanoli, E., G. M. DeFerrari, M. Stramba-Badiale, S. S. Hull, R. D. Foreman, and P. J. Schwartz. Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ.Res.* 68: 1471-1481, 1991.

30. Wang, Y., J. F. X. Jones, R. D. Jeggo, M. de Burgh Daly, D. Jordan, and A. G. Ramage. Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguus in anesthetized rats. *J.Physiol.(London)* 526: 157-165, 2000.

31. Woods, R. L. Cardioprotective functions of atrial natriuretic peptide and B-type natriuretic peptide : a brief review. *Clin Exp Pharmacol Physiol* 31: 791-794, 2004.

32. Woods, R. L. and M. J. M. Jones. Atrial, B-type and C-type natriuretic peptides cause mesenteric vasoconstriction in conscious dogs. *Am.J.Physiol.* 276: R1443-R1452, 1999.

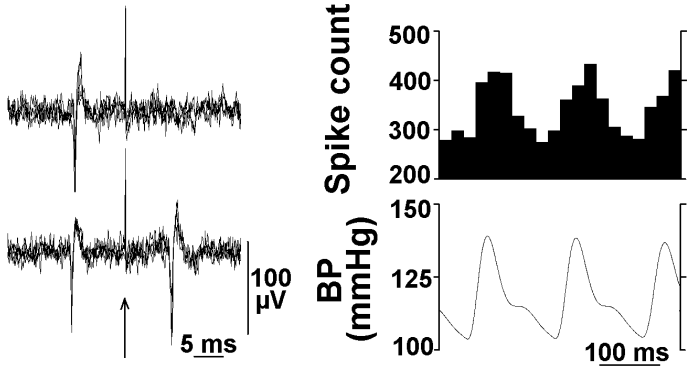


Figure 1 : Identification of a cardiac vagal motoneuron (CVM). Left: collision test for antidromic activation from cardiac vagal branch (4 superimposed oscillographic traces). The stimulus (artifact at arrow) was triggered to occur 8.7 ms (upper trace) or 9 ms (lower trace) after a spontaneous spike (*left* side of both traces). The antidromic response (right side, lower trace) of the CVM to cardiac branch stimulation was cancelled by collision of the action potential if the stimulus delay was 8.7ms or less (upper trace). Right : Barosynchronicity : upper trace shows an arterial pulse-triggered histogram constructed from of the spontaneous activity of the same CVM (10236 cycles, 20ms bins), and the mean arterial pulse wave (lower trace).

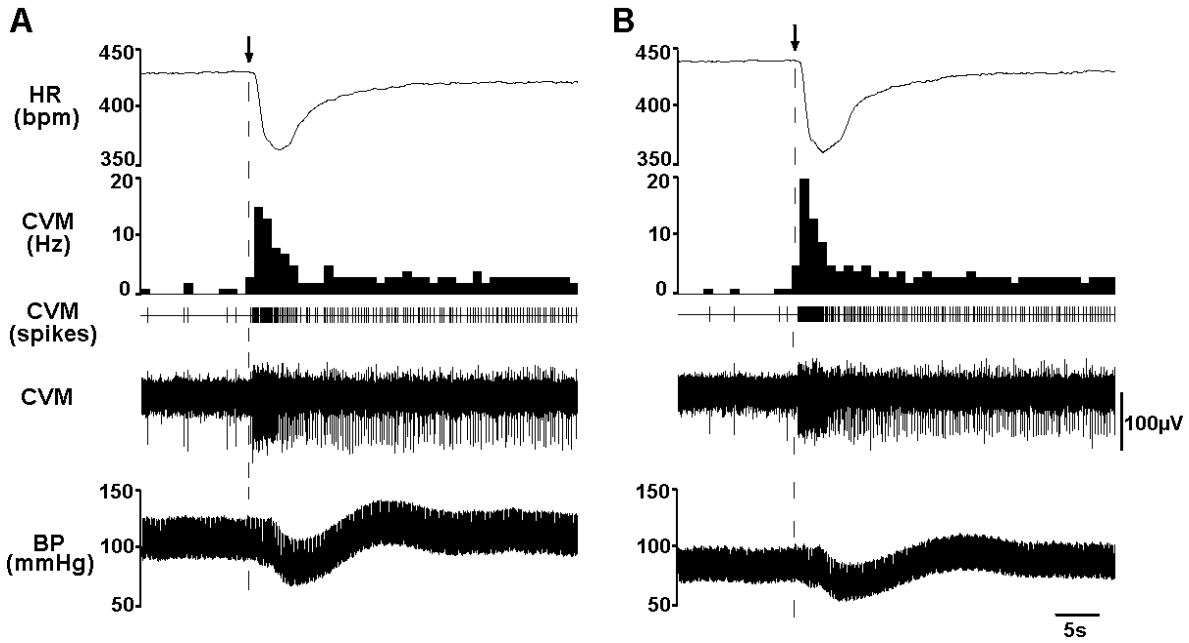


Figure 2 : Representative chart records from one experiment showing the CVM and cardiovascular responses to a 1µg PBG bolus (at arrows) before (A) and during (B) BNP infusion. Traces from above: heart rate (HR), CVM firing rate, counted CVM spikes, CVM activity (raw signal), arterial blood pressure (BP).

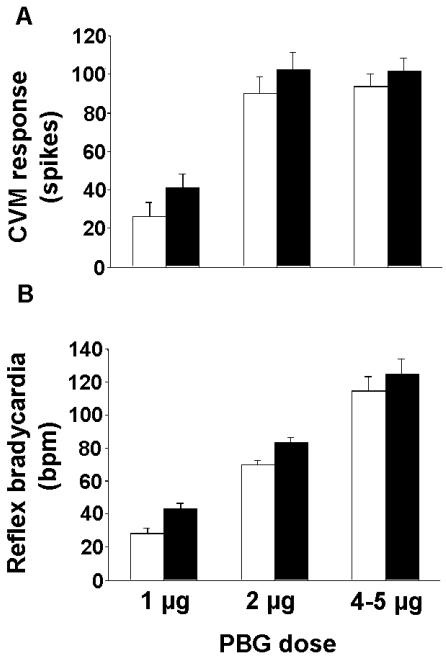


Figure 3 : Bar graphs of grouped data showing mean CVM responses (A) and reflex bradycardia (B) to three doses of PBG. Open columns show responses before BNP and solid columns show responses during BNP.
The mean CVM and bradycardic responses to PBG (all doses) were both significantly greater during BNP infusion than before ($p<0.01$ in each case). Error bars indicate SED ; n-values: 1µg, 5 units; 2µg, 5 units; 4-5µg, 3 units.

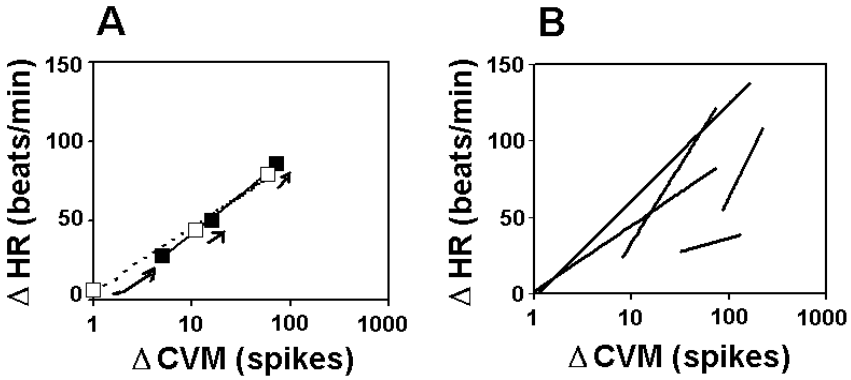


Figure 4 : Left figure shows a plot of the bradycardia (ΔHR) *versus* the logarithm of the CVM response of 1 animal to three doses of PBG before (open symbols) and during BNP infusion (closed symbols). Arrows link responses to the same PBG dose. Right figure shows on the corresponding plot, regression lines for all responses to PBG (before and during BNP) of 5 CVM in 5 rats (r^2 values: 0.98, 0.83, 0.98, 0.91, 0.92).

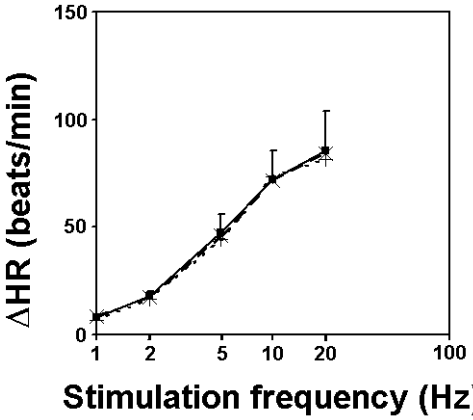
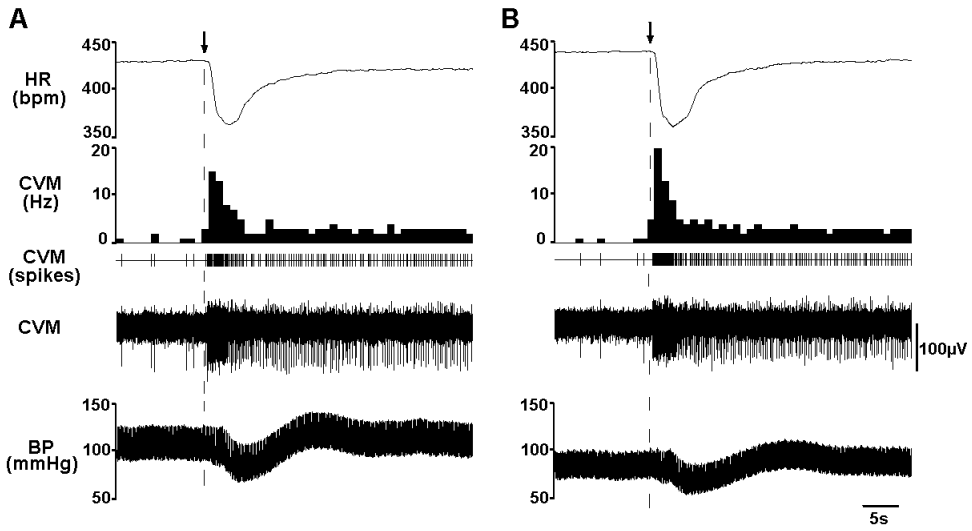
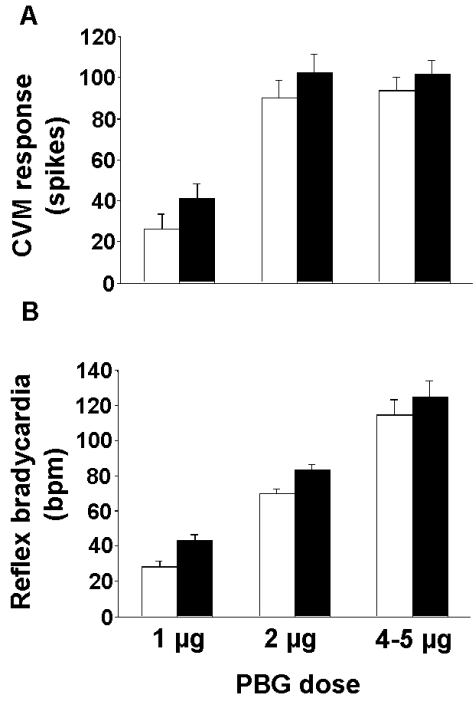


Figure 5 : Bradycardic responses (ΔHR) to supramaximal stimulation of the right cervical vagal at frequencies between 1 and 20 Hz. Note the logarithmic scale on the abscissa. Symbols indicate: before (filled squares, continuous lines), during (X, dashed lines) and after (+, dotted lines) BNP infusion.Error bars indicate the largest SEM of any series at each frequency. The regression lines of each data set were not significantly different from each other with respect to slope or intercept.



ms2 Figure 2. Representative chart records from one experiment showing the CVM and cardiovascular responses to a 1µg PBG bolus (at arrows) before (A) and during (B) BNP infusion. Traces from above: heart rate (HR), CVM firing rate, counted CVM spikes, CVM activity (raw signal), arterial blood pressure (BP).



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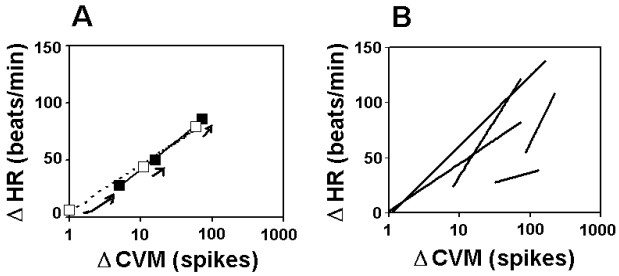

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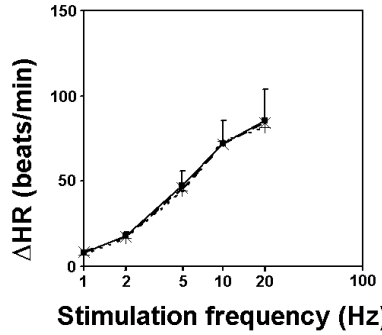

ms2 Figure 5. Bradycardic responses (ΔHR) to supramaximal stimulation of the right cervical vagal at frequencies between 1 and 20 Hz. Note the logarithmic scale on the abscissa. Symbols indicate: before (filled squares, continuous lines), during (X, dashed lines) and after (+, dotted lines) BNP infusion. Error bars indicate the largest SEM of any series at each frequency. The regression lines of each data set were not significantly different from each other with respect to slope or intercept.

III. Discussion

BNP effect on BJ reflex. PBG is injected close to the right atrium to evoke the BJ reflex. Thus, PBG causes dose-related decreases in HR and increases in CVM activity (**ms2 figure 2**). BNP significantly enhances the BJ reflex bradycardia following PBG administration (**ms2 figure 3**). Thus, dose-related decreases in HR are greater under BNP infusion. BNP infusion also increased the CVM response.

Site of action. As a peptide hormone, BNP is unlikely to penetrate the blood-brain-barrier. Thus, it could act only on the afferent or the efferent reflex pathways. Two facts prove that the BNP effect is not on the efferent limb:

a) Following vagotomy, BNP has no effect on the bradycardia induced by vagal stimulation (**ms2 figure 5**). The relation between the firing of the CVM axon and the cardiac ganglion is unchanged by the BNP.

b) The log-linear relation between the change in HR and in CVM activity (i.e. the cell body firing) following PBG boluses is unchanged by administration of BNP (**ms2 figure 4**).

Thus, a subtractive logic suggests that the site of action of BNP should be on the afferent pathway of the reflex, presumably at the level of the first order neurons. Despite the fact that a peptide is unlikely to penetrate the blood-brain barrier, BNP could act on the neurons of the sensory circumventricular organs. However, a selective activation of the cardiopulmonary chemoreceptors is the most likely.

Conclusion. The BJ reflex is triggered by factors affecting coronary flow, such as reperfusion after ischemia (121), coronary thrombolysis therapy, and during coronary angiography (93). By contrast, there is evidence that cardiac vagal activity is impaired in conditions such as myocardial infarction, hypertension (134), or CHF (40). Reductions in sensitivity of cardiac sensory receptors may contribute to the pathophysiology of acute or chronic cardiovascular disorders (93). In humans, there is a range of events where activation of the cardiopulmonary chemoreceptors occurs coincident with natriuretic peptide release. BNP release into the circulation is augmented in patients with unstable angina (72), during angioplasty (80), after MI (37), and in chronic conditions associated with ventricular dysfunction and cardiac failure (105). NP may be cardioprotective by enhancing parasympathetic activity during times of compromised myocardial blood flow (130; 136). The present study demonstrates that the BNP enhances the gain of the neural arc of the BJ reflex in rat. This is done, most likely, by an action on the first-order neurons of the reflex.

GENERAL DISCUSSION

A – CONCLUSION

I. Methodology

The 2 drugs studied here lowered ventricular stress either by lowering filling pressures (BNP and clonidine) or by lowering impedance to ventricular ejection (clonidine). In addition to these effects linked primarily to sympatho-inhibition, these drugs recruited cardiac vagal activity. These drugs were studied at central level using *in-vivo* electrophysiological methods. This is not a commonly used method of analysing the cardiac baroreflex. Cheaper, non-invasive and simpler methods where HR is coupled with BP are usually preferred. Despite their advantages in clinical use, these methods do not measure the cardiac vagal activity directly and centrally. Therefore, techniques dealing with BP and HR have some drawbacks that make them inappropriate for the present study. Firstly, they only allow a total cardiac baroreflex loop gain estimation, providing less information about the mechanism and site of action of the studied drugs within the reflex pathway. Thus, effectively opening the neural arc by performing an *in-vivo* recording of the SUA of CVM seems to be the best way to analyse the reflex vagal control of the heart. Secondly, these spontaneous methods might be less accurate, because small non-baroreflex variations of RR-interval might be wrongly correlated to SBP variations, and be interpreted as a parasympathetic effect. A pressure ramp generating method, where clear, well-determined rises in pressure can be easily correlated with consequent fast RR-interval responses, appears the best way to analyze of the cardiac vagal influence on the heart.

In ms2, by stimulating the cut vagus, the site of action of BNP was studied, either at the level of the projection of the CVM on the heart or somewhere upstream. HR response to the stimulation did not change with BNP (**ms2 figure 5**). This was in line with the results presented in **ms2 figure 4**, where heart response was related to the unitary activity of the CVM. The agreement of these results shows the importance of recording the single unit activity of the CVM, which is well correlated with the whole nerve activity. CVM activity could be a marker of the overall parasympathetic control of the heart.

II. Physiology

Nitroprusside : The present work tested the behaviour of the CVM during decreases of pressure generated by bolus injections of nitroprusside. As expected, the CVM activity decreased during the pressure fall. This cardiac vagal deactivation is in agreement with the sympathetic activation observed elsewhere in the same conditions (126). In one case, the activity of the CVM was not completely suppressed, even if BP fell to a value where the baroreceptors are normally silenced. This raised the question whether the CVM could have a baro-independent baseline activity.

BP lability : The cardiac baroreflex operates as a rapid beat-by-beat control system to adjust HR and cardiac output to maintain BP around a set point. This set point is regulated by other systems. Second by second BP variations are subsequently corrected by baroreflex compensatory mechanisms. A reduced cardiac baroreflex gain impairs the compensatory response and results in greater beat-by-beat BP lability. Accordingly, several studies reported a coexistence of increased BP lability, decreased HR variability and depressed cardiac baroreflex gain (90; 113). Patients with impaired autonomic reflex have greater intra-operative BP lability compared with autonomically intact patients (19; 82). Increased BP lability is associated with a higher risk of end-organ damage. In addition, many of the intravenous anesthetics and all inhaled anesthetics impair parasympathetic reflex responses (103). Thus, hypertensive patients under anesthesia are likely to have substantial postoperatory BP lability following surgical stress or blood loss. In the present work (ms1), BP lability and HR variability presented an inverse variation in anesthetized rats in response to clonidine. This is confirmed by previous studies (39).

III. Pharmacology

Clonidine : In a previous study done on hypertensive patients the clonidine reduced the BP lability (113), presumably by increasing the HR variability (39; 91). In order to see what is the autonomic mechanism of this issue, an analysis of the cardiac vagal activity was needed. The electrophysiological analysis done in this work in rats showed that clonidine improved the gain of the cardiac baroreflex measured at the heart level, but not significantly (**ms1 figure 8F**). However, the SBP-CVM relationship was increased significantly. Thus, the cardiac vagal activity is increased by clonidine. This could explain at least partly the lowered BP lability in hypertensive patients following clonidine administration. Another mechanism of action of clonidine could be the changed firing pattern of the CVM to a phasic pattern. This changed firing pattern suggests also that clonidine might have a direct action on CVM.

BNP : The mechanism of action of another cardioprotective substance supposed to increase the cardiac vagal activity, the BNP, was also studied. Results showed that the BNP improved the cardiac vagal activity (**ms2 figure 3**). However, results confirmed that the peptide did not have a direct effect on the CVM. The increased response was possibly due to an effect of BNP on the afferent arm of the reflex. This parasympathetic enhancement, together with a previously demonstrated diminished effect on the sympathetic tone, could explain why BNP shifts the so-called “sympatho-vagal balance” towards vagal dominance (138).

Overall, the present study allowed a better understanding of the central mechanism of action of two cardio-vascular protective drugs, clonidine and BNP. Both drugs improved the gain of two important cardiovascular reflexes, the cardiac baroreflex and the BJ reflex, respectively. The cardiac vagal activity was increased by both drugs, either through a direct action on the CVM, or by an enhanced input.

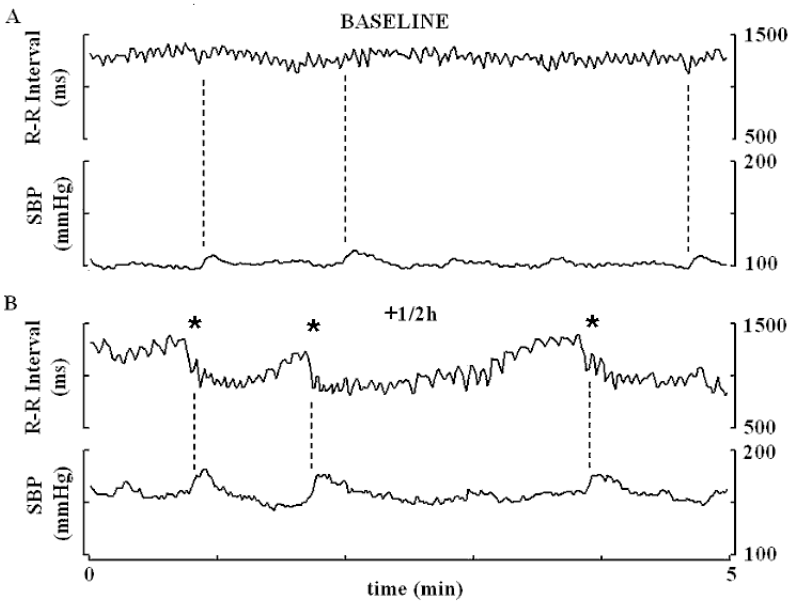

Figure 28. Typical recordings of R-R interval and SBP in a young patient, before surgery (baseline) and 1/2h after extubation.

During recovery (B), in the young patient pressure lability is larger as compared to baseline (A). The nadir of HR (here the higher R-R interval) is identical or lower than observed at baseline. However, much larger variations of HR are observed as compared to baseline. Great decreases in the R-R interval (tachycardia) are associated with rises in BP (dashed lines) whereas the pressure lability is as compared to baseline. This suggests bursts of sympathetic activation and/or cardiac vagal de-activation versus a background of high cardiac vagal activity (stars). Decreases in R-R interval are large because a high baseline cardiac activity allows cardiac vagal de-activation to occur. These decreases in R-R interval are partly responsible for a higher pressure lability after extubation in the young patient as compared to baseline. (Cividjian, submitted)

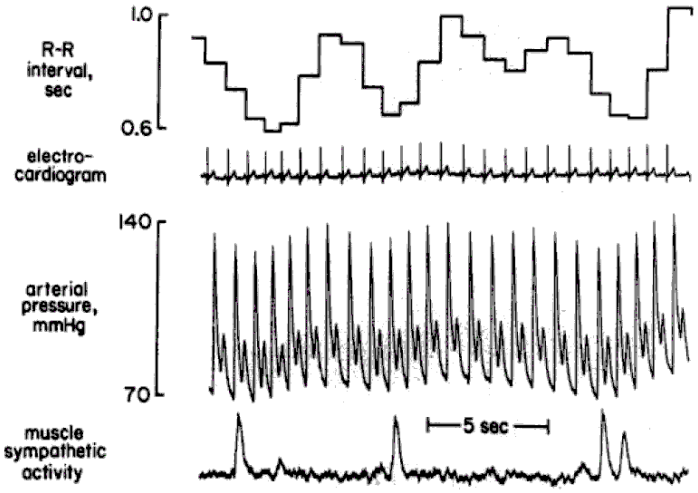

Figure 29. Sympathetic nervous activity, BP, ECG trace and RR interval in an healthy supine volunteer at rest

Note that minimal increase in pressure leads to bradycardia of large amplitude. Note the co-existence of low sympathetic nervous activity with high cardiac vagal activity. Figure adapted from (41).

B – PERSPECTIVES

How does the finding pertaining to clonidine (ms 2) relate to previous work from various groups, including ours ? The key matter pertains to the:

1) *grouped pattern of action potentials of the CVM* (“doublet”). **Figures 1** and **2** have to be viewed along this grouped pattern. In an hypertensive patient, under baseline conditions, or following major surgery, a “fixed heart rate” is apparent. Simultaneously, pressure lability is high. By contrast, in an healthy volunteer lying supine at rest, or in an hypertensive patient treated with clonidine a high HR variability co-exists with a very stable pressure trace. The first explanation is that a high cardiac vagal activity fully stabilizes pressure. In this respect, grouped pattern may be key to produce a more than proportional bradycardia upon minimal pressure rises. Thus a much prolonged diastolic time may lead to normalization of pressure within one beat (see Appendix 2).

2) *increased CVM activity coupled to increased sinus arrhythmia and reduced pressure lability* (**figure 23**). Under condition of constant and low pressure lability, clonidine induces a further reduction in pressure lability which is directly linked to the increased CVM activity and increased arrhythmia. The view of a direct link between CVM activity and pressure lability does hold here. This view may not hold in other settings : **figure 28** shows a low sympathetic activity, a low pressure lability and a large sinus arrhythmia in a healthy supine patient before leaving the ward to the operating room. By contrast, during the postoperative period, a higher pressure lability is observed with simultaneous increase in pressure and HR (dashed lines). When pressure is stable, a large sinus arrhythmia re-appears. The tentative conclusion is that pressure lability is: i) a function of a low sympathetic nervous activity and ii) a function of a high CVM activity which generates a large sinus arrhythmia in response to any minimal increase in BP (see appendix 2). However, the presence of any increased sympathetic activity blunts the buffering effect of the cardiac vagal activity (**figure 29, 30**).

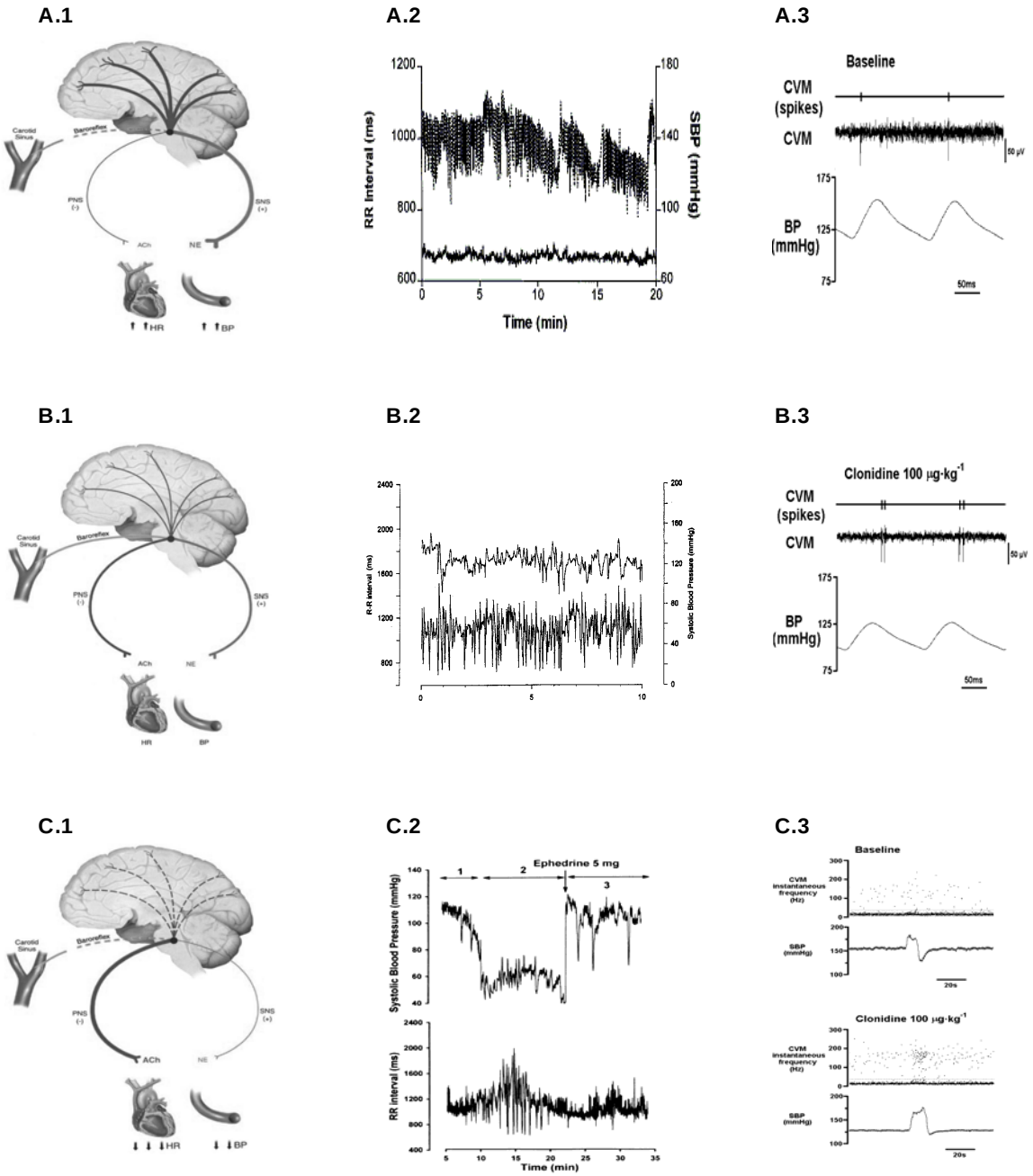

Figure 30. Relationship between BP lability, HR variability, and sympathetic/parasympathetic interaction. Hypothesis regarding CVM discharge pattern

Baroreflex failure (71) is an association of increased sympathetic activity and reduced cardiac vagal activity (figure A1). Such pattern may be observed in the postoperative period of hypertensive patients recovering from major surgery where high BP lability and low HR variability ("fixed HR") is observed (114) (figure A2). By analogy, this pattern is observed in the anesthetized rat following major thoracic and neurosurgery where 75% of the CVM are silent. When the CVM are active, the firing pattern occurs as a single spike (figure A3).

By contrast, when the sympathetic system and the parasympathetic system function normally (figure B1, (71)), this entails a normal functioning of the cardiac baroreflex. In the healthy supine volunteer, a normal circulatory pattern entails a low pressure lability and a high HR variability (figure B2; Gratadour, unpublished data). By analogy, cardiac vagal activation by clonidine in the anesthetized rat increases CVM activity with the appearance of volleys of action potentials ("doublet", "triplet"; figure B3). These volleys of action potential presumably generate large, non linear release of acetylcholine on the sino-atrial node. Presumably, this leads to a large bradycardia following a minor spontaneous increase in pressure (see RR interval trace in healthy volunteer : figure B2).

Exacerbation of parasympathetic activity (figure C1, (71)) may lead to vagal syncope in a patient under spinal anesthesia. During syncope, the HR is oscillating on a beat-by-beat basis between 90 bpmin to 30 bpmin (instantaneous frequency; observe RR interval trace in figure C2 (48)). Upon evoked rises in BP following intra-aortic balloon inflation, the occurrence of doublets is exacerbated (figure C3). The demonstration of such a pattern of CVM activation remained to be done during vagal syncope.

C – FUTURE STUDIES

I. Methodology

SBP-CVM relationship: In order to assess where does the increase of the SBP-CVM slope come from after clonidine administration, this slope could be normalized to the set-point firing rate for baseline and clonidine groups. If increased CVM activity is the only source of SBP-CVM relationship increase, then the normalized SBP-CVM relationship should be the same for baseline and clonidine groups.

SBP-RR relationship: An unexpected finding of this study was the fact that SBP-CVM slope increase, while the SBP-RR interval relationship is not significantly increased. This awaits a further analysis. A study performed on a set of rats with no electrodes mounted on the cardiac branch should clarify why the increase in SBP-RR interval relationship is not significant.

Saturation of CVM: Presumed CVM units analysed in a previous study increased their frequency to a maximum beyond which firing frequency was not increased even though the HR response increased (2). This suggests a saturation phenomenon (chap. IV.3.3). Since cells in this study were not fully antidromically identified a new analysis could be done, on the experimental set-up described in the present study. This should clarify whether there is a saturation phenomenon and the mechanism by which HR increases when CVM activity is saturated.

Respiratory pattern of CVM: When calculating the slope of the SBP-CVM relationship, the respiratory pattern of the CVM, if present, was not taken in consideration (chap. IV.3.4). Indeed, a momentary silencing of the CVM activity due to an inspiratory inhibition during a pressure rise could affect the SBP-CVM slope. This could be accounted for by discarding CVM where a strong respiratory modulation is observed. In this case, care should be taken when describing the sample population, since these respiratory modulated CVM might represent a specific category of neurons.

Relation between CVM and sympathetic neurons: The experimental set-up of the present study could help to understand whether there is any connection between the activity of CVM and the sympathetic activity (chap. IV.3.5). The interaction between the sympathetic system and the parasympathetic system should be addressed centrally. Thus interactions between presympathetic motoneurons and CVM should be addressed using systemic (clonidine, alpha-methylparatyrosine, etc) and local (microejection) interventions.

II. Pharmacology

Clonidine, site of action: In order to further investigate the site of action of the clonidine, experiments similar to BNP vagal stimulation experiments (ms2) should be performed. Thus, analysing the effects of stimulating the right cut vagus on the HR before and after clonidine administration, could infer whether clonidine acts at the level of parasympathetic arm projection on the heart or upstream.

Clonidine, BNP site of action: Iontophoretical application and pneumatic ejection of clonidine and BNP at different levels of the neural arc (NA, NTS) were not performed in this study. However, this could elucidate whether these drugs have a direct action on the CVM, or on neurons projecting from the NTS to the NA, or on the baroreceptors, or on all together.

Defence reaction : CVM were shown to be inhibited during stimulation of the dorsal periaqueductal grey matter (PAGd). (59). However, this demonstration falls short as the CVM were not antidromically identified. Furthermore the n is low (n=3). Thus the demonstration remains to be completed. Of course various drugs known to interfere with the defence reaction e.g.clonidine, granisetron (122), would be of interest.

Experimental setting: Cardiac vagal activity is considered a key target to manipulate the cardiovascular system (131). The experimental setting described in this work will allow pharmacological investigation of different drugs known to have a cardiovascular effect, like newer centrally acting agents (rilmelidine, monoxidine) and other centrally acting agents (angiotensin antagonists, M1 antagonists : pirenzepine, etc). Thus, a better understanding of the mechanism and level of action of these drugs in the cardiac baroreflex arc could be achieved.

APPENDIX

I. Difficulties encountered during the experiments

Given the difficulty of the experiment, success rate has to be considered in order to prevent any systematic error (**table 3**). Indeed, the rate of success of the second series of experiments was seriously affected by a systematic incorrect mounting of electrodes on the cardiac branch.

Table 3. Success rate in different sets of CVM experiments

Series of exp	Total nb. of rats	Cells recorded	Success rate
A (CLO)	22	5	23%
E+S (AT)	72	10	14%
E (BNP)	25	8	32%
E (CLO+AT)	15	5	33%

To prevent such difficulties the following has to be taken into account:

- A saline infusion should be run through the arterial catheter, in order to prevent clotting and to replace blood losses due to surgery, anesthesia and ventilation. However, this infusion should be controlled at a rate of 2-3ml/h. This volume infusion is to be increased in case of important blood losses. Care should be taken not to overload the animal, at the expense of oedema, cardiac vagal deactivation and CVM silencing.

- Overheating due to excessive use of thermocautery should be avoided, in order to preserve animal's physiological condition. An excessive heating of the medulla could also induce cerebral oedema. Pouring cold saline on the cauterised tissue would reduce such problems.

- The PTCA catheter, preferably lubricated with silicone, with the balloon tightly deflated around the catheter lumen, should be introduced through the right femoral artery. The advance should be done slowly, especially at the iliac bifurcation. At this level, a perforation of the aorta due to the sharp and tough end of the catheter could be lethal. The amplitude of the pressure rise upon balloon inflation is a function of the position of the catheter in the abdominal vs thoracic aorta. In order to avoid any

difficulty during recording itself, the balloon should be placed at the right position at the beginning of the experiment. Any change in the position of the balloon once a cell is found would lead to loosing the cell. A more rostral position, however, is preferred to a more caudal one: in case of an excessive pressure rise (> 50 mmHg) the balloon could be slowly moved caudally toward the abdominal aorta.

- The electrodes used for antidromical stimulation should be placed with great care on the cardiac branch (**figure 14**). The cardiac branch itself should be treated with great caution. The branch should be kept wet (e.g. by putting a small piece of wet tissue along it), because drying might impair the physiological condition of the nerve. A pair of electrodes made from teflon-coated silver wire, bared at the tips, is placed under the cardiac branch, keeping it in mild tension. Putting the electrodes around the nerve might, theoretically, improve the chances to have a good contact. However, this should be avoided, because there is a great chance to damage the branch. This would minimize the rate of success of the experiment. *We experienced a very low success rate due to such a wrong placement of electrodes combined with absence of saline over the cardiac branch.* The wires of the electrodes are tied to a rib to avoid pulling on the nerve. A small sheet of polyethylene inserted beneath the branch permits a better isolation from the underlying tissue. The whole setup is secured in contact with silicon gel.

- The surgery of the medulla should be done carefully. Any vascular breach on the medulla surface is difficult to manage. Cauterising at this level is difficult, and if successful eventually degenerates in oedema. Thus carrying on the experiment is impossible.

- The carbon-fiber electrode used for SUA recording, filled with NaCl 2M solution should be temporarily covered with silicone on the top, in order to prevent saline evaporation.

- Whenever a silent cell is found, occasional balloon generated pressure ramps might activate the cell: then a collision test could be done.

II. Working hypothesis regarding the beat-by-beat functioning of the cardiac baroreflex

Does the cardiac vagal activity mimic an intra-aortic balloon ?

A key question behind this work is the buffering of the pressure lability by the sinus arrhythmia. Upon a spontaneous pressure rise during $systole_n$ there is a lengthening of the $diastole_n$ and an increase in the stroke $volume_{n+1}$. This larger stroke volume should lead to a rise in SBP during $systole_{n+1}$. However, in healthy volunteers, the SBP observed during $systole_{n+1}$ is lower than SBP observed during $systole_n$.

How can this be explained ? The first hypothesis to handle these contradictory facts (a lower SBP_{n+1} with a longer $diastole_n$) is to suppose that the compliance of the aorta and large vessels (*Windkessel*) is immediately increased : this is unlikely to be the case as a) the *Windkessel* is relatively rigid b) the sluggishness of the sympathetic system does not allow an immediate vasodilatation either at the level of the *Windkessel* or at the level of small arteries. Thus the only option left is to suppose that the output from the *Windkessel* during the prolonged $diastole_n$ is larger than the input into the *Windkessel* caused by the stroke $volume_{n+1}$. This larger increase in output as compared to the enlarged stroke $volume_{n+1}$ allows a lower DBP_n and a lower SBP_{n+1}. The stroke $volume_{n+1}$ “sees” an emptied aorta, with a mechanism similar to the one used during deflation of an intraaortic balloon immediately before systole. Aortic counterpulsation mimics cardiac vagal activity : diastolic $pressure_n$ is so lowered that the next SBP_{n+1} is lower than SBP_n. Thus, the cardiac parasympathetic system plays within one beat with the only variable which can be immediately adjusted : the total diastolic $time_n$. Such an hypothesis would require, in healthy volunteers or dogs, the placement of Doppler flow probes at the aortic root and at the various major vessels (carotids, subclavian and femoral arteries) to measure regional flows under baseline, atropine low and high dose or clonidine conditions. *If the technology is precise enough*, an increase should be observed in regional flows during $diastole_n$ as compared to aortic flow during $systole_{n+1}$ during baseline, atropine low dose or clonidine conditions. By contrast, shortening of $diastole_n$, after atropine high dose, would not allow the outgoing regional flows to be larger than the aortic $flow_{n+1}$.

References

1. **Agarwal SK and Calaresu FR**. Reciprocal connections between nucleus tractus solitarii and rostral ventrolateral medulla. *Brain Res* 523: 305-308, 1990.
2. **Agarwal SK and Calaresu FR**. Electrical stimulation of nucleus tractus solitarius excites vagal preganglionic cardiomotor neurons of the nucleus ambiguus in rats. *Brain Res* 574: 320-324, 1992.
3. **Agarwal SK, Gelsema AJ and Calaresu FR**. Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. *Am J Physiol* 257: R265-R270, 1989.
4. **Agarwal SK, Gelsema AJ and Calaresu FR**. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am J Physiol* 258: R1271-R1278, 1990.
5. **Anonymous**. The sixth report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Int Med* 157: 2413-2445, 1997.
6. **Anrep GV and Rossler R.** Respiratory variations of the heart rate. II The central mechanism of the respiratory arrhythmia and the interrelationships between the central and reflex mechanisms. *Proc Roy Soc* 119: 218-231, 1935.
7. **Aronson S, Fontes ML, Miao Y and Mangano DT**. Risk index for perioperative renal dysfunction/failure: critical dependence on pulse pressure hypertension. *Circulation* 115: 733-742, 2007.
8. **Berntson GG, Bigger JT, Eckberg DL, Grossmann P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH and Van Der Molen MW**. Heart rate variability: origins, methods, and interpretive caveats. *Psychophys* 34: 623-648, 1997.
9. **Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A and Mancia G**. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol* 254: H377-H383, 1988.
10. **Blinder KJ, Gatti PJ, Johnson TA, Lauenstein JM, Coleman WP, Gray AL and Massari VJ**. Ultrastructural circuitry of cardiorespiratory reflexes: there is a monosynaptic path between the nucleus of the solitary tract and vagal preganglionic motoneurons controlling atrioventricular conduction in the cat. *Brain Res* 785: 143-157, 1998.
11. **Borison HL and Domjan D**. Persistence of the cardio-inhibitory response to brain stem ischaemia after destruction of the area postrema and the dorsal vagal nuclei. *J Physiol* 211: 263-277, 1970.
12. **Borst C and Karemaker J**. Time delays in the human baroreceptor reflex. *J Auton Nerv Syst* 9: 399-409, 1983.
13. **Bouairi E, Neff R, Evans C, Gold A, Andresen MC and Mendelowitz D**. Respiratory sinus arrhythmia in freely moving and anesthetized rats. *J Appl Physiol* 97: 1431-1436, 2004.
14. **Bousquet P, Feldman J and Schwartz J**. Central cardiovascular effects of alpha adrenergic drugs : differences between catecholamines and imidazolines. *J Pharmacol Exp Ther* 230: 232-236, 1984.
15. **Bristow JD, Honour AJ, Pickering GW, Sleight P and Smyth HS**. Diminished baroreflex sensitivity in high blood pressure. *Circulation* 34: 48-54, 1969.
16. **Brown DL and Guyenet PG**. Cardiovascular neurons of brain stem with projections to spinal cord. *Am J Physiol* 247: R1009-R1016, 1984.
17. **Brown GL and Eccles JC**. The action of a single vagal volley on the rhythm of the hear beat. *J Physiol (London)* 82: 211-241, 1934.
18. **Brunner-La Rocca HP, Esler MD, Jennings GL and Kaye DM**. Effect of cardiac sympathetic nervous activity on mode of death in congestive heart failure. *Eur Heart J* 22: 1136-1143, 2001.
19. **Burgos LG, Ebert TJ, Asiddao C, Turner LA, Pattison CZ, Wang-Cheng R and Kampine JP**. Increased intraoperative cardiovascular morbidity in diabetics with autonomic neuropathy. *Anesthesiology* 70: 591-597, 1989.
20. **Casadei B, Meyer T, Coats AJS, Conway J and Sleight P**. Baroreflex control of stroke volume in man : an effect mediated by the vagus. *J Physiol (London)* 448: 539-550, 1992.
21. **Cividjian A, Rentero N, McAllen RM and Quintin L**. A simple method for generating a blood pressure-unit activity relationship for central cardiovascular neurons in the rat. *Experimental Physiology* 87: 535-538, 2002.
22. **Cividjian A, Martinez JY, Combourieu E, Precloux P, Beraud AM, Rochette Y, Cler M, Bourdon L, Escarment J and Quintin L**. Beat-by-beat cardiovascular index to predict unexpected intraoperative movement in anesthetized unparalyzed patients: a retrospective analysis. *J Clin Monit Comput* 21: 91-101, 2007.
23. **Cividjian A, Rentero N, Pequignot JM and Quintin L**. Effect of catecholamine depletion on increased blood pressure lability upon emergence from halothane anesthesia in rats. *Journal of Anesthesia (Tokyo)* 2008.
24. **Coleman TG**. Arterial baroreflex control of heart rate in the conscious rat. *Am J Physiol* 238: H515-H520, 1980.
25. **Coleridge HM and Coleridge JCG**. Cardiovascular afferents involved inthe control of peripheral vessels. *Annual Review of Physiology* 42: 413-427, 1980.
26. **Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, Godwin J, Qizilbash N, Taylor JO and Hennekens CH**. Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* 335: 827-838, 1990.
27. **Colucci WS**. Neseritide for the treatment of decompensated heart failure. *Journal of Cardiac Heart Failure* 7: 92-100, 2001.

28. **Cowley AW, Liard J-F and Guyton AC**. Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. *Circ Res* 32: 564-576, 1973.

29. **Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, Polson JW, Potts PD and Tagawa T**. Central mechanisms underlying short- and long-term regulation of the cardiovascular system. *Clin Exp Pharmacol Physiol* 29: 261-268, 2002.

30. **DeBoer RW, Karemaker JM and Strackee J**. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *Am J Physiol* 253: H680-H689, 1987.

31. **Dexter F, Yang T and Levy MN**. Analysis of vagally induced sinus arrhythmia. *J Theor Biol* 159: 507-511, 1992.

32. **Di Rienzo M, Bertinieri G, Mancia G and Pedotti A**. A new method for evaluating the baroreflex role by a joint pattern analysis of pulse interval and systolic blood pressure series. *Med Biol Eng Comp* 23: 313-314, 1985.

33. **Dodd J and Role LW**. The autonomic nervous system. In: Principles of neural science, edited by Kandel ER, Schwartz JH and Jessell TM. Prentice-Hall, 1991, p. 761-775.

34. **Eckberg DL**. Nonlinearities of the human carotid baroreceptor-cardiac reflex. *Circ Res* 47: 208-216, 1980.

35. **Eckberg DL, Drabinsky M and Braunwald E**. Defective cardiac parasympathetic control in patients with heart disease. *N Engl J Med* 285: 877-883, 1971.

36. **Eckberg DL, Kifle YT and Roberts VL**. Phase relationship between normal human respiration and baroreflex responsiveness. *J Physiol (London)* 304: 489-502, 1980.

37. **Espiner EA, Richards AM, Yandle TG and Nicholls MG**. Natriuretic hormones. *Endocrinol Metab Clin North Am* 24: 481-509, 1995.

38. **Faris I, Iannos J, Jamieson G and Ludbrook J**. Comparison of methods for eliciting the baroreceptor-heart rate reflex in conscious rabbits. *Clin Exp Pharmacol Physiol* 7: 281-291, 1980.

39. **Ferrari AU, Daffonchio A, Albergati F and Mancia G**. Inverse relationship between heart rate and blood pressure variabilities in rats. *Hyper* 10: 533-537, 1987.

40. **Flapan AD, Nolan J, Neilson JMM and Ewing DJ**. Effect of captopril on cardiac parasympathetic activity in chronic cardiac failure secondary to coronary artery disease. *Am J Cardiol* 69: 532-535, 1992.

41. **Fritsch JM, Eckberg DL, Graves LD and Wallin BG**. Arterial pressure ramps provoke linear increases of heart period in humans. *Am J Physiol* 251: R1086-R1090, 1986.

42. **Gandevia SC, Killian K, McKenzie DK, Allen GMGRB and Hales JP**. Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. *J Physiol (London)* 470: 85-107, 1993.

43. **Geis GS, Kozelka J W and Wuster RD**. Organisation and reflex control of vagal cardiomotor neurons. *Journal of the Autonomic Nervous System* 3: 437-450, 1981.

44. **Ghignone M, Calvillo O and Quintin L**. Anesthesia and Hypertension : the effect of clonidine on perioperative hemodynamics and isoflurane requirements. *Anesthesiology* 67: 3-10, 1987.

45. **Gilbey MP, Jordan D, Richter DW and Spyer KM**. Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *J Physiol (London)* 356: 65-78, 1984.

46. **Godwin SJ, Tortelli CF, Parkin ML and Head GA**. Comparison of the baroreceptor-heart rate reflex effects of moxonidine, rilmenidine and clonidine in conscious rabbits. *J Auton Nerv Syst* 72: 195-204, 1998.

47. **Gonon FG**. Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Nsci* 24: 19-28, 1988.

48. **Gratadour P, Viale JP, Parlow J, Sagnard P, Bagou G, Annat G, Hughson RL and Quintin L**. Sympathovagal effect of spinal anesthesia assessed by the spontaneous cardiac baroreflex. *Anesthesiology* 87: 1359-1367, 1997.

49. **Grkovic I, Fernandez K, McAllen RM and Anderson CR**. Misidentification of cardiac vagal pre-ganglionic neurons after injections of retrograde tracer into the pericardial space in the rat. *Cell Tissue Res* 321: 335-340, 2005.

50. **Gunn CG, Sevelius G, Puiggari J and Myers FK**. Vagal cardiomotor mechanisms in the hindbrain of the dog and cat. *Am J Physiol* 214: 258-262, 1968.

51. **Guyenet P**. Role of the ventral medulla oblongata in blood pressure regulation. In: Central Regulation of Autonomic Functions, edited by Loewy AD and Spyer KM. Oxford: Oxford University Press, 1990, p. 145-167.

52. **Habler HJ, Janig W and Michaelis M**. Respiratory modulation in the activity of sympathetic neurones. *Progress in Neurobiology* 43: 567-606, 1994.

53. **Herring N, Zaman JA and Paterson DJ**. Natriuretic peptides like NO facilitate cardiac vagal neurotransmission and bradycardia via a cGMP pathway. *Am J Physiol Heart Circ Physiol* 281: H2318-H2327, 2001.

54. **Hill-Smith I and Purves RD**. Synaptic delay in the heart : an ionophoretic study. *J Physiol (London)* 279: 31-54, 1978.

55. **Hohnloser SH, Klingenheben T, van de LA, Hablawetz E, Just H and Schwartz PJ**. Reflex versus tonic vagal activity as a prognostic parameter in patients with sustained ventricular tachycardia or ventricular fibrillation. *Circulation* 89: 1068-1073, 1994.

56. **Hopkins DA, Gootman PM, Gootman N, Di Russo SM and Zeballos ME**. Brainstem cells of origin of the cervical vagus and cardiopulmonary nerves in the neonatal pig (Sus scrofa). *Brain Res* 306: 63-72, 1984.

57. **Hull SS, Vanoli E, Adamson PB, Verrier RL, Foreman RD and Schwartz PJ**. Exercise training confers anticipatory protection from sudden death during acute myocardial ischemia. *Circulation* 89: 548-552, 1994.

58. **Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, Ganiats TG, Goldstein S, Gregoratos G, Jessup ML, Noble RJ, Packer M, Silver MA, Stevenson LW, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Jacobs AK, Hiratzka LF, Russell RO and Smith SC, Jr.** ACC/AHA Guidelines for the Evaluation and Management of Chronic Heart Failure in the Adult: Executive Summary A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): Developed in Collaboration With the International Society for Heart and Lung Transplantation; Endorsed by the Heart Failure Society of America. *Circulation* 104: 2996-3007, 2001.

59. **Inui K and Nosaka S**. Target site of inhibition mediated by midbrain periaqueducal grey matter of baroreflex vagal bradycardia. *J Neurophysiol* 70: 2205-2214, 1993.

60. **Izzo PN, Deuchars J and Spyer KM**. Localization of cardiac vagal preganglionic motneurones in the rat : immunocytochemical evidence of synaptic inputs containing 5-hydroxytryptamine. *J Comp Neurol* 327: 572-583, 1993.

61. **Jewett DL**. Activity of single efferent fibres in the cervical vagus nerve of the dog, with special reference to possible cardio-inhibitory fibres. *J Physiol (London)* 175: 321-357, 1964.

62. **Jones JFX, Wang Y and Jordan D**. Heart rate responses to selective stimulation of cardiac vagal C fibres in anesthetized cats, rats and rabbits. *J Physiol (London)* 489: 203-214, 1995.

63. **Jones JF**. Vagal control of the rat heart. *Exp Physiol* 86: 797-801, 2001.

64. **Jones JF**. A moving-invisible-needle in a haystack experiment. *Exp Physiol* 92: 1003, 2007.

65. **Jordan J, Tank J, Shannon JR, Diedrich A, Lipp A, Schroder C, Arnold G, Sharma AM, Biaggioni I, Robertson D and Luft FC**. Baroreflex buffering and susceptibility to vasoactive drugs. *Circulation* 105: 1459-1464, 2002.

66. **Jose AD and Taylor RR**. Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. *J Clin Invest* 48: 2019-2030, 1969.

67. **Julius S and Gudbrandsson T**. Early association of sympathetic overactivity, hypertension, insulin resistance, and coronary risk. *J Cardiovasc Pharmacol* 20 Suppl 8: S40-S48, 1992.

68. **Kalia M and Mesulam MM**. Brain stem projections of sensory and motor components of the vagus complex in the cat. *J Comp Neurol* 193: 435-508, 1980.

69. **Katona PG, Lipson D and Dauchot PJ**. Opposing central and peripheral effects of atropine on parasympathetic cardiac control. *Am J Physiol* 232: H146-H151, 1977.

70. **Katona PG, Poitras JW, Barnett GO and Terry BS**. Cardiac vagal efferent activity and heart period in the carotid sinus reflex. *Am J Physiol* 218(4): 1030-1037, 1970.

71. **Ketch T, Biaggioni I, Robertson R and Robertson D**. Four faces of baroreflex failure: hypertensive crisis, volatile hypertension, orthostatic tachycardia, and malignant vagotonia. *Circulation* 105: 2518-2523, 2002.

72. **Kikuta K, Yasue H, Yoshimura M, Morita E, Sumida H, Kato H, Kugiyama K, Ogawa H, Okumura K, Ogawa Y and Nakao K**. Increased plasma levels of B-type natriuretic peptide in patients with unstable angina. *Am Heart J* 132: 101-107, 1996.

73. **Kikuya M, Hozawa A, Ohokubo T, Tsuji I, Michimata M, Matsubara M, Ota M, Nagai K, Araki T, Satoh H, Ito S, Hisamichi S and Imai Y**. Prognostic significance of blood pressure and heart rate variabilities : the Ohasama study. *Hyper* 36: 901-906, 2000.

74. **Koh J, Brown TE, Beightol LA and Eckberg DL**. Contribution of tidal lung inflation to human RR interval and arterial pressure fluctuations. *J Auton Nerv Syst* 68: 89-95, 1998.

75. **Koh J, Brown TE, Beightol LA, Ha CY and Eckberg DL**. Human autonomic rhythms: vagal cardiac mechanisms in tetraplegic subjects. *J Physiol (London)* 474: 483-495, 1994.

76. **Kong S, Liu JH, Ramage AG and Wang Y**. Cardiac vagal preganglionic neurones in the intermediate zone of the brainstem in anaesthetized cats. *Exp Physiol* 92: 1023-1028, 2007.

77. **Korner PI, Shaw J, West MJ and Oliver JR**. Central nervous system control of baroreceptor reflexes in the rabbit. *Circ Res* 31: 637-652, 1972.

78. **Krayer O**. The history of the Bezold-Jarisch effect. *Naunyn* 240: 361-368, 1961.

79. **Kunze DL**. Reflex discharge patterns of cardiac vagal efferent fibres. *J Physiol (London)* 222: 1-15, 1972.

80. **Kyriakides ZS, Markianos M, Michalis L, Antoniadis A, Nikolaou NI and Kremastinos DT**. Brain natriuretic peptide increases acutely and much more prominently than atrial natriuretic peptide during coronary angioplasty. *Clin Cardiol* 23: 285-288, 2000.

81. **La Rovere MT, Bigger JT, Marcus FI, Mortara A and Schwartz PJ**. Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 351: 478-484, 1998.

82. **Latson TW, Ashmore TH, Reinhart DJ, Klein KW and Giesecke AH**. Autonomic reflex dysfunction in patients presenting for elective surgery is associated with hypotension after anesthesia induction. *Anesthesiology* 80: 326-337, 1994.

83. **Laubie M, Schmitt H and Drouillat M**. Action of clonidine on the baroreceptor pathway and medullary sites mediating vagal bradycardia. *Eur J Pharmacol* 38: 293-303, 1976.

84. **Lee TM, Kuo JS and Chai CY**. Central integrating mechanism of the Bezold-Jarisch and baroceptor reflexes. *Am J Physiol* 222: 713-720, 1972.

85. **Levy MN, Iano T and Zieske H**. Effects of repetitive bursts of vagal activity on heart rate. *Circ Res* 30: 186-195, 1972.

86. **Levy MN, Yang T and Wallick DW**. Assessment of beat-by-beat control of heart rate by the autonomic nervous system : molecular biology techniques are necessary, but not sufficient. *J Cardiovasc Electrophysiol* 4: 183-193, 1993.

87. **Lipski J**. Antidromic activation of neurones as an analytical tool in the study of the central nervous system. *J Neurosci Methods* 4: 1-32, 1981.

88. **Machado BH and Brody MJ**. Effect of nucleus ambiguus lesion on the development of neurogenic hypertension. *Hyper* 11: I135-I138, 1988.

89. **Machado BH and Brody MJ**. The nucleus ambiguus region participates in arterial pressure regulation. *Neurosci Lett* 135: 91-94, 1992.

90. **Mancia G, Bertinieri G, Cavallazzi A, Di Rienzo M, Parati G, Pomidossi G, Ramirez AJ and Zanchetti A**. Mechanisms of blood pressure variability in man. *Clinical and Experimental Hypertension (Theory and Practice)* A7: 167-178, 1985.

91. **Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G and Bertinieri G**. Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. *Circ Res* 53: 96-103, 1983.

92. **Mancia G and Mark AL**. Arterial baroreflexes in humans. In: Handbook of physiology, Bethesda,MD: American Physiological Society, 1983, p. 755-793.

93. **Mark AL**. The Bezold-Jarisch reflex revisited : clinical implications of inhibitory reflexes originating in the heart. *J Am Coll Cardiol* 1: 90-102, 1983.

94. **Massari VJ, Dickerson LW, Gray AL, Lauenstein JM, Blinder KJ, Newsome JT, Rodak DJ, Fleming TJ, Gatti PJ and Gillis RA**. Neural control of left ventricular contractility in the dog heart: synaptic interactions of negative inotropic vagal preganglionic neurons in the nucleus ambiguus with tyrosine hydroxylase immunoreactive terminals. *Brain Res* 802: 205-220, 1998.

95. **Massari VJ, Johnson TA and Gatti PJ**. Cardiotopic organization of the nucleus ambiguus ? an anatomical and physiological analysis of neurons regulating atrioventricular conduction. *Brain Res* 679: 227-240, 1995.

96. **McAllen RM and Spyer KM**. The location of cardiac vagal preganglionic motoneurones in the medulla of the cat. *J Physiol (London)* 258: 187-204, 1976.

97. **McAllen RM and Spyer KM**. The baroreceptor input to cardiac vagal motoneurones. *J Physiol (London)* 282: 365-374, 1978.

98. **McAllen RM and Spyer KM**. Two types of vagal preganglionic motoneurones projecting to the heart and lungs. *J Physiol (London)* 282: 353-364, 1978.

99. **McKitrick D J and Calaresu FR**. Nucleus ambiguus inhibits activity of cardiovascular units in RVLM. *Brain Res* 742: 203-210, 1996.

100. **McKitrick DJ and Calaresu FR**. Reciprocal connection between nucleus ambiguus and caudal ventolateral medulla. *Brain Res* 770: 213-220, 1997.

101. **Mendelowitz D**. Advances in parasympathetic control of heart rate and cardiac function. *NIPS* 14: 155-161, 1999.

102. **Mortara A, La Rovere MT, Pinna GD, Prpa A, Maestri R, Febo O, Pozzoli M, Opasich C and Tavazzi L**. Arterial baroreflex modulation of heart rate in chronic heart failure : clinical and hemodynamic correlates and prognostic implications. *Circulation* 96: 3450-3458, 1997.

103. **Muzi M and Ebert TJ**. A comparison of baroreflex sensitivity during isoflurane and desflurane anesthesia in humans. *Anesthesiology* 82: 919-925, 1995.

104. **Muzi M, Goff DR, Kampine JP, Roerig DL and Ebert TJ**. Clonidine reduces sympathetic activity but maintains baroreflex responses in normotensive humans. *Anesthesiology* 77: 864-871, 1992.

105. **Nicholls MG**. The natriuretic peptides in heart failure. *J Intern Med* 235: 515-526, 1994.

106. **Nosaka S, Yamamoto T and Yasunaga K**. Localization of vagal cardioinhibitory preganglionic neurons within rat brain stem. *J Comp Neur* 186: 79-92, 1979.

107. **Nosaka S, Yasunaga K and Tamai S**. Vagal cardiac preganglionic neurons : distribution, cell types and reflex discharges. *Am J Physiol* 243: R92-R98, 1982.

108. **O'Leary DM and Jones JF**. Discharge patterns of preganglionic neurones with axons in a cardiac vagal branch in the rat. *Exp Physiol* 88: 711-723, 2003.

109. **Osculati G, Giannattasio C, Seravalle G, Valagussa F, Zanchetti A and Mancia G**. Early alterations of the baroreceptors control of heart rate in patients with acute myocardial infarction. *Circulation* 81: 939-948, 1990.

110. **Osterziel KJ, Hanlein D, Willenbroock R, Eichhorn C, Luft F and Dietz R**. Baroreflex sensitivity and cardiovascular mortality in patients with mild to moderate heart failure. *Brit Heart J* 73: 517-522, 1995.

111. **Pagani M, Lombardi F, Guzzetto S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, Turiel M, Baselli G, Cerutti S and Malliani A**. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 59: 178-193, 1986.

112. **Parati G, Pomidossi G, Casadei R, Groppelli A, Trazzi S, Di Rienzo M and Mancia G**. Role of heart rate variability in the production of blood pressure variability in man. *J Hypertension* 5: 557-560, 1987.

113. **Parlow J, Begou G, Sagnard P, Cottet-Emard JM, Levron JC, Annat G, Bonnet F, Ghignone M, Hughson RL, Viale JP and Quintin L**. Cardiac baroreflex during the postoperative period in patients with hypertension : effect of clonidine. *Anesthesiology* 90: 681-692, 1999.

114. **Parlow J, Viale JP, Annat G, Hughson RL and Quintin L**. Spontaneous cardiac baroreflex in humans : comparison with drug-induced responses. *Hyper* 25: 1058-1068, 1995.

115. **Pickering AE, Boscan P and Paton JF**. Nociception attenuates parasympathetic but not sympathetic baroreflex via NK1 receptors in the rat nucleus tractus solitarii. *J Physiol* 551: 589-599, 2003.

116. **Pickering TG, Gribbin B and Sleight P**. Comparison of the reflex heart rate response to rising and falling arterial pressure in man. *Cardiovasc Res* 6: 277-283, 1972.

117. **Porter R**. Unit response evoked in the medulla oblongata by vagus nerve stimulation. *J Physiol (London)* 168: 717-735, 1963.

118. **Potter EK**. Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimuli in the dog. *J Physiol* 316: 177-190, 1981.

119. **Rentero N, Cividjian A, Trevaks D, Pequignot JM, Quintin L and McAllen RM**. Activity patterns of cardiac vagal motoneurons in rat nucleus ambiguus. *Am J Physiol* 283: R1327-R1334, 2002.

120. **Sander D, Kukla C, Klingelhofer J, Winbeck K and Conrad B**. Relationship between circadian blood pressure patterns and progression of early carotid artherosclerosis : a 3 year follow up study. *Circulation* 102: 1536-1541, 2000.

121. **Schultz HD and Ustinova EE**. Cardiac vagal afferent stimulation by free radicals during ischaemia and reperfusion. *Clin Exp Pharmacol Physiol* 23: 700-708, 1996.

122. **Sevoz-Couche C, Comet MA, Hamon M and Laguzzi R**. Role of nucleus tractus solitarius 5-HT3 receptors in the defense reaction-induced inhibition of the aortic baroreflex in rats. *J Neurophysiol* 90: 2521-2530, 2003.

123. **Sleight P, West MJ, Korner PI, Oliver JR, Chalmers JP and Robinson JL**. The action of clonidine on the baroreflex control of heart rate in conscious animals and man and on single aortic baroreceptor discharge in the rabbit. *Arch Int Pharmacodyn* 214: 4-11, 1975.

124. **Smyth HS, Sleight P and Pickering GW**. Reflex regulation of arterial pressure during sleep in man. *Circ Res* 24: 109-121, 1969.

125. **Spyer KM**. Neural mechanisms involved in cardiovascular control during affective behavior. *Trends Neurosci* 12: 506-513, 1989.

126. **Sun MK and Guyenet P**. Effect of clonidine and gamma-aminobutyric acid on the discharges of medullo-spinal sympathoexcitatory neurons in the rat. *Brain Res* 368: 1-17, 1986.

127. **Sun MK and Guyenet PG**. Arterial baroreceptor and vagal inputs to sympathoexcitatory neurons in rat medulla. *Am J Physiol* 252: R699-R709, 1987.

128. **Takanaga A, Hayakawa T, Tanaka K, Kawabata K, Maeda S and Seki M**. Immunohistochemical characterization of cardiac vagal preganglionic neurons in the rat. *Auton Neurosci* 106: 132-137, 2003.

129. **Thomas CJ, Head GA and Woods RL**. Similar baroreflex bradycardic actions of atrial natriuretic peptide and B and C types of natriuretic peptides in conscious rats. *J Hypertension* 17: 801-806, 1999.

130. **Thomas CJ, May CN, Sharma AD and Woods RL**. ANP,BNP,CNP enhance bradycardic responses to cardiopulmonary chemoreceptor activation in conscious sheep. *Am J Physiol* 280: R282-R288, 2001.

131. **Townend JN and Littler WA**. Cardiac vagal activity : a target for intervention in heart disease. *Lancet* 1: 937-938, 1995.

132. **Vanoli E, DeFerrari GM, Stramba-Badiale M, Hull SS, Foreman RD and Schwartz PJ**. Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ Res* 68: 1471-1481, 1991.

133. **Verberne AJ and Guyenet PG**. Medullary pathway of the Bezold-Jarisch reflex in the rat. *Am J Physiol* 263: R1195-R1202, 1992.

134. **Vesalainen RK, Kaila TJ, Kantola IM, Tahvanainen KUO, Airaksinen KEJ, Kuusela TA and Eckberg DL**. Low-dose transdermal scopolamine decreases blood pressure in mild essential hypertension. *J Hypertension* 16: 321-329, 1998.

135. **Watkins L and Maixner W**. Hypotensive effect of clonidine is not mediated by enhanced baroreflex gain in rats. *J Cardiovasc Pharmacol* 21: 264-271, 1993.

136. **Wennberg PW, Miller VM, Rabelink T and Burnett JC, Jr.** Further attenuation of endothelium-dependent relaxation imparted by natriuretic peptide receptor antagonism. *Am J Physiol* 277: H1618-H1621, 1999.

137. **Westerhof BE, Gisolf J, Stok WJ, Wesseling KH and Karemaker J**. Time-domain cross-correlation baroreflex sensitivity : performance on the EUROBAVAR data set. *J Hypertension* 22: 1371-1380, 2004.

138. **Woods RL**. Cardioprotective functions of atrial natriuretic peptide and B-type natriuretic peptide : a brief review. *Clin Exp Pharmacol Physiol* 31: 791-794, 2004.

139. **Yasuma F and Hayano J**. Respiratory sinus arrhythmia: why does the heartbeat synchronize with respiratory rhythm? *Chest* 125: 683-690, 2004.

ACTIVATION PARASYMPATHIQUE CENTRALE MISE EN EVIDENCE PAR ENREGISTREMENT DES MOTONEURONES CARDIAQUES VAGAUX CHEZ LE RAT

L'innervation parasympathique du cœur est responsable de la régulation à court terme de la pression artérielle (PA), par le biais du contrôle battement par battement de la fréquence cardiaque. Une diminution de l'activité vagale est considérée comme indice de mauvais pronostic. La projection vagale du noyau ambigu sur le cœur constitue un chemin commun pour *le baroréflexe cardiaque* et l*e chemoreflexe de von Bezold Jarisch* (BJ). Des mises au point récentes des techniques d'enregistrement extracellulaire des motoneurones cardiaques vagaux (CVM) chez le rat ont rendu un peu plus aisé l'exploration de ces deux réflexes. Ceci permet une meilleure compréhension des mécanismes et de sites d'action. 1) Un agoniste α-2 adrénergique, la *clonidine*, hypotenseur central, agit sur la régulation à court-terme de la PA. Le présent travail a montré une augmentation de l'activité unitaire des CVMs, et de la pente du baroréflexe cardiaque au niveau central (relation PA-CVM) quand la clonidine est administrée systémiquement, en dose croissante (10-100µg/kg i.v.). Une analyse approfondie de l'activité des neurones a révélé un nouveau mécanisme d'action de la clonidine via des décharges rapides (« doublet »). 2) Une peptide natriuretique de type B (*BNP*), utilisée dans le traitement de l'insuffisance cardiaque, a augmenté significativement la bradycardie et l'activité des CVMs lors de l'activation du réflexe BJ. La bradycardie, proportionnelle à l'activation vagale, fait conclure par logique soustractive que le site d'action du BNP est sur le bras afférent du réflexe. Une activation parasympathique centrale représenterait une solution dans le traitement des problèmes cardiovasculaires.

Mots clés : motoneuron cardiaque vagal, vagus, bradycardie, baroréflexe, chemoreflexe, von Bezold-Jarisch, clonidine, BNP, rat, variabilité de la pression artérielle, activité unitaire.

CENTRAL PARASYMPATHETIC ACTIVATION ASSESSED BY CARDIAC VAGAL MOTONEURON RECORDINGS IN RAT

The parasympathetic supply to the heart is responsible for the short-term regulation of blood pressure (BP), by controlling the heart rate (HR) on a beat-by-beat basis. Decreased cardiac vagal activity is considered an index of poor outcome. The vagal projection of the nucleus ambiguus on the heart is the common pathway for different cardiac reflexes, like the *cardiac baroreflex* and the *von Bezold-Jarisch (BJ) chemoreflex*. Recent advances in extracellular recording methods of cardiac vagal motoneurons (CVM) in rat made easier the exploring of these two reflexes. Thus, a better understanding of the mechanisms and sites of action of two drugs could be achieved. 1). An α-2 adrenergic agonist, *clonidine*, a central hypotensive, influences the short-term regulation of BP. The present work shows an increase of the activity of CVM, together with the slope of the cardiac baroreflex at central level (BP-CVM relationship) when clonidine is administered systemically, in cumulative doses (10-100µg/kg i.v.). A deeper analysis of the CVM activity revealed a possible new mechanism of action of clonidine by fast firing ("doublet"). 2). B-type natriuretic peptide (BNP), used in the treatment of the heart failure, increased significantly the bradycardia and the CVM activity during the activation of the BJ reflex. The bradycardia is proportional to the vagal activation. This proves, by subtractive logic, that the BNP site of action is on the afferent arm of the reflex. Cardiac vagal activation is a solution in the treatment of cardiovascular disorders.

Key words: cardiac vagal motoneuron, vagus, bradycardia, baroreflex, chemoreflex, von Bezold-Jarisch, clonidine, BNP, rat, blood pressure variability, single unit activity.