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CENTRAL AUTONOMIC CONTROL IN SPONTANEOUSLY HYPERTENSIVE RATS: A STUDY ON PHASIC PHENOMENA DURING RAPID-EYE-MOVEMENT SLEEP

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Summary

The cardiovascular regulation undergoes wide changes in the different states of sleep-wake cycle. In particular, the relationship between spontaneous fluctuations in heart period and arterial pressure clearly shows differences between the two sleep states. In non rapid-eye-movement sleep, heart rhythm is under prevalent baroreflex control, whereas in rapid-eye-movement sleep central autonomic commands prevail (Zoccoli et al., 2001). Moreover, during rapid-eye-movement sleep the cardiovascular variables show wide fluctuations around their mean value. In particular, during rapid-eye-movement sleep, the arterial pressure shows phasic hypertensive events which are superimposed upon the tonic level of arterial pressure. These phasic increases in arterial pressure are accompanied by an increase in heart rate (Sei & Morita, 1996; Silvani *et al.*, 2005). Thus, rapid-eye-movement sleep may represent an "autonomic stress test" for the cardiovascular system, able to unmask pathological patterns of cardiovascular regulation (Verrier et al. 2005), but this hypothesis has never been tested experimentally.

The aim of this study was to investigate whether rapid-eye-movement sleep may reveal derangements in central autonomic cardiovascular control in an experimental model of essential hypertension. The study was performed in Spontaneously Hypertensive Rats, which represent the most widely used model of essential hypertension, and allow full control of genetic and environmental confounding factors.

In particular, we analyzed the cardiovascular, electroencephalogram, and electromyogram changes associated with phasic hypertensive events during rapid-eye-movement sleep in Spontaneously Hypertensive Rats and in their genetic Wistar Kyoto control strain. Moreover, we studied also a group of Spontaneously Hypertensive Rats made phenotypically normotensive by means of a chronic treatment with an angiotensin converting enzyme inhibitor, the Enalapril maleate, from the age of four weeks to the end of the experiment. All rats were implanted with electrodes for electroencephalographic and electromyographic recordings and with an arterial catheter for arterial pressure measurement. After six days for postoperative recovery, the rats were studied for five days, at an age of ten weeks.

The study indicated that the peak of mean arterial pressure increase during the phasic hypertensive events in rapid-eye-movement sleep did not differ significantly between Spontaneously Hypertensive Rats and Wistar Kyoto rats, while on the other hand Spontaneously Hypertensive Rats showed a reduced increase in the frequency of theta rhythm and a reduced tachicardia with respect to Wistar Kyoto rats. The same pattern of changes in mean arterial pressure, heart period, and theta frequency was observed between Spontaneously Hypertensive Rats and Spontaneously Hypertensive Rats treated with Enalapril maleate. Spontaneously Hypertensive Rats do not differ from Wistar Kyoto rats only in terms of arterial hypertension, but also due to multiple unknown genetic differences. Spontaneously Hypertensive Rats were developed by selective breeding of Wistar Kyoto rats based only on the level of arterial pressure. However, in this process, multiple genes possibly unrelated to hypertension may have been selected together with the genetic determinants of hypertension (Carley *et al.*, 2000). This study indicated that Spontaneously Hypertensive Rats differ from Wistar Kyoto rats, but not from Spontaneously Hypertensive Rats treated with Enalapril maleate, in terms of arterial pH and theta frequency. This feature may be due to genetic determinants unrelated to hypertension. In sharp contrast, the persistence of differences in the peak of heart period decrease and the peak of theta frequency increase during phasic hypertensive events between Spontaneously Hypertensive Rats and Spontaneously Hypertensive Rats treated with Enalapril maleate demonstrates that the observed reduction in central autonomic control of the cardiovascular system in Spontaneously Hypertensive Rats is not an irreversible consequence of inherited genetic determinants. Rather, the comparison between Spontaneously Hypertensive Rats and Spontaneously Hypertensive Rats treated with Enalapril maleate indicates that the observed differences in central autonomic control are the result of the hypertension per se.

This work supports the view that the study of cardiovascular regulation in sleep provides fundamental insight on the pathophysiology of hypertension, and may thus contribute to the understanding of this disease, which is a major health problem in European countries (Wolf-Maier *et al.*, 2003) with its burden of cardiac, vascular, and renal complications.

1. Cardiovascular regulatory mechanisms

Cardiovascular regulation has multiple levels of control organized hierarchically: bottom up local tissue blood flow control mechanisms; autonomic nervous system (autonomic reflexes) and finally central nervous system.

Interactions between local, reflex, and central vascular regulatory mechanisms are ubiquitary and complex. Mechanisms involved are often non-linear (Malpas, 2002; Ursino & Magosso, 2003), so that it is possible to have divergent results at the level of the cardiovascular end-variables measured in similar experiments due to quantitative differences in controller or effector responses that shift the balance among interacting regulatory mechanisms. Furthermore, there are genetic differences between species and groups that may affect the balance among the controls involved and that may explain some inconsistencies among experimental results, even if the basic features of cardiovascular controls appear constant (Silvani & Lenzi, 2005).

Mean arterial pressure is the product of two variables: cardiac output and total peripheral resistance, which is the sum of the resistance to flow, offered by all the systemic blood vessels. Changes in total resistance are mainly due to changes in the resistance of arterioles.

1.1 Local mechanisms in the control of the circulation

At the lowest level of the cardiovascular regulatory mechanisms there are local control mechanisms. Vascular resistance changes as a function of the local physical and chemical environment. Therefore, blood flow is relatively independent of perfusion pressure (autoregulation), and is coupled to the local rate of energy utilization (flow-metabolism coupling). This coupling also depends on partial pressures of oxygen and carbon dioxide (chemical regulation).

1.1.1 Intrinsic regulation of heart activity

Under most conditions, the amount of blood pumped by the heart each minute is determined almost entirely by the rate of blood flow into the heart from the veins (venous return). Each peripheral tissue of the body controls its own local blood flow, and all the local tissue flows combine and return by way of the veins to the right atrium. The heart, in turn, automatically pumps this incoming blood into the arteries, so that it can flow around the circuit again.

This capacity of the heart to adapt to increasing volumes of inflowing blood is called Frank-Starling mechanism: within physiological limits, the heart pumps all the quantity of blood that returns to it by the way of the veins.

When an extra amount of blood flows into the ventricles, the cardiac muscle itself is stretched to greater length. This in turn causes the muscle to contract with increased force because the actin and myosin filaments are brought to a more nearly optimal degree of overlap for force generation. So that, the ventricle, because of its increased pumping, automatically pumps the extra blood into the arteries (Guyton & Hall, 2005b).

1.1.2 Local control of blood flow in response to tissue needs

Each tissue has the capacity to control its own local blood flow in proportion to its metabolic needs.

Local blood flow control can be divided into two phases: long-term control and acute control. Long-term control means slow, controlled changes in flow over a period of days or weeks, and it is achieved by an increase or decrease in the physical sizes and numbers of actual blood vessels supplying the tissues. This type of regulation is important when the long-term metabolic demands of a tissue changes. Thus, if a tissue becomes chronically overactive and therefore requires chronically increased quantities of oxygen and other nutrients, the arterioles and capillary vessels usually increase both in number and size to match the need of the tissue-unless the circulatory system has become pathological or too old to respond (Guyton & Hall, 2005c).

Acute control is achieved by rapid changes in local vasodilatation or vasoconstriction of the arterioles, metarterioles, and precapillary sphincters, occurring within seconds to minutes to provide very rapid maintenance of appropriate local tissue blood flow. There are two theories for the acute control: the vasodilatators theory and the nutrient lack theory. The first one said that the greater the rate of metabolism or the less the

availability of oxygen or some other nutrients to a tissue, the greater the rate of formation of vasodilator substances (i.e. adenosine, carbon dioxide, adenosine phosphate compounds, histamine, potassium ions) in the tissue cells. Vasodilatators substances diffuse trough tissues to precapillary sphincters, metarterioles, and arterioles to cause dilatation.

The latter theory said that oxygen and other nutrients are required to cause vascular muscle contraction. Without adequate oxygen, the blood vessels simply would relax and therefore naturally dilate. In addition, increased utilization of oxygen in the tissues for increased metabolism could decrease the availability of oxygen in the smooth muscle fibers in the local blood vessels, and this too, would cause local vasodilatation. In fact, smooth muscle requires oxygen to remain contracted, and the strength of contraction of the sphincters would increase with an increase in oxygen concentration. So that, when the oxygen concentration in the tissue rises above a certain level, the precapillary and metarteriole sphincters presumably would close until the tissue cells consume the excess oxygen. But when the excess oxygen is gone and the oxygen concentration falls low enough, the sphincters would open once more to begin the cycle again.

Either vasodilatators substance theory or nutrient lack theory could explain acute local blood flow regulation in response to the metabolic needs of the tissues and probably the truth lies in a combination of the two mechanisms (Guyton & Hall, 2005c).

In certain tissues, blood flow is adjusted to the existing metabolic activity of the tissue. But, within less than a minute, the blood flow in most tissues returns almost to the normal level even thought the arterial pressure is kept elevated. This mechanism is commonly referred to as ‘autoregulation’ of blood flow. Why blood flow remains constant in presence of an altered perfusion pressure may be explained by the metabolic regulation or by the myogenic mechanism. The first one suggests that when the arterial pressure becomes too great, the excess flow provides too much oxygen and too many other nutrients to the tissues. These nutrients cause the blood vessel to constrict and the flow to return nearly to normal despite the increased pressure. The latter is based on the observation that sudden stretch of small blood vessels causes the smooth muscle of the vessel wall to contract for a few seconds. So that, when high arterial pressure stretches the vessels, this in turn causes reactive vascular constriction that reduces blood flow nearly back to normal. Conversely, at low pressure, the degree of stretch of the vessel is less, so that the smooth muscle relaxes and allows increased flow (Berne & Levy, 2005d; Guyton & Hall, 2005c).

1.2 Autonomic nervous system in the control of the cardiovascular function

The intermediate level of cardiac and vascular control is exerted by autonomic reflexes, which originate from peripheral tissues and from the cardiovascular system itself. The neural control of the cardiovascular system is accomplished by the autonomic outflow to the heart, through sympathetic and parasympathetic drives, and to the vessels by sympathetic drive. Autonomic outflow also comprises either reflex contribution of peripheral factors (baroreceptors, chemoreceptors, and thermoreceptors) or central commands that change as a function of the behavioural state, such as the wake-sleep states, the defence reaction, and emotional states.

To the homeostasis contributes the integrated reflex control of the cardiovascular and ventilatory functions that allows an effective buffering of alterations in systemic arterial pressure, arterial blood gas concentration, and body temperature (Silvani & Lenzi, 2005).

The nervous system controls the circulation almost entirely through the autonomic nervous system; by far the most important part of the autonomic nervous system for regulating the circulation is the sympathetic nervous system; the parasympathetic nervous system also contributes specifically to regulation of heart function (Guyton & Hall, 2005d).

1.2.1 Sympathetic innervation of blood vessels

In most tissues, sympathetic nerve fibers innervate all the vessels except the capillaries, precapillary sphincters, and metarterioles. The innervation of small arteries and arterioles allows to increase resistance to blood flow and thereby to decrease rate of blood flow through tissues; the innervation of large vessels, particularly veins, makes it possible to decrease the volume of the vessels; so that blood is pushed into the heart and in this way sympathetic stimulation may play a role in the regulation of heart pumping (Guyton & Hall, 2005d).

These vasoconstrictor fibers are distributed to essentially all segments of the circulation, but more to some tissues than others. They are most abundant in the

kidneys, skin, intestines, spleen, relatively sparse in the coronary and cerebral vessels and skeletal muscle (Boulpaep, 2003; Guyton & Hall, 2005d).

The transmitter secreted at the endings of the vasoconstrictor nerves is almost entirely norepinephrine. Norepinephrine acts directly on the α adrenergic receptors on the membrane of vascular smooth-muscle cells to cause vasoconstriction (Boulpaep, 2003; Guyton & Hall, 2005d).

1.2.2 Control of the heart by parasympathetic and sympathetic nerves

The pumping effectiveness of the heart is controlled by both the divisions of the autonomic nervous system, which abundantly supply the heart. Sympathetic and parasympathetic nerves tonically influence the cardiac pacemaker, the sinoatrial node: the sympathetic one enhances automaticity, whereas the parasympathetic one inhibits it. Changes in heart rate usually involve a reciprocal action of these two divisions of the autonomic nervous system: the heart rate usually increases with a combined decrease in parasympathetic activity and an increase in sympathetic activity; the heart rate decreases with the opposite changes in autonomic neural activity, even if parasympathetic tone ordinarily predominates in healthy, resting individuals (Berne & Levy, 2005c; Guyton & Hall, 2005b).

Parasympathetic pathways

The cardiac parasympathetic fibers originate in the medulla oblongata, in particular in the cells that lie in the dorsal motor nucleus of the vagus or in the nucleus ambiguous. The precise location of the parasympathetic fibers varies from species to species (Berne & Levy, 2005c). Most of the vagal ganglion cells are spotted in epicardial fat pads near the sinoatrial and atrioventricular nodes (Berne & Levy, 2005c) and not much to the ventricles, where the power contraction of the heart occurs. This explains the effect of vagal stimulation mainly to decrease heart rate rather than to decrease greatly the strength of heart contraction (Guyton & Hall, 2005b).

The right and left vagi are differently distributed to cardiac structures, even if the distribution of the efferent vagal fibers is overlapping. The right vagus nerve affects the sinoatrial node particularly and the stimulation of this nerve slows sinoatrial nodal firing and can even stop the firing for a few seconds, but then the heart usually ‘escapes’ and

beats at a rate of 20 to 40 beats per minute as long as the parasympathetic stimulation continues; the left vagus nerve mainly inhibits atrioventricular conduction tissue to produce various degrees of atrioventricular block (Berne & Levy, 2005c; Guyton & Hall, 2005b).

The vagus normally exerts an intense tonic, parasympathetic activity on the heart via acetylcholine released by the postganglionic fibers. Acetylcholine is rapidly hydrolyzed by the cholinesterase enzyme, which are abundant the sinoatrial and atrioventricular nodes. Owing to this rapid breakdown of the enzyme, the effects of any given vagal stimulation decay very quickly, when vagal stimulation is discontinued. Furthermore, the effects of vagal activity on sinoatrial and atrioventricular nodal function have a very short latency (50-100ms), because the released acetylcholine quickly activates special acetylcholine-regulated K^+ channels in the cardiac cells that do not need an intermediate second messenger system. In this way, brief latency and rapid decay of response permits these nerves to exert a beat by beat control of sinoatrial and atrioventricular nodal functions (Boulpaep, 2003; Berne & Levy, 2005c).

Sympathetic pathways

The cardiac sympathetic fibers originate in the intermediolateral columns of the upper five or six thoracic and lower one or two cervical segments of the spinal cord. When the postganglionic cardiac sympathetic fibers approach the base of the heart along the adeventitial surface of the great vessels, these fibers are distributed to the various chambers as an extensive epicardial plexus. Then they penetrate the myocardium, usually accompanying the coronary vessels and innervate sinoatrial node, atria and ventricles(Berne & Levy, 2005c).

As with the vagus nerve, the left and right sympathetic fibers are distributed to different areas of the heart. Sympathetic input from the right cardiac nerve has more effect on the heart rate than input from the left cardiac nerve, because it dominates the innervation of the sinoatrial node. On the other hand, sympathetic input from the left cardiac nerve has more effect on contractility. At rest, their firing rate is less than that of the vagus nerve (Boulpaep, 2003).

Norepinephrine is released by the postganglionic sympathetic neurons and acts on postsynaptic β_1-adrenergic receptors of pacemaker cells in the sinoatrial node, as well as on similar receptors of myocardial cells in the atria and ventricles. The β_1 adrenoceptor,

via the G-protein G_s, acts via the cyclic adenosine monophosphate-protein kinase A pathway to phosphorilate multiple effector molecules in both pacemaker cells and cardiac myocytes (Boulpaep, 2003).

The effects of sympathetic stimulation decay only gradually after stimulation is stopped, in contrast to the abrupt termination of the response to vagal activity. Nerve terminals take up most of the norepinephrine released during sympathetic stimulation, and much of the remainder is carried away by the bloodstream. All these processes are slow. Furthermore, at the beginning of sympathetic stimulation, the facilitatory effects on the heart attain steady-state values much more slowly than do the inhibitory effects of vagal stimulation.

The onset of the cardiac response to sympathetic stimulation is slow for two main reasons: first, norepinephrine is released at a relatively slow rate from the cardiac sympathetic nerve terminals; second, the cardiac effects of the neurally released norepinephrine are mediated mainly via a slow second messenger system (principally the adenylyl cyclase system). As a result, sympathetic activity modifies heart rate and atrioventricular conduction much more slowly than does vagal activity. Hence, unlike vagal activity, sympathetic activity can not exert beat by beat control of cardiac function (Berne & Levy, 2005c).

1.3 Regulation of cardiac output

Cardiac output is defined as the volume of blood pumped by each ventricle per minute. It is also the volume of blood flowing through either the systemic or the pulmonary circuit per minute. In mathematical terms, cardiac output can be expressed as the product of stroke volume, the blood volume ejected by each ventricle with each beat, and heart rate, the number of beats per minute. Cardiac output may be varied by changing the stroke volume or the heartbeat's frequency.

Various factors can produce changes in force of contraction, but there are three main elements. The first one is the end-diastolic volume, the volume of blood in the ventricles just before contraction. The relationship between the end-diastolic volume and stoke volume is determined by the Frank-Starling mechanism: at any given heart rate, an increase in venous return, the flow of blood from the veins to the heart,

automatically forces an increase in cardiac output by increasing end-diastolic volume and thus stroke volume.

The second factor related to stroke volume is the sympathetic activity to the ventricles. Norepinephrine acts on β_1-adrenergic receptors in myocardial cells to increase the strength of contraction at any given end-diastolic volume. The increase of contractility induces to a more complete ejection of the end-diastolic ventricular volume and then to an increase in stroke volume.

The third factor that influences stroke volume is afterload, the arterial pressure against which the ventricles pump. An increase in afterload tends to reduces stroke volume (Widmaier *et al.*, 2004).

Cardiac output is also influenced by heart rate, but this relation is much more complex because a change in heart rate may alter also other factors (contractility, afterload, end-diastolic volume). So that since the stroke volume tends to decrease as heart rate increases, the cardiac output vs. heart rate function shows a characteristic inverted U shape. However, this relationship varies quantitatively among subjects and among physiological states in any given subject (Berne & Levy, 2005a).

1.4 Short and long-regulation of arterial pressure

The arterial baroreceptor play a key role in short-term adjustments of blood pressure in response to a relatively abrupt changes in blood volume, cardiac output, or peripheral resistance (as in exercise or in alarm reaction). However, long-term control of blood pressure, over days or weeks, is determined by the fluid balance of the individual. The most important organ in the control of body fluid volume and hence of blood pressure is the kidney.

Short-regulation of arterial pressure

There are multiple nervous control mechanisms that operate all the time to maintain the arterial pressure at or near normal. By far the best known of the nervous mechanisms for arterial pressure control is the baroreceptor reflex, a negative feedback reflex mechanism.

Changes in arterial pressure induce deformation of the vessels and then changes in the arterial wall tension, which are sensed by baroreceptors. Baroreceptors are stretch receptors, located in the wall of each internal carotid artery slightly above the carotid bifurcation (carotid sinus) and in the wall of the aortic arch. Impulses that arise in the carotid sinus travel up the carotid sinus nerve (nerve of Hering) to the glossopharyngeal nerve and, via the latter, to the nucleus of the tractus solitarius in the medulla. Signals from the aortic baroreceptors are transmitted through the vagus nerves also to the same tractus solitarius of the medulla.

The frequency of firing of the baroreceptor nerve terminals is enhanced by an increase in arterial blood pressure and diminished by a reduction in arterial blood pressure. An increase in impulse frequency, as occurs with a rise in arterial pressure, inhibits the vasoconstrictor center of the medulla and excites the vagal parasympathetic center. The net effects are peripheral vasodilatation and decreased heart rate and strength of heart contraction. So that, activation of the baroreceptors by high pressure causes the arterial pressure to decrease because of both a decrease in peripheral resistance and a decrease in cardiac output. Conversely, low pressure has opposite effects, reflexly causing the pressure to rise back toward normal values (Berne & Levy, 2005d; Guyton & Hall, 2005d).

The arterial baroreceptors provide powerful moment-to-moment control of arterial pressure and tend to reset in 1 to 2 days to the pressure level to which they are exposed, so they have little or no importance in long term regulation of mean arterial pressure. Experimental studies, however, have suggested that the baroreceptors do not completely rest and may therefore contribute to long-term blood pressure regulation, especially by influencing sympathetic nerve activity of the kidneys (Malpas, 2004).

In hypertension, baroreceptor sensitivity decreases, because the carotid sinuses become stiffer as a result of the high arterial pressure. In fact, the set point of the baroreceptor is raised in hypertension, such that the threshold is increased and the pressure receptors are less sensitive to changes in transmural pressure.

As would be expected, sino-aortic denervation can produce temporary or prolonged hypertension (Berne & Levy, 2005d; Guyton & Hall, 2005d).

Long-regulation of arterial pressure

The baroreceptor mechanisms are of great importance for the moment-to-moment stabilization of arterial pressure, but because they do not possess sufficient strength and because they reset in time to the prevailing level of arterial pressure, they cannot provide a sustained negative feedback signal to provide long-term regulation of arterial pressure in face of sustained stimuli (Cowley, 1992). The body, however, also has powerful mechanisms for regulating arterial pressure week after week and month after month, in particular the renin-angiotensin cascade, which is a neurohumoral mechanism that regulates the effective blood volume.

Renin is a protein enzyme released by the kidneys in response to a reduction in arterial pressure. This enzyme is synthesized and stored in an inactive form (prorenin) in the juxtaglomerular cells, modified smooth muscle cells located in the walls of the afferent arterioles immediately proximal to the glomeruli, in the kidneys. When arterial pressure falls, intrinsic reactions in the kidneys themselves cause many of the prorenin molecules in the juxtaglomerular cells to split and release renin. Renin enters in the bloodstream, but a small amount remains in the local fluid of the kidneys.

Renin acts enzymatically on a globulin called angiotensinogen, another plasma protein, to release a peptide, angiotensin I. Angiotensin I has bland vasoconstrictor proprieties, and it is eventually converted to angiotensin II in the endothelium of lung vessels by an enzyme called angiotensin converting enzyme (ACE). Angiotensin II persists in the blood only 1 or 2 minutes after which it is inactivated by angiotensinases.

Angiotensin II is a powerful vasoconstrictor in many areas of the body: vasoconstriction of the arterioles increases the total peripheral resistance, thereby raising the arterial pressure; moreover, angiotensin II stimulates aldosterone secretion by the adrenal gland, which increases both salt and water reabsorption by the kidneys tubules, thus increasing the total body extracellular fluid volume and leading secondarily to long-term elevation of arterial pressure (Guyton *et al.*, 1972). Thus the renin-angiotensin system is an automatic feedback mechanism that helps maintain arterial pressure at or near the normal level even when salt intake is changed; conversely, opposite effects take place, when salt intake is decreased below normal (Guyton & Hall, 2005a).

1.5 Central nervous system in the control of the circulation

The highest level of cardiovascular control is exerted by the central nervous system, which imposes autonomic commands (Spyer, 1994) on cardiac, vascular, and ventilatory effectors and overriding the levels of local and reflex regulation. Therefore, autonomic commands mediate a feedforward control mechanism that prevails temporally over negative feedback controls. Central nervous system, with central autonomic commands, anticipates the needs of the organism, increasing blood pressure and heart rate so that a blood pressure drop is prevented during a subsequent physical activity. As in physical exercise or in defence reaction, central autonomic commands contribute to adapt cardiovascular regulation to changing behavioural needs (Berne & Levy, 2005b).

1.5.1 Physical exercise

In humans or trained animals, at the beginning of physical exercise, the vagal nerve impulses to the heart are inhibited, whereas the sympathetic discharge is increased. The simultaneous inhibition of parasympathetic areas and activation of sympathetic areas augment heart rate and myocardial contractility. As a result, the tachycardia and enhanced contractility increase cardiac output.

Concomitantly with cardiac stimulation, the sympathetic nervous system changes vascular resistance in the periphery. In splanchnic regions, skin, kidneys and inactive muscles, vasoconstriction increases vascular resistance and diverts blood away from these areas. Blood flow to the myocardium increases, whereas flow to the brain is unchanged. Skin blood flow initially decreases during exercise, and then it increases as body temperature rises with increments in the duration an intensity of exercise.

The major cardiovascular adjustment to exercise occurs in the vasculature of the active muscles. Local formation of vasoactive metabolites causes a decrease in the total peripheral resistance and enables an increase in venous return and cardiac output.

At rest only a small percentage of the capillaries are perfused, but during exercise nearly all of the capillaries contain flowing blood (capillary recruitment). In this way, the surface area available for exchange of gases, water, solutes is increased many times.

Arterial pressure starts to rise with the onset of the exercise as a result of the increase in cardiac output. Therefore, the increase in cardiac output is proportionally greater than the decrease in total vascular resistance. Furthermore, the vasoconstriction produced in the inactive tissues by the sympathetic nervous system is fundamental for maintenance of normal or increased blood pressure. Experiments show that sympathectomy or drug-induced block of the adrenergic sympathetic nerve fibers determine hypotension during exercise (Berne & Levy, 2005b).

Central commands interact with baroreceptor reflex in controlling the cardiovascular system at the onset of exercise. The operating point of the arterial baroreflex is not fixed, but is variable over a wide range of pressures and is determined by a variety of inputs from the peripheral and central nervous systems. The initial increase in heart rate and sympathetic nerve activity, during physical exercise, is mediated by central command. This command operates by resetting the operating point of the arterial baroreflex to a higher pressure (DiCarlo & Bishop, 2001; Silvani & Lenzi, 2005). Other experiments show a reduction in the baroreflex sensitivity at the operating point as a result of vagal withdrawal rather than an increase in sympathetic activity (Ogoh *et al.*, 2005).

1.5.2 Defence reaction

A complex set of somatic and autonomic responses, called defence reaction, is triggered by dangerous situations that may require the organism to fight or flight. This reaction may also be experimentally caused by electrical stimulation of the mesencephalic tectum in rats (Schenberg *et al.*, 1993) and involves an increase in sympathetic activity to heart and vessels. In this way, the organism undergoes an anticipatory increase in arterial pressure rather than waiting for the baroreflex to buffer the hypotension, which would follow muscle activity during fight or flight and would hamper the effectiveness of such vital behaviours (Guyton & Hall, 2005d).

The defence reaction obtained by electrical stimulation in rats (Schenberg *et al.*, 1993), shows that the baroreflex interacts with central commands in determining the cardiovascular changes that characterize this condition. Electrical stimulation produced stimulus intensity-dependent behaviours including freezing at lower intensities and flight at higher intensities. Simultaneous increases of mean arterial blood pressure and heart rate is seen at the beginning of the flight response, after which heart rate rapidly

falls to baseline levels, consistently with a late baroreflex dampening, whereas the mean arterial blood pressure remained at an hypertensive level until the end of the stimulus. Experiments with sinoaortic baroreceptor denervated rats corroborate these data. The denervation strengthens flight tachycardia and prevents its later reset, but mean blood arterial pressure responses of baroreceptor denervated rats do not differ from non denervated rats. The sustained hypertension, thus, appears to be mediated by mechanisms other than the mere baroreceptor reflex deactivation.

1.6 Spontaneous fluctuations in heart period and blood pressure

Spontaneous oscillations in cardiovascular variables, such as arterial pressure and heart rate, can be observed in experimental animals and in human and fluctuate on a beat to beat basis. These fluctuations have traditionally been ignored or, at best, treated as noise to be averaged out. It is unknown whether these fluctuations subserve a physiological need; however they are generated and shaped by cardiovascular and respiratory systems and by their control mechanisms. In this way these fluctuations give information on the physiology and pathophysiology of the systems that generate and modulate them. The variability in cardiovascular signals reflects the homeodynamic interplay between perturbations to cardiovascular function and the dynamic response of the cardiovascular regulatory systems (Appel *et al.*, 1989).

The information conveyed by spontaneous cardiovascular variability is valuable because it may be obtained non-invasively, without perturbing the behavioural state of the subject; it pertains to the working point of the cardiovascular and respiratory systems; and it allows non-linear regulatory systems to be approximated by simpler linear systems, because of the small amplitude of spontaneous oscillations. However, it is important to underline that not all changes in cardiovascular variability are indicative of changes in autonomic function (Malpas, 2002).

The relationship between heart period, the reciprocal of heart rate, and blood pressure changes as a function of the frequency range of the cardiovascular fluctuations analyzed. Spontaneous oscillations of heart period and arterial pressure can be analysed by mathematical tools to extract the main frequency-related information. This is

generally contained within three principal bands: a high-frequency band, related to the respiratory rate; a low-frequency band, centred around 0.1 Hz in human subjects (Malliani *et al.*, 1991; Sleight *et al.*, 1995) and 0.4 Hz in rats (Brown *et al.*, 1994); and a very-low-frequency band, spanning the leftmost part of the spectrum, but typically around 0.05 Hz (Cevese *et al.*, 2001).

Variability in blood pressure around the breathing rate can be explained on the basis of the cyclic variation in intrathoracic pressure associated with breathing, which affect cardiac output via changes in venous return and thus blood pressure (Malpas, 2002). Therefore, the high-frequency variability of blood pressure is not substantially modified in patients with denervated human heart (Bernardi et al., 1989).

Rhythmic variations in heart rate, occurring at the frequency of respiration, are called respiratory sinus arrhythmia and are detectable in most subjects. At rest, heart rate tends to accelerate during inspiration and decelerates during expiration (Saul et al., 1989). Recordings from the autonomic nerves to the heart reveal that neural activity increases in the sympathetic fibers during inspiration, whereas neural activity in the vagal fibers increases during expiration (Kollai & Koizumi, 1979). Vagal nervous system is known to participate in respiratory sinus arrhythmia, both by means of a central coupling with the neural centres that control breathing and through the baroreflex (Malpas, 2002). So that, respiratory oscillations in heart rate and blood pressure are not determined only by the baroreceptor reflex. Accordingly, after sino aortic deafferentation in cats, rats or dogs, the spectral power density of blood pressure fluctuations in the high-frequency band is not substantially changed (Di Rienzo et al., 1991; Cerutti et al., 1994). In human subjects, respiratory sinus arrhythmia can actually contribute to respiratory arterial pressure fluctuations. Therefore, respiratory sinus arrhythmia does not represent simple baroreflex buffering of arterial pressure (Taylor & Eckberg, 1996).

2. Sleep

Sleep is a complex amalgam of physiologic and behavioural processes, the control mechanisms of which are manifested at every level of biological organization, from genes and intracellular mechanisms to networks of cell populations, and to all central neuronal systems at the organismic level, including those that control movement, arousal, autonomic functions, behaviour and cognition (Pace-Schott & Hobson, 2002). It is a state of immobility with greatly reduced responsiveness, which can be distinguished from coma or anaesthesia by its rapid reversibility. When it is prevented, the body tries to recover the lost amount: the existence of sleep 'rebound' after deprivation demonstrates that sleep is not simply a period of reduced activity or alertness regulated by circadian or ultradian rhythms (Carskadon & Dement, 2005; Siegel, 2005).

In mammals and birds, there are two types of sleep: non rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep. They are defined in terms of electrophysiological signs that are detected with a combination of electroencephalography (EEG), electroculography (EOG) and electromyography (EMG), the measurement of which in humans is collectively termed polysomnography (Rechtschaffen & Kales, 1968).

2.1 From wakefulness to sleep

When relaxed with eyes closed, the majority of humans show an EEG of rhythmic α activity (in the range of 8-13 Hz) and the EMG shows tonic activity of a relatively high level. When a person is awake, control of eye movements is voluntary: the waking EOG tracing generally consists of rapid eye movements and eye blinks, when the eyes are open, and few or no eye movements, when the eyes are closed. Involuntary slow, rolling eye movements, with eyes closed, often characterize the EOG in the seconds to minute preceding the EEG change to stage I sleep (Carskadon & Rechtschaffen, 2005).

The precise definition of the onset of sleep does not exist, because a change in EEG pattern is not always associated with an individual's perception of sleep. The onset of sleep under normal circumstances in normal adult humans is through NREM sleep (Carskadon & Dement, 2005) and NREM and REM sleep alternate in each of the four or five cycles that occur in each night. Early in the night, NREM sleep is deeper and occupies a disproportionately large amount of time, especially in the first cycle, when the REM epoch might be short or aborted. Later in the night, NREM sleep is shallow, and more of each cycle is devoted to REM.

The cyclic organization of sleep varies within and between species. In fact, the period length of each REM–NREM epoch increases with brain size across species, and the depth and proportion of the NREM phase in each cycle increases with brain maturation within species. NREM sleep complexity is a function of brain systems, such as the thalamocortical circuitry, that reach their maximum development in mature humans only to decline in post-mature age (Pace-Schott & Hobson, 2002).

In humans, the average length of the first NREM-REM sleep cycle is approximately 70 to 100 minutes; the average length of the second and later cycles is approximately 90 to 120 minutes. Across the night, the average period of the NREM-REM cycle is approximately 90 to 110 minutes (Carskadon & Dement, 2005).

2.1.1 NREM sleep

A shorthand definition of NREM sleep is a relatively inactive, yet actively regulating brain in a movable body (Carskadon & Dement, 2005). NREM sleep or slow wave sleep (SWS) is easily distinguishable from both wakefulness and REM sleep by high voltage and synchronous EEG rhythms (Steriade, 2005).

In human, NREM sleep is divided into four stages, corresponding to increasing depth of sleep, as indicated by progressive dominance of the EEG by high-voltage, low-frequency ('synchronized') wave activity. Such low-frequency waves dominate the deepest stages of NREM (stages III and IV). The four NREM stages (stages I, II, III, IV) roughly parallel a depth of sleep continuum, with arousal thresholds generally lowest in stage I and highest in stage IV sleep. NREM sleep is associated with fragmentary mental activity (Carskadon & Dement, 2005).

In the *stage I* there is a relatively low voltage, mixed-frequency EEG activity; vertex sharp waves are common during stage I sleep occurring at the beginning of the night. The EEG activity is generally in the θ range (3-7 Hz); muscle tone is maintained during all NREM sleep stages and registers as low-amplitude EMG activity (Carskadon & Rechtschaffen, 2005), but there is a gradual decline in muscle tone (hypotonia) during NREM sleep compared with that during wakefulness (Chase & Morales, 2005).

In the *stage II* there is a relatively low voltage, mixed-frequency EEG activity. There are two specific EEG patterns that occur sporadically on this mixed-frequency background and enable to distinguish stage II from stage I: the sleep spindle and the K-complex. Sleep spindles have a waxing and waning spindle shape, composed of waves in the range of 12-14 Hz, with a duration of about 0.5-1.5 seconds (Carskadon & Rechtschaffen, 2005). Sleep spindles are a common feature of mammalian sleep and are generated in the thalamus, but the cerebral cortex plays a major role in their synchronization and virtually simultaneous appearance over widespread thalamic and cortical areas (Steriade, 2005). The K-complex consists of a "well delineated negative sharp wave which is immediately followed by a positive component. The total duration of the complex should exceed 0.5 second" (Rechtschaffen & Kales, 1968). K-complexes occur spontaneously during stage II sleep and are also evoked in response to auditory stimuli. The EMG during stage II sleep is tonically active, generally at low amplitude relative to wakefulness.

The EEG of stages III and IV sleep is defined by the presence of high-voltage, slow wave activity. Stage III is composed of waves of 2 Hz or slower and in stage IV such waves predominate (more than 50% of the epoch); EMG is tonically active, but at a low level (Carskadon & Rechtschaffen, 2005) (see Figures 2.1 and 2.2).

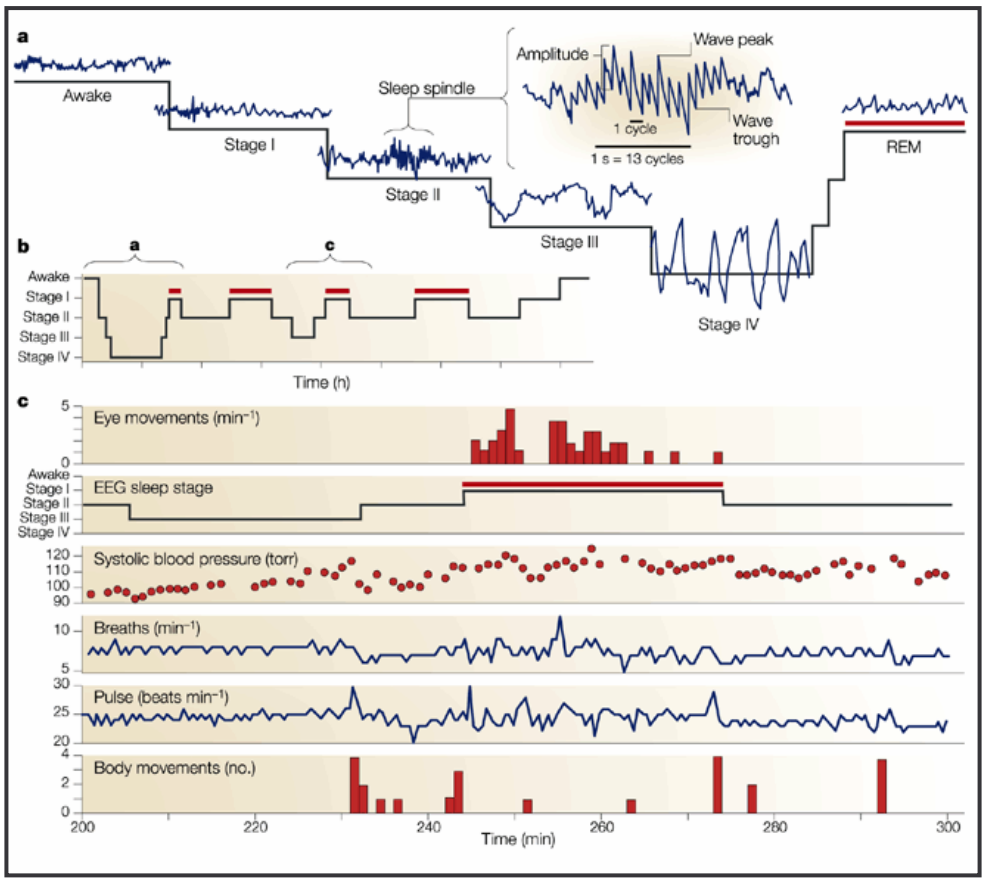

Figure 2.1. Sleep cycle. Panel *a* shows the characteristic waveforms of the different sleep stages; panel *b* illustrates changes over the course of a night's sleep; panel *c* shows changes in peripheral physiology associated with these stages. REM sleep is indicated by red bars. Panel *a* depicts, in detail, features of an early-night sleep cycle in which NREM reaches its greatest depth at stage III and IV (delta) sleep, whereas panel *c* depicts a late-night cycle in which NREM descends only to stage III. The constant period length of the NREM–REM cycle indicates that it is timed by a reliable oscillator, the amplitude of which varies according to extrinsic factors. From (Pace-Schott & Hobson, 2002).

2.1.2 REM sleep

A shorthand definition of REM sleep is a highly activated brain in a paralyzed body (Carskadon & Dement, 2005).

Staging REM sleep (or paradoxical sleep) requires the coincidence of specific activities in all three electrographic measures: 'activated' or desynchronized EEG, bursts of rapid eye movements in the EOG and suppression of EMG activity. The REM sleep EEG pattern is characterized as one of relatively low voltage, mixed frequency. An EEG pattern called sawtooth waves, with frequency in θ range, is fairly common during REM sleep, particularly in proximity to eye movements. Activity in the α range

(1-2 Hz slower than waking α activity) may also be seen in the REM sleep EEG (Carskadon & Rechtschaffen, 2005).

The desynchronization of the EEG pattern during REM sleep and also during waking means the disruption of high-amplitude and synchronous EEG waves and the replacement of low-frequency oscillation by fast rhythm with lower amplitude. However, during REM sleep and also during wakefulness, the spontaneously occurring fast rhythms (20 to 50 Hz, called β and γ) are synchronized over restricted distances in the cortex as well as among cortical areas and related thalamic nuclei. Thus, it is preferable to use the term 'activation' than 'desynchronization' to define the brain electrical activity during REM sleep and waking (Steriade, 2005).

The paradox that a similar EEG activity characterizes two states of vigilances (wakefulness and REM sleep) that are at the opposite sides of the sleep-waking cycle suggests that, with regard to brain cellular activities, waking and REM sleep are closer than is usually believed. In this regard, both states show distinct differences from NREM sleep, which is characterized by widely syncronized brain electrical activity (Steriade, 2005).

Other striking features of REM sleep are ponto-geniculo-occipital (PGO) spikes in feline (Jouvet, 1967) and rhythmic θ activity (4-8 Hz), originating in the hippocampus, in many primates, cats, dogs and rodents. In these latter it can be recorded with implanted epidural electrodes over the parietal or occipital cortex (Zepelin *et al.*, 2005).

An universal feature of REM sleep in intact organism is the tonic suppression of skeletal muscle tone (atonia) and reflexes via a circuit that involves pontine activation of medullary inhibitory centers by neurotransmitter glycine and culminates in postsynaptic hyperpolarization of brainstem and spinal motoneurons. Superimposed on this background of tonic motor inhibition occasional twitches and jerks of distal muscles can be seen. Twitches occur due to excitatory processes (postsynaptic potentials) that impinge on motoneurons; but, even during these periods of excitatory potentials, motoneurons continue to be inhibited by glycine. Therefore, during REM sleep, there is tonic inhibition of motoneurons, as well as phasically occurring brief periods of motoneuron excitation (Chase, 1983; Chase & Morales, 2005). In human twitches appear as very short-lived EMG elevations, usually in proximity to eye movement bursts and in other species, paws, face, whiskers show twitches during REM sleep.

REM sleep is not divided into stages, although tonic and phasic types of REM sleep are often distinguished. The marker of REM sleep phasic activity is the burst of rapid eye movements, which are often accompanied by muscle twitches and cardiorespiratory irregularities (see Figures 2.1 and 2.2).

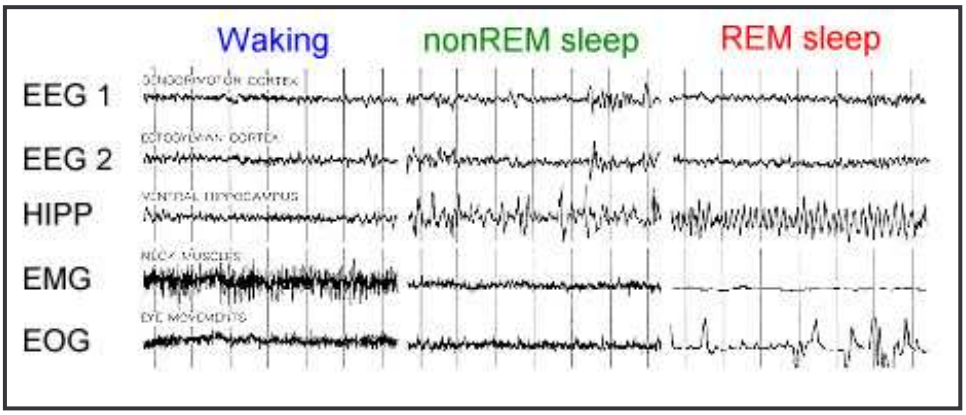

Figure 2.2. Two cortical EEG, hippocampal EEG, electromyogram (EMG) and electro-oculogram (EOG) during wakefulness, NREM sleep and REM sleep in cats. From (Jouvet, 1967).

2.2 Homeostasis and physiologic regulation in sleep

In mammals, sleep is under homeostatic control. The principle of homeostasis in physiology was defined by W.B. Cannon in 1929 as "the coordinated physiologic processes which maintain most of the steady states in the organism" (Cannon, 1929). Physiologic homeostasis is effected by feedforward and feedback operations that predictively and reactively minimize the influences of internal and external disturbances on the organism. This means that it is necessary to verify whether effector activity maintains (homeostasis) or impairs (poikilostasis) the stability, the normal range, of the fundamental variables of the interstitial and cellular compartments (temperature, water volume, electrolytes, osmolarity, pH, nutrients, oxygen, carbon dioxide) that underlie cellular survival. The homeostasis of such variables is the eventual result of continuous adjustments of "instrumental" variables (heart rate, ventilation, blood pressure, muscle force, vascular resistance, cardiac output, stroke volume) that are directly affected by the activity of somatic and visceral effectors.

Homeostatic regulation of physiologic function depends on the sleep-wake cycle and this dependency is the result of changes in the functional dominance of different brain structures in different behavioural states (Parmeggiani, 2005).

A basic difference between NREM sleep and REM sleep is the functional similarity of the former in different species and the functional variety and variability of the latter within and between species. The basic somatic features of NREM sleep are the assumption of a thermoregulatory posture and a decrease in muscle activity; the basic autonomic feature of this state is the functional prevalence of parasympathetic influences over the sympathetic activity. These peculiarities are indicative of closed-loop operations that automatically maintain homeostasis at a lower level of energy expenditure compared with that in wakefulness and the leading neural structures are diencephalic. Conversely, the basic somatic features of REM sleep are muscle atonia, rapid eye movements and myoclonic twitches; the basic autonomic feature of REM sleep is the great variability in sympathetic activity and phasic changes in tonic parasympathetic discharge (Parmeggiani, 2005). The somatic and visceral phenomena of REM sleep are characterized by the greatest variability as a result of open-loop operations of central origin, which impair the homeostasis and the leading neural structures are rhomboencephalic (Parmeggiani, 1980).

This basic functional dichotomy may be also applied to the nervous control of body temperature and of circulatory and respiratory functions.

2.3 Control of body temperature

During NREM sleep thermoregulatory mechanisms are operative as they are in wakefulness, despite some state-differences in the threshold and gain of effector responses to thermal loads and down-regulation, together with energy expenditure, of body and hypothalamic temperatures. Homeothermy, in mammals, is controlled by preoptic-hypothalamic integrative mechanisms that drive subordinate brainstem and spinal somatic and visceral mechanisms that elicit thermoregulatory effector responses (Parmeggiani, 2005).

The thermoregulatory responses to ambient thermal loads are present during NREM sleep and absent or depressed during REM sleep. During NREM sleep cat's posture, for

example, clearly varies in relation to ambient temperature, while the drop in postural muscle tone during REM sleep is unrelated to ambient temperature (Parmeggiani & Rabini, 1970). Warm temperature notwithstanding, tachypnea in cat and heat-exchange vasodilatation in cat, rabbit and rat disappear and sweating in humans decreases during REM sleep. In a similar way with a cold temperature, shivering and piloerection in cat, heat-exchange vasoconstriction in cat, rabbit and rat are suppressed during REM sleep. Events in REM sleep are not only simply the result of state-dependent changes in threshold and gain of the different thermoregulatory responses; REM sleep is characterized by effector activity that is not only functionally inconsistent with the aim of temperature regulation but also lacks any proportional relationship with the intensity of the thermal stimulus. The conclusion is that the temperature of the body changes according to its thermal inertia, like in a poikilothermic organism (Parmeggiani, 2005).

2.4 Circulatory function

Sleep states exert a major impact on circulatory function and this is a direct consequence of the significant variations in the brain states that occur in the normal cycling between NREM and REM sleep.

On the whole, NREM sleep is depicted by a relative autonomic stability and functional coordination between respiration, pumping action of the heart, and maintenance of arterial blood pressure (Verrier *et al.*, 2005). The cardiovascular changes in NREM sleep are consistent with the changes in ventilation and thermoregulation in a condition of postural and motor quiescence (Parmeggiani, 2005).

NREM sleep is characterized by a down regulation of cardiovascular activity of variable intensity depending on the species and its previous level in wakefulness (Parmeggiani, 2005), with vagal nerve dominance and heightened baroreceptor gain (Verrier *et al.*, 2005). In fact, relative to the values in wakefulness, NREM sleep entails hypotension, bradycardia, and a reduction in cardiac output and systemic vascular resistance (Mancia, 1993). The hypotension is markedly attenuated by surgical sympathectomy and can hence be largely ascribed to a reduction in sympathetic vasomotor tone as it has been highlighted for skeletal muscle vessels in human subjects (Somers et al., 1993); whereas the bradycardia is due mainly to an increase in vagal

nerve activity (Mancia, 1993). The decrease in arterial blood pressure occurs in cats and rats, although less consistent, but not in rabbits. In cats, heart rate decreases moderately, whereas stroke volume is practically unchanged. There is a significant decrease in heart rate in rats, but the decrease is less substantial significant in rabbits. See (Parmeggiani, 2005) for a recent review. In humans there is a tonic decrease in arterial blood pressure (Coccagna *et al.*, 1971), although it has varying intensity in different individuals (Mancia, 1993).

At the level of the heart, an increase in baroreceptor reflex sensitivity has been frequently, although not constantly, reported in human subjects in NREM sleep (Conway *et al.*, 1983). Moreover, in experimental model widely different in terms of species and developmental age, for example adult rat (Zoccoli *et al.*, 2001; Silvani *et al.*, 2003) or newborn lamb (Silvani *et al.*, 2005), the role of the baroreceptor reflex in controlling heart rhythm has been demonstrated to be higher during NREM sleep than either during wakefulness or REM sleep. This distinctiveness of NREM sleep may in part underlie the greater stability in blood pressure observed during this state.

The tonic changes in heart rate during NREM sleep may be ascribed either to baroreflex resetting or to the effects of central autonomic commands (Silvani & Lenzi, 2005). The latter may be prominent, as heart rate still decreases from wakefulness to NREM sleep in rats after sinoaortic denervation (Sei *et al.*, 1999).

During NREM sleep, a near sinusoidal modulation of heart rate variation occurs due to a coupling with respiratory activity and cardiorespiratory centers in the brain and the result is what is termed normal respiratory sinus arrhythmia. During inspiration, heart rate accelerates briefly to accommodate increased venous return, resulting in increased cardiac output, whereas during expiration, a progressive slowing in rate ensues (Verrier *et al.*, 2005). Respiratory sinus arrhythmia is prominent during NREM sleep, indicating a high degree of parasympathetic tone (Mancia, 1993).

To summarize, NREM sleep with its autonomic stability provide a relatively salutary neurohumoral background during which heart has an opportunity of metabolic restoration.

Different phenomena characterize REM sleep in both humans and animals. Endogenous brain activation, typical of this state, is accompanied by a prominent variability of heart rate and arterial blood pressure (Verrier *et al.*, 2005). This variability is an important feature of REM sleep and it is generally associated with bursts of rapid

eye movements, myoclonic twitches and breathing irregularities (see Figure 2.3) (Verrier *et al.*, 1996). Surges in cardiac-bound sympathetic and parasympathetic activity provoke accelerations and pauses in heart rhythm, in association with alterations in ponto-geniculo-occipital activity and θ rhythm that are signs of phasic central nervous system activation in REM sleep (Verrier *et al.*, 2005).

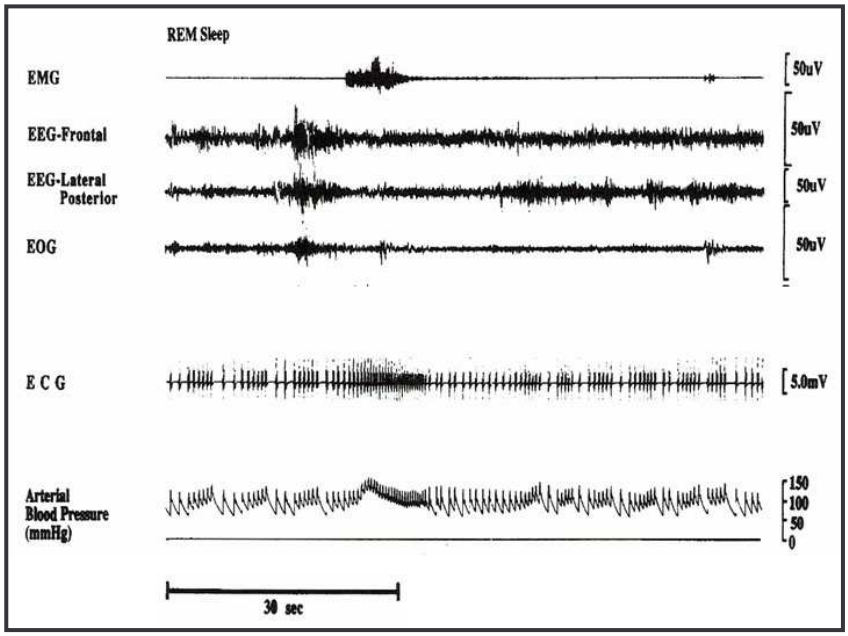

Figure 2.3. Surges in heart rate and in blood pressure during REM sleep with phasic muscle twitch. EMG: electromyogram; EOG: electro-oculogram; ECG: electrocardiogram. Modified from (Verrier *et al.*, 1996).

At cardiac level, heart rate fluctuates strikingly, with marked episodes of tachycardia and bradycardia (see Figure 2.4) (Verrier *et al.*, 1996) and baroreceptor gain is reduced.

However, such variability is not only the direct result of central changes in the regulation of the autonomic outflow; these changes also activate indirectly a number of feedback loops by affecting the peripherally controlled variables. So, the interaction between the central variability of visceral control during REM sleep and the central effects of activated reflexes are main factors in the generation of the instability of cardiovascular regulation in REM sleep. In this regard, circulation in different species is affected by similar central influences in REM sleep, although the eventual pattern of change in cardiovascular variables also depends on species-specific differences in the

operation of feedback loops and autoregulation. See (Parmeggiani, 2005) for a recent review.

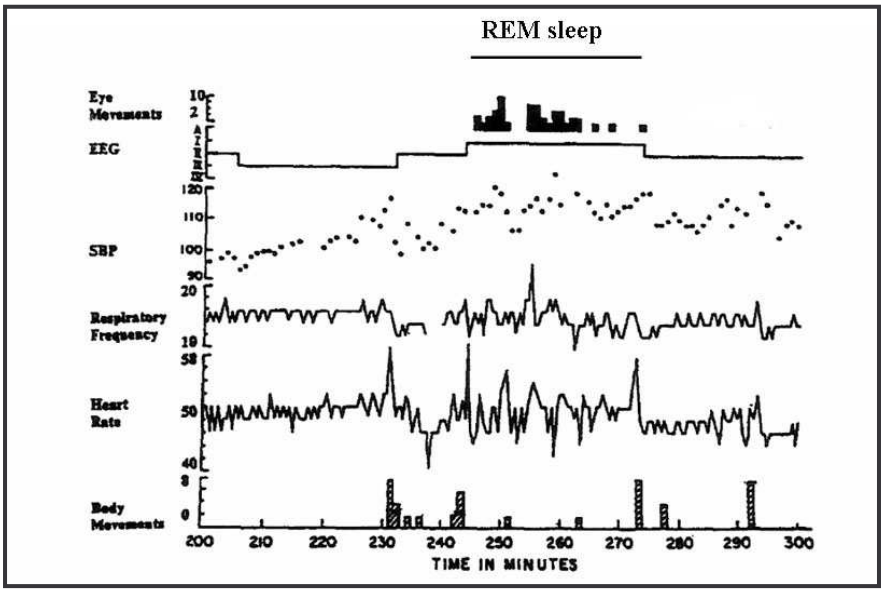

Figure 2.4. Minute-by-minute fluctuations in cardiovascular variables in REM sleep in human healthy subject. EEG: electroencephalogram; SBP: systolic blood pressure. Note that during REM sleep (minute 242-273), two sustained bursts of eye movements (filled bars, top line) were associated with marked irregularities in SBP, respiratory frequency and heart rate. From (Verrier *et al.*, 1996).

The notion that central autonomic commands act on the heart and blood vessels during REM sleep has been recently quantified by assessing the role played by central commands and by the baroreflex in the control of heart rhythm during sleep (Zoccoli *et al.*, 2001; Silvani *et al.*, 2003; Silvani *et al.*, 2005). In particular, parallel changes in heart period and blood pressure (e.g., a pattern of hypertension and cardiac slowing) indicate that heart rhythm control is mainly exerted by the baroreceptor reflex, whereas opposite changes in the two variables (e.g., a pattern of hypertension and tachycardia, like that observed during pressure surges in REM sleep) indicates that central autonomic commands prevail over the baroreflex in controlling heart rhythm (Zoccoli *et al.*, 2001).

In adult rats, central autonomic commands on the heart and blood vessels prevail in REM sleep as a whole over the control exerted by the baroreceptor reflex (Zoccoli *et al.*, 2001; Silvani *et al.*, 2003).

During REM sleep the activity of the brain increases and also cerebral blood flow increases. So, during REM sleep, there is flow-metabolism coupling. On the contrary, changes in other peripheral beds respond to sympatovagal balance and local activity changes that are sleep dependent and may conflict with the functional logic of the organs. Cerebral circulation during REM sleep shares the regulatory mechanism of other brain-activated states, but the disrupted integrated control of the remaining peripheral beds is unique to REM sleep (Franzini, 1992, 2005).

To summarize autonomic regulation of the cardiovascular system during REM sleep can disrupt cardiorespiratory homeostasis.

2.4.1 Phasic hypertensive events during REM sleep

The control pattern of peripheral vascular beds in REM sleep is deeply affected by central autonomic commands and by reflexes other than the baroreflex (Baccelli *et al.*, 1974; Parmeggiani, 1980).

Changes in regional sympathetic activity during REM sleep may take place in the absence of baroreflex resetting and of reflexes elicited by muscle atonia, and may thus represent the result of central autonomic commands issued by brainstem structure. See (Silvani & Lenzi, 2005) for a review.

Likewise, central autonomic commands underlie the phasic hypertensive events (arterial pressure surges), which are superimposed upon the tonic level of arterial pressure in REM sleep. Several investigators have reported REM-induced increases in heart rate in experimental animals: cats (Mancia *et al.*, 1971) (see Figure 2.5); rats (Sei & Morita, 1996) (see Figure 2.6), where heart rate tends to rise during the pressure surges and increases significantly thereafter; mice (Campen *et al.*, 2002) (see Figure 2.7), lambs (Fewell, 1993; Silvani *et al.*, 2005) and also in human subjects (Coccagna *et al.*, 1971) (see Figure 2.8). In these latter the arterial pressure may exceed the highest values recorded during wakefulness (Coccagna *et al.*, 1971).

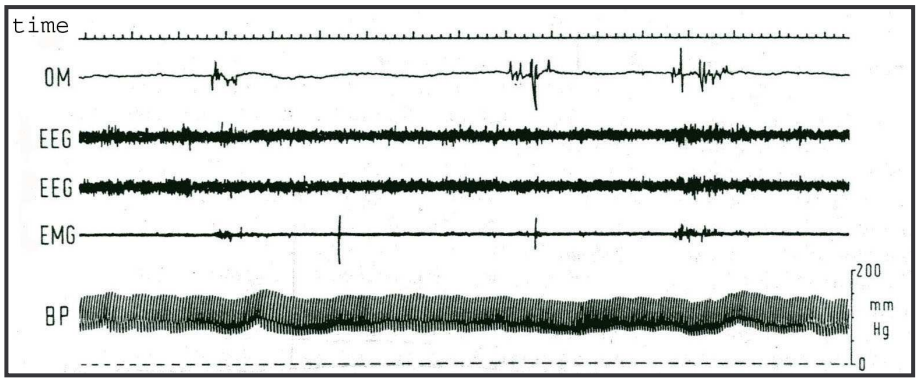

Figure 2.5. Original tracings from a REM sleep episode in cat, showing several phasic manifestations. Time (s); OM: ocular movements; EEG: electroencephalograms; EMG: electromyogram; BP: arterial blood pressure. Modified from (Mancia *et al.*, 1971).

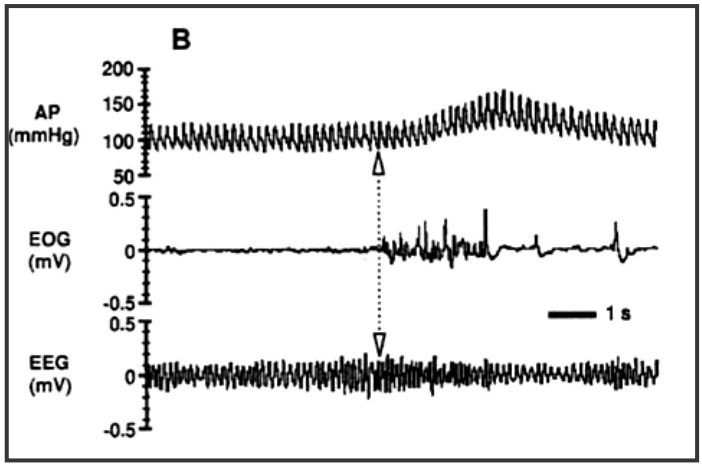

Figure 2.6. Recordings of Arterial Pressure (AP), electro-oculogram (EOG) and electroencephalogram (EEG) signals during REM sleep in rat. Open arrows with dotted line indicate the trigger point for the summation of each signal (beginning point of eye movement burst). Modified from (Sei & Morita, 1996).

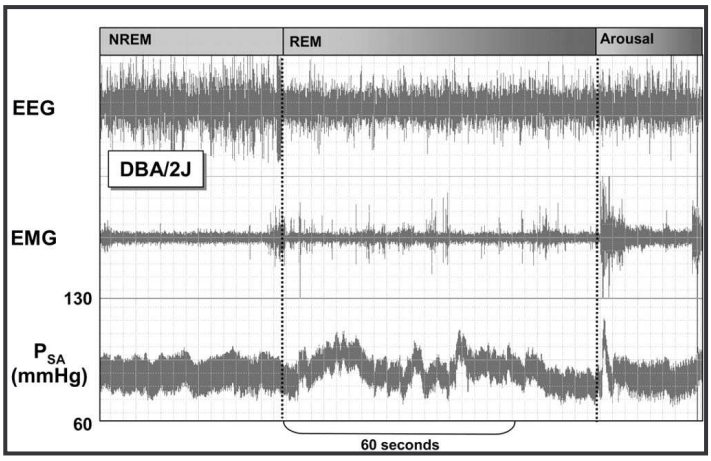

Figure 2.7. Representative tracings of arterial blood pressure (P_{SA}) responses during NREM and REM sleep in a DBA/2J mouse. At the onset of REM sleep, the P_{SA} of the DBA/2J mouse shows surges of 10 mmHg or more above NREM levels during REM sleep. REM, rapid eye movement sleep; NREM, non-REM sleep. Modified from (Campen *et al.*, 2002).

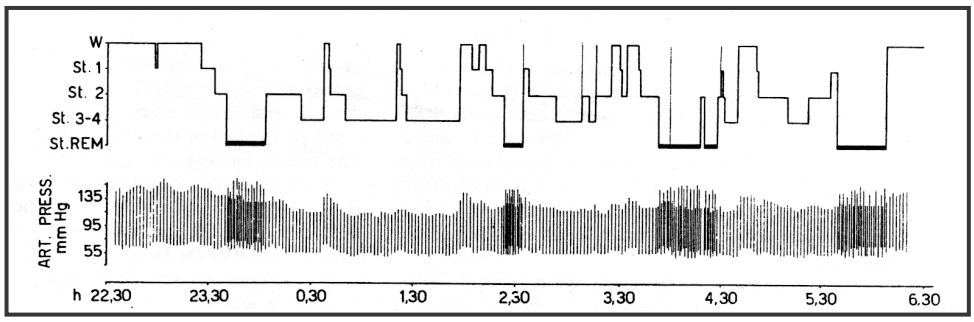

Figure 2.8. Histogram of sleep and trend of the maximal and minimal arterial pressure in a man. During REM sleep pressure values are indicated every 30 seconds (instead of every 60 seconds during NREM sleep) to show the phasic pressure variations. St. refers to sleep stage and W to waking state. Modified from (Coccagna *et al.*, 1971).

During arterial pressure surges, peripheral vascular resistance increases and also coronary vascular resistance (Fewell, 1993), despite of the greater cardiac metabolic demand during the hypertensive events. The increase in muscle vascular resistance (Mancia *et al.*, 1971) is abolished by sympathectomy but not by limb deafferentation, proving that local reflexes are not necessary for its origin (Baccelli *et al.*, 1974).

The baroreflex may play a role in shaping blood pressure surges in REM sleep, as suggested by the finding that muscle sympathetic nerve activity increases before the surges, but abruptly ceases during their course (Somers *et al.*, 1993) (see Figure 2.9).

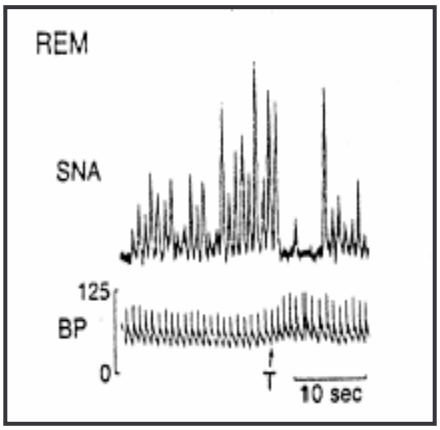

Figure 2.9. Recording of Sympathetic-Nerve Activity (SNA) and Mean Blood Pressure (BP) in muscle blood vessel in human subject. There was a frequent association between REM twitches (momentary periods of restoration of muscle tone, denoted by T on the tracing) and abrupt inhibition of sympathetic-nerve discharge and increased in blood pressure. Modified from (Somers *et al.*, 1993).

At cardiac level, a tachycardia occurs in lambs at the beginning of the arterial pressure surges, indicating that central autonomic commands prevail on the heart as well as on blood vessels at surge onset. Later, heart period and mean arterial pressure are both increased over baseline values, highlighting that the baroreflex effect on the heart prevails late in the course of the surges, in spite of enduring central control on blood vessel (Silvani et al., 2005) (see Figure 2.10).

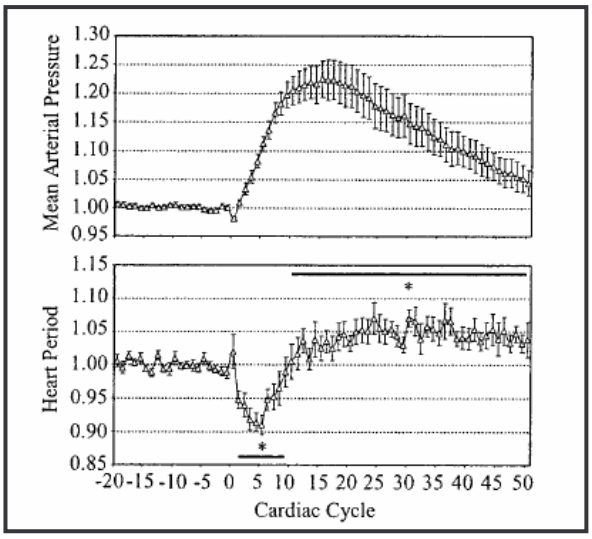

Figure 2.10. Beat-to-beat changes in Mean Arterial Pressure and Heart Period during phasic blood pressure surges in REM sleep in newborn lambs. Beat-to-beat values of heart Period and Mean Arterial Pressure were divided by their baseline values and averaged with the cardiac cycles corresponding with the onset of the Mean Arterial Pressure surges coinciding. Mean values ± SEM are shown. Heart Period started to decrease at the onset of the surge; an increase in Heart Period is evident later in the course of the Mean Arterial Pressure surge, consistent with baroreflex control. *$p<0.05$ for the difference between Heart Period (average value over the cardiac cycles identified by horizontal lines) and its baseline value. n = 7. From (Silvani *et al.*, 2005).

In dogs heart rate surges are accompanied by a rise in mean arterial pressure and are followed by a rate deceleration that is apparently baroreceptor mediated. Because the sequence is completely abolished by interruption of sympathetic neural input to the heart (Kirby & Verrier, 1989), the acceleration does not appear to be dependent on withdrawal of parasympathetic nerve activity (Dickerson *et al.*, 1993).

The rate accelerations are linked to central nervous system activation as reflected in a concomitant increase in incidence and frequency of hippocampal θ waves, ponto geniculo-occipital (PGO) activity, and eye movements (Rowe *et al.*, 1999) (see Figure 2.11). The hippocampus is one of the important limbic structures, and shows a rhythmical sinusoidal EEG (θ wave) during REM sleep, especially in rodents. This appearance of a θ wave is indicative of the pronounced activity of the limbic system during REM sleep. In cats and rats, the appearance of theta waves is characteristic of arousal, orienting activity, alertness and REM sleep (Sei & Morita, 1996; Rowe *et al.*, 1999).

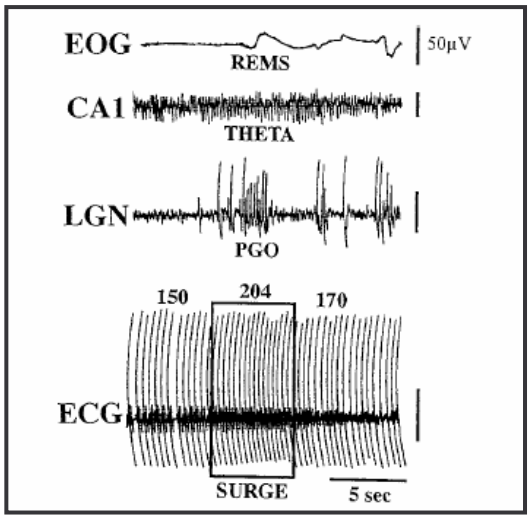

Figure 2.11. Representative polygraphic recording of a heart rate surge during REM sleep associated with eye movements, fast theta activity in the hippocampus, and a burst of pontogeniculooccipital (PGO) spikes. Before and after this heart rate surge, hippocampal field potentials, although exhibiting rhythmic activity in the theta range, were of variable amplitude and frequency. In contrast, hippocampal theta activity stabilized, and its frequency increased in association with the surge. No PGO spikes occurred during the 6 s preceding the surge, and single spikes dominated the control period after the surge. The channels recorded were electromyogram, electrooculogram (EOG), transcortical, hippocampal theta rhythm (CA1), electrocardiogram (ECG), and PGO waves of the lateral geniculate nucleus (LGN). During this surge, heart rate increased from 150 to 204 beats/min or 26.4%. From (Rowe *et al.*, 1999).

In rats, Sei and Morita (1996) reported an association between θ activity, eye movements, and increased heart rate and blood pressure. They demonstrated a significant increase in theta frequency at 1 s before eye movement activity. However, they did not find a consistent correlation with increased mean arterial pressure or heart rate, which, when it occurred, was delayed by 7 s (see Figure 2.12).

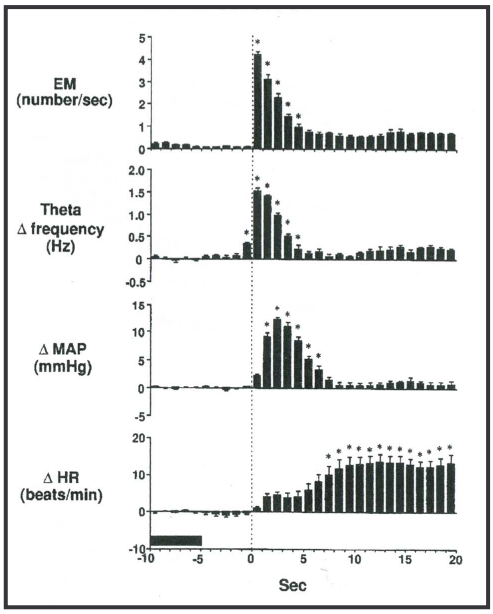

Figure 2.12. Changes in eye movement (EM) number, theta frequency, mean arterial pressure (MAP) and heart rate (HR) in 1s bins. The onset of EM burst (time 0) was taken as a reference point, indicated by a dotted line. The relative changes in theta frequency, MAP and HR were calculated from the baseline values obtained from the 5s period indicated by a closed horizontal bar at bottom. *<0.05, one-way ANOVA with Bonferroni-Dunn test, compared with the baseline vaue. Modified from (Sei & Morita, 1996).

2.5 Respiratory function

Sleep is a time when the respiratory tranquillity of resting wakefulness is replaced by conspicuous respiratory variability due to changes in both the drive to the ventilatory pump muscles and the upper airway opening muscles. Breathing during wakefulness is controlled by several factors, including voluntary and behavioural elements, chemical factors, and mechanical signals from the lung and chest wall. During sleep, there is loss of voluntary control and a decrease in the usual ventilatory response to both low oxygen and high carbon dioxide levels (Douglas, 2005).

The transition from wakefulness to NREM sleep is characterized by the inactivation of the telencephalic control mechanism, typical of the wakefulness, and the release of the automatic control mechanism of the reticular formation of the brain stem. This transition (stages 1 and 2 of NREM sleep in humans) is characterized by breathing instability and the appearance of respiratory and circulatory periodic phenomena.

Regular breathing sets in with deep NREM sleep (stages 3 and 4 in humans), when breathing is driven by the automatic control mechanism. Ventilation during NREM is lower than during wakefulness, and the deeper the NREM sleep stages, the lower is the ventilation. Mean inspiratory flow is decreased, whereas there are no consistent changes in inspiratory duration and cycle duration: the result is a decrease in tidal volume according to the metabolic rate (Krieger, 2005; Parmeggiani, 2005).

The phenomena of REM sleep point to a profound alteration in the activity of the automatic control mechanism of respiration. REM sleep is characterized by erratic, shallow breathing with irregularities both in amplitude and in frequency synchronous to REM bursts that are most probably of central origin and related to REM sleep processes (Krieger, 2005). The irregularity consists of sudden changes in both respiratory amplitude and frequency, at times interrupted by central apneas lasting 10 to 30 seconds, and is different from the regular periodic breathing at sleep onset. In animals, this irregular pattern persists during hypoxia, hypercapnia, and metabolic alkalosis, as well as after vagotomy and chemodenervation. For these reasons, it has been suggested that the breathing pattern is not dependent on chemical regulation processes but is produced by activation of the behavioural respiratory control system by REM sleep processes (Krieger, 2005). The frequency of breathing increases, tidal volumes decrease, and minute ventilation decreases; furthermore in humans, metabolic rate increases in REM sleep, presumably because of a large increase in cerebral metabolism (Orem & Kubin, 2005).

2.6 Sleep as autonomic stress test

Sleep implies profound autonomic changes in the activity of the central nervous system, altering the neural integration of cardioventilatory control. Moreover, sleep limits the variability in local metabolic needs associated with behavioural engagement with the external environment. However, cardiovascular challenges modify the sleep process, which in turn may further modify the regulatory capacity (Silvani & Lenzi, 2005). The mean level and fluctuations around the mean of cardiovascular variables depend on the sleep-wake state and are not of exclusive physiological interest. Rather, the features of cardiovascular regulation during sleep (Verrier *et al.*, 1996) and their

transition to the regulatory pattern of morning awakening (George, 2000) may play a role in pathophysiology of myocardial infarction, stroke and sudden death.

Whereas sleep is generally considered to be a relatively tranquil state dominated by the relative autonomic stability of NREM sleep, the onset of REM sleep is associated with marked perturbations in the interplay between the two divisions of the autonomic nervous system (Verrier, 2000).

NREM sleep is generally salutary with respect to ventricular arrhythmogenesis: activation of the vagus nerve reduces heart rate, increases cardiac electrical stability and reduces cardiac metabolic activity (Verrier *et al.*, 2005) Anyway, in case of severe coronary disease or acute myocardial infarction, hypotension during NREM sleep can lead to myocardial ischemia because of the inadequate coronary perfusion pressure and thereby provoke arrhythmias and myocardial infarction (Mancia, 1993).

The abrupt increases in vagus nerve tone that can occur during periods of REM sleep can result in significant pauses in heart rhythm or bradyarrhythmias. Moreover, the increase in sympathetic nerve activity that occurs at the onset of REM sleep (Somers *et al.*, 1993) provides a potent stimulus for ventricular tachyarrhythmias because of the arrhythmogenic influence of neurally released catecholamines. Experimentally-induced increases in sympathetic nerve activity can encourage cardiac vulnerability in the normal and ischemic heart. Thus, surges in sympathetic and parasympathetic nerve activity during REM sleep, which are well tolerated in normal individuals, may result in arrhythmias, myocardial ischemia and myocardial infarction in those with heart disease (Verrier *et al.*, 2005).

For all of these considerations, sleep may constitute an “autonomic stress test” for the cardiovascular system, and could prove helpful in identifying individuals at risk for sudden cardiac death, because a pathological pattern of cardiovascular regulation may become evident earlier during sleep than in wakefulness (Verrier *et al.*, 1996).

3. Hypertension

Blood pressure in human populations is distributed normally and the cutoff point for high blood pressure is arbitrary. The diagnosis of hypertension in adult is made when average of two or more diastolic blood pressure measurements on at least two subsequent visits is 90 mmHg or more or when the average of multiple systolic blood pressure readings on two or more subsequent visits is consistently greater than 140 mmHg. The current classification results from two international guidelines, developed by the Sixth Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure of U.S.A. (JNC VI) and the World Health Organization-International Society of Hypertension (WHO-ISH), and indicates several levels of risk on the basis of systolic and diastolic pressure values (see Table 3.1) (Oparil, 2000).

	BLOOD PRESSURE (mmHg)	
	Systolic	**Diastolic**
Optimal	<120	<80
Normal	<130	<85
High-Normal	130-139	85-89
Hypertension		
Stage 1	140-159	90-99
Stage 2	160-179	100-109
Stage 3	≥180	≥110

Table 3.1. Classification of blood pressure for adults. From (Oparil, 2000).

3.1 Prevalence

The prevalence of hypertension depends on both racial composition of the population studied and the criteria used to define the condition.

The prevalence of blood pressure increases with age: it is a common health problem in the geriatric population, afflicting approximately 65% of the population in the 65-74 year old group. Blacks have a higher prevalence of hypertension than whites (38%

versus 29%) and men have a higher overall prevalence of hypertension than women (33% than 27%), up to approximately age 50; after that age, hypertension is more common in women (Oparil, 2000); this increase is presumably related to the hormonal changes of menopause (Williams, 1995).

In European countries systemic hypertension, with its burden of cardiac, vascular, and renal complications is a major health problem and its prevalence is 44% (Wolf-Maier et al., 2003).

3.2 Essential Hypertension

Individuals in whom generalized or functional abnormalities may be the cause of hypertension are defined as having primary, essential, or idiophatic hypertension. More than 95% of all cases of hypertension are of unknown cause. The main difficulty in uncovering the mechanism responsible for the hypertension in these patients is attributable to the variety of systems that are involved in the regulation of arterial pressure- peripheral and/or central adrenergic, renal, hormonal, and vascular- and to the complexity of the interrelations of these systems.

Primary hypertension tends to cluster in families and represents a collection of genetically based diseases and/or syndromes with a number of underlying inherited biochemical and pathophysiologic factors. High blood pressure, in most cases, results from a complex interaction of genetic, environmental and demographic factors and the development of the disease is slow and gradual. By the time that blood pressure become elevated, the initiating factors may no longer be apparent because they may have been ‘normalized’ by multiple compensatory interactions (Oparil, 2000).

Individuals in whom a specific structural organ or gene defect is responsible for hypertension are defined as having a secondary form of hypertension. Systemic hypertension of known etiology accounts for fewer than 5% of all cases of systemic hypertension. Common causes of secondary hypertension are renal diseases, endocrine diseases, neurologic disorders and also some drugs and chemicals. Importance of identifying patients with secondary hypertension is that they can sometimes be cured by surgery or by specific medical treatment (Williams, 1995; Oparil, 2000).

3.3 Mechanisms of primary hypertension

Since persistent hypertension can develop only in response to an increase in cardiac output or a rise in peripheral resistance, defects may be present in one or more of the multiple factors that affects these two variables. The interplay of various derangements in factors affecting cardiac output and peripheral resistance may precipitate the disease, and these abnormalities may differ in both type and degree in different patients (see Figure 3.1) (Kaplan, 2001).

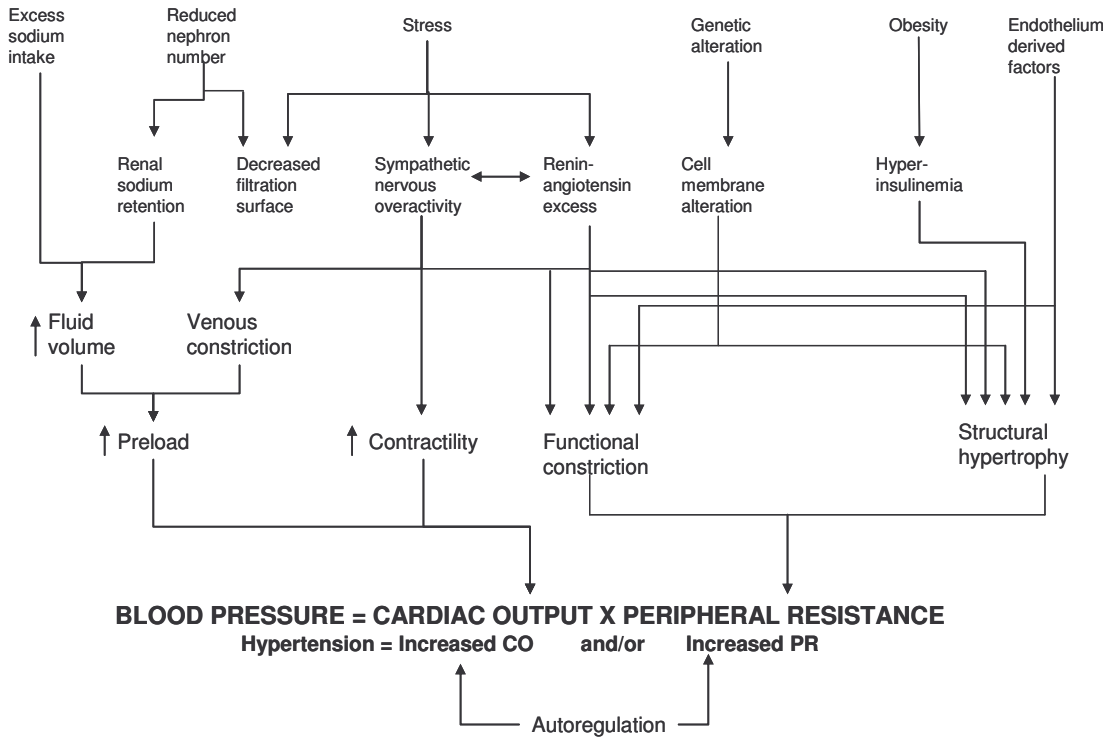

Figure 3.1. Some of the factors involved in the control of blood pressure. Modified from (Kaplan, 2001).

Genetic predisposition

Genetic alterations may initiate the cascade to permanent hypertension. In studies of twin and family members in which the degree of familial aggregation of blood pressure levels is compared with the closeness of genetic sharing, the genetic contributions have been estimated to range from 30 to 60 percent (Harrap, 1994).

Three rare forms of hypertension have been found to be caused by a monogenic abnormality (Luft, 1998); in addition, polymorphism of genes involving the rennin-

angiotensin system, aldosterone synthesis, and adrenergic receptors has been noted to be more common in hypertensive than normotensive patients (Kaplan, 2001).

Renal retention of excess dietary sodium

A lot of evidences support a role for sodium in the genesis of hypertension: some of that excess sodium must be retained by the kidneys. More than enough ways are available to incite renal retention of even a very small bit of the excess sodium typically ingested that could eventually expand body fluid volume. Variations in sensitivity to sodium may explain why only some people respond to excess sodium and others do not.

The retention could arise in a number of ways, comprising (Kaplan, 2001):

1. a decrease in filtration surface by a congenital or acquired deficiency in nephron number or function;
2. a regulation of the normal pressure-natriuresis relationship wherein a rise in pressure invokes an immediate increase in renal sodium excretion, thereby shrinking fluid volume and returning the pressure to normal;
3. nephron heterogeneity: a subpopulation of nephrons that is ischemic either from afferent arteriolar vasoconstriction or from an intrinsic narrowing of the lumen. Rennin secretion from this subgroup of nephrons is tonically elevated and this increased renin secretion interferes with the compensatory capacity of intermingled normal nephrons to adaptively excrete sodium and, consequently, perturbs overall blood pressure homeostasis;
4. an acquired inhibitor of the sodium pump or other abnormalities in sodium transport;
5. defective responsiveness to atrial natriuretic hormone.

Vascular hypertrophy

Multiple vasoactive substances act as growth factors for vascular hypertrophy. These pressor-growth promoters may result in both vascular contraction and hypertrophy simultaneously, but perpetuation of hypertension involves hypertrophy (Kaplan, 2001).

Sympathetic nervous hyperactivity

In primary human hypertension, measurement of regional sympathetic activity using electrophysiologic (sympathetic nerve recording) and neurochemical (measurement of

norepinephrine spillover) techniques has demonstrated activation of the sympathetic nervous outflows to the heart, kidneys, and skeletal muscle vasculature, particularly in younger patients. This sympathetic activation contributes to blood pressure elevation, and to the development of atherosclerosis, cardiovascular hypertrophy, and cardiac arrhythmias. The specific causes of the increased sympathetic activity in primary hypertension remain largely unknown, although genetic influences are evident and behavioural and lifestyle factors appear to be involved (Esler, 2000).

The majority of evidence currently favours a pivotal role for the autonomic nervous system in the etiology of primary hypertension. A variety of confirmations support the concept of sympathetic hyperactivity and parasympathetic underactivity as central in the etiology of not only early and borderline hypertension, but also in the maintenance of sustained essential hypertension (see Figure 3.2) (Brook & Julius, 2000).

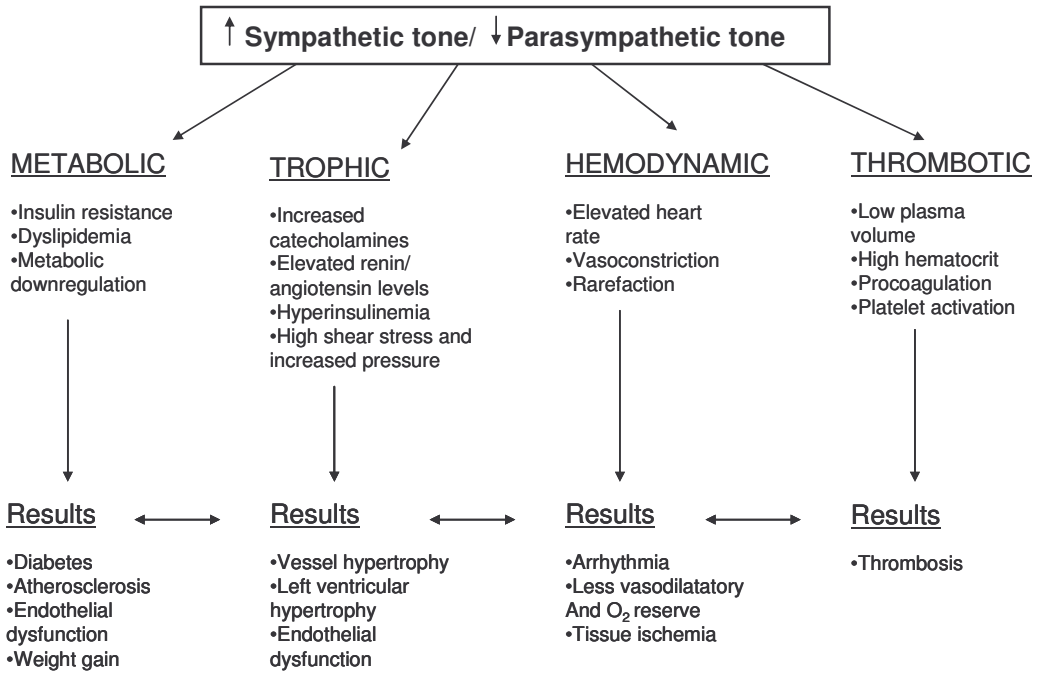

Figure 3.2. Autonomic imbalance. Modified from (Brook & Julius, 2000).

Primary hypertension conveys an increased risk of cardiovascular morbidity and mortality. The common finding of an autonomic imbalance in these patients contributes to the etiology of hypertension itself, but also to the cardiac risk and resulting adverse

consequences. A high sympathetic tone in particular is responsible for many of the metabolic, hemodynamic, trophic, and rheologic abnormalities that cluster in patients with high blood pressure (Brook & Julius, 2000).

Both sympathetic overactivity and parasympathetic underactivity contribute to the state of relative tachycardia in hypertension. Elevated plasma catecholamines and intraneural recordings also support the etiology to be neurogenic. High heart rate can increase morbidity and mortality by several mechanisms. Decreased parasympathetic tone, along with an activated sympathetic tone, is conducive to cardiac arrhythmias in both human and animal models. The relationship between elevated sudden death risk and high heart rate is likely explained by the fact that tachycardia leads to increased myocardial tissue oxygen demand. This often occurs in conjunction with left ventricular hypertrophy in hypertension, further increasing cardiac oxygen needs. In the setting of conditions common to hypertension (an elevated cardiac afterload, coronary atherosclerosis, endothelial dysfunction, reduced coronary vasodilatory reserve), high oxygen demand drastically predisposes to cardiac ischemia. Finally, a high heart rate has experimentally been shown to directly cause coronary atherosclerosis in animal models. The reverse is also true. Reducing heart rate 30% by ablating the sinoaortic node lowered the number and severity of coronary lesions by 50% in a high-cholesterol–fed animal model (Beere *et al.*, 1984). Higher coronary artery shear forces induced by tachycardia, particularly at artery branches, are likely responsible for this increased rate of atherosclerotic lesion formation. These findings suggest that the common condition of a high heart rate in patients with high blood pressure is not a benign condition. The autonomic imbalance in these patients causes this relative tachycardia. Therefore, methods to reduce heart rate by restoring normal autonomic nervous system tone should prove to be protective against cardiovascular consequences (Brook & Julius, 2000).

Baroreceptor reflex

As regard the baroreflex sensibility, it has been demonstrated that in primary hypertension, both in human subjects (Lucini *et al.*, 1994) and in animal models (Oosting *et al.*, 1997a; Nagai *et al.*, 2003), the gain of cardiac effector is reduced. The baroreceptor reflex plays no role in long-term regulation of arterial pressure, because baroreceptors were thought to reset in 1 or 2 days toward whatever pressure level they

are exposed. However, very recent studies suggested that baroreceptors may not completely reset, at least not with regard to renal sympathetic nerve activity, and thus that baroreceptors may indeed be able to contribute to long-term mean arterial pressure (Persson, 2005).

Renin-Angiotensin system

Both as a direct pressor and as a growth promoter, the renin-angiotensin mechanism may also be involved in the pathogenesis of hypertension. Renin is an enzyme secreted by the juxtaglomerular cell of the kidney and linked with aldosterone in a negative feedback loop. A variety of factors can modify its rate of secretion, in particular changes in dietary sodium intake. The end product of the action of renin on its substrate is the generation of the peptide angiotensin II. The response of target tissues to this peptide is only determined by the prior dietary electrolyte intake. The range of plasma renin activities observed in hypertensive subjects is broader than in normotensive individuals. So that, some hypertensive patients have been defined as having low-renin and others as having high-renin essential hypertension.

- Low-renin primary hypertension. About 20% of patients who by all other criteria have primary hypertension have suppressed plasma renin activity and expanded extracellular fluid volumes. Some studies have suggested that the adrenal cortex of some of these patients has an increased sensitivity to angiotensin II as the underlying mechanism. On a diet with normal or high sodium content, aldosterone production will not be suppressed normally, leading to a mild degree of hyperaldosteronism with its resulting increased sodium retention, volume expansion, and increase in blood pressure.
- Non-modulating primary hypertension. About 25-30% of the hypertensive population has an adrenal defect opposite to that observed in low-renin patients, a reduced adrenal response to sodium restriction. In these individuals, sodium intake does not modulate either adrenal or renal vascular responses to angiotensin II. Hypertensives in this group have been termed non-modulators because of the absence of the sodium-mediated modulation of target tissue responses to angiotensin II; they have plasma renin activity levels that are normal to high if measured when the patient is on a low-salt diet, and have

hypertension that is salt sensitive because of a defect in the kidney's ability to excrete sodium appropriately.

- High-renin primary hypertension. Approximately 15% of patients with primary hypertension have plasma renin activity levels above the normal range. Plasma renin probably plays an important role in the pathogenesis of the elevated arterial pressure in these patients. Elevated renin levels and blood pressure may both be secondary to an increase in adrenergic system activity. It seems that, in patients with angiotensin-dependent high-renin hypertension whose arterial pressures are lowered by an angiotensin II antagonist, the mechanism responsible for the increase in renin and, therefore, for the hypertension is the non-modulating defect (Williams, 1995).

Obesity

Hypertension is more common among obese individuals and adds to their increased risk for ischemic heart disease. Even small amounts of weight gain are associated with a marked increase in the incidence of hypertension and coronary mortality (Kaplan, 2001).

Sleep apnea

One of the contributors to the hypertension in obese persons is sleep apnea. Snoring and sleep apnea are often associated with hypertension, which may in turn be induced by increased sympathetic activity and endothelin release in response to hypoxemia during apnea (Kaplan, 2001).

Other conditions

A number of environmental factors have been implicated in the development of hypertension, including salt intake, alcohol intake, physical inactivity, smoking, haematological findings, hyperuricemia, insulin resistance, endothelial cell dysfunction, family size, occupation, crowding (Williams, 1995; Kaplan, 2001).

3.4 Effects of hypertension

If untreated about 50% of hypertensive patients die of coronary heart disease or congestive failure, about 33% percent of stroke, and 10-15% of renal failure (Kaplan, 2001).

To what concern cardiac involvement, hypertension places increased tension on the left ventricular myocardium that is manifested as stiffness and hypertrophy, which accelerates the development of atherosclerosis within the coronary vessels. Combination of increased demand and lessened supply increases the likelihood of myocardial ischemia and thereby leads to a higher incidence of myocardial infarction, sudden death, arrhythmias, and congestive failure in hypertensives.

Renal dysfunction too unperceivable to be recognized may be responsible for the development of most cases of primary hypertension. Increased renal retention of salt and water may be a mechanism initiating primary hypertension, but retention is so small that it escapes detection. As hypertension-induced nephrosclerosis proceeds, the plasma creatinine level begins to rise and eventually, renal insufficiency with uremia may develop, thus making hypertension a leading cause of end-stage renal disease.

To what concern cerebral involvement, hypertension may accelerate cognitive decline with age. Systolic hypertension, in particular, is a major risk factor for initial and recurrent stroke and for transient ischemic attacks caused by extracranial atherosclerosis (Kaplan, 2001).

3.5 Treatment of hypertension

The goal of antihypertensive therapy is to reduce overall cardiovascular risk and thus cardiovascular morbidity and mortality.

Non drug therapeutic intervention is probably indicated in all patients with sustained hypertension and probably in most with labile hypertension. The general measures employed include: relief of stress, dietary management, regular aerobic exercise, weight reduction and control of other risk factors contributing to the development of atherosclerosis.

Monotherapy with most antihypertensive drugs effectively controls blood pressure in fewer than 50% of patients. In general there are six classes of drugs: diuretics, antiadrenergic agents, vasodilatators, calcium entry blockers, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor antagonists (Williams, 1995; Oparil, 2000).

The drug used in this work was Enalapril (Adams *et al.*, 1990), an angiotensin converting enzyme inhibitor. Enalapril (see Figure 3.3) is a prodrug that is converted by deesterification to a converting enzyme inhibitor, enalaprilat. Enalapril is converted to the active metabolite by hydrolysis, primarily in the liver. Peak concentrations of enalaprilat occur 3–4 hours after dosing with Enalapril, and the half-life of enalaprilat is about 11 hours (Benowitz, 2001).

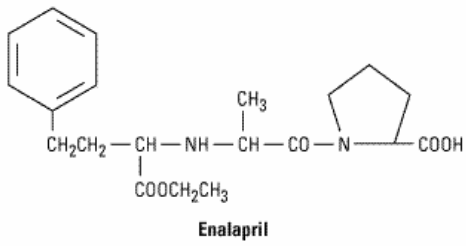

Figure 3.3. Chemical structure of Enalapril (Benowitz, 2001).

Enalaprilat inhibits the enzyme converting angiotensin I into angiotensin II, the angiotensin converting enzyme (ACE). This agent is useful because it not only inhibits the generation of a potent vasoconstrictor (angiotensin II) but also may retard the degradation of a potent vasodilator (bradykinin), alter prostaglandin production and can modify the activity of the adrenergic nervous system. It is especially useful in renal or renovascular hypertension (Williams, 1995).

Angiotensin II inhibitor lowers blood pressure principally by decreasing peripheral vascular resistance. Cardiac output and heart rate are not significantly changed. Unlike direct vasodilator, this agent does not result in reflex sympathetic activation and can be used safely in persons with ischemic heart disease. The absence of reflex tachycardia may be due to downward resetting of the baroreceptors or to enhanced parasympathetic activity. Although converting enzyme inhibitor is most effective in conditions associated with high plasma renin activity, there is no good correlation among subjects between plasma renin activity and antihypertensive response, and renin profiling is

unnecessary. ACE inhibitors have a particularly useful role in treating patients with diabetic nephropathy because they diminish proteinuria and stabilize renal function (even in the absence of lowering of blood pressure).

Severe hypotension can occur after initial doses of any ACE inhibitor in patients who are hypovolemic due to diuretics, salt restriction, or gastrointestinal fluid loss. Other adverse effects common to all ACE inhibitors include acute renal failure, hyperkalemia, dry cough sometimes accompanied by wheezing, and angioedema. Hyperkalemia is more likely to occur in patients with renal insufficiency or diabetes. Bradykinin and substance P seem to be responsible for the cough and angioedema seen with ACE inhibition (Benowitz, 2001).

3.6 Animal model of hypertension: the Spontaneously Hypertensive Rat

Hypertension is a multifactorial, polygenic disease that involves complex interactions between genetically determined homeostatic control mechanisms and environmental factors, and its exploration thus requires availability of experimentally manipulable animal models. The ideal animal model for hypertension research should have human-like cardiovascular anatomy, hemodynamics, and physiology and develop human disease characteristics and complications in accelerated fashion. Inevitably, no species can consistently answer all of these needs, and experimental design and other constraints often dictate the choice of animal models for specific research applications.

In human primary hypertension, multiple genes contribute to the individual disease phenotype; as a result, no single genetic defect can explain development of primary hypertension. Growth of experimental models of hypertension allowed dissection and isolation of various factors associated with regulation of blood pressure, inheritance of hypertensive traits, and cellular responses to injury (Lerman *et al.*, 2005).

The major part of the experimental hypertension research has been carried out in rodents, namely, in the rat (Folkow, 1990), which is very convenient for the study of cardiovascular physiology and which has a particular potential for exploration of polygenic hypertension. In the rat, the wide spectrum of experimental hypertension models so far available differs in the contribution of genetic and environmental factors

to the elevation of blood pressure. In general, development of homozygous hypertensive rat strains is achieved by selective breeding of animals displaying the desired phenotype over several generations (Lerman *et al.*, 2005).

One of the first strains of inherited hypertension was the genetically hypertensive rat developed from the New Zealand strain (Smirk & Hall, 1958). Other important hypertensive rat strains include the salt-sensitive Dahl and the Sabra model, which exhibits gender-specific quantitative trait loci for salt susceptibility on chromosome 1 SS1a and SS1b in men and SS1b in women (Yagil *et al.*, 2003). Other important models include the Lyon genetic hypertensive rat, a model of low-renin hypertension that shows hypersensitivity of preglomerular vessels to AngII (Sassard *et al.*, 2003), and Milan SHR, which has a mutation in the gene coding for adducin, a skeletal protein involved in transepithelial sodium transport (Cusi *et al.*, 1993). Many of these models indeed mimic the human form of low-renin hypertension (Lerman *et al.*, 2005).

In most phenotype-driven models, hypertension is associated with cardiac hypertrophy, endothelial dysfunction, and renal functional impairment (proteinuria, decreased creatinine clearance), but cardiac insufficiency and end-stage renal disease are not consistently observed. The outcomes seem to depend on the underlying origin, genetic background, and possibly species differences, as well as on the degree of hypertension (Lerman *et al.*, 2005).

The Spontaneously Hypertensive Rats (SHR) strain, the current paradigm for essential hypertension research, was developed in a breeding program based solely on selection by elevated blood pressure in the Wistar Kyoto rats (WKY) by Okamoto in 1964 (Okamoto & Aoki, 1963). The WKY strain was established as a normotensive control strain for the SHR by inbreeding of the normotensive Wistar colony (from which the SHR originally emerged) by brother/sister mating (Friese *et al.*, 2005). In particular, a 48% allelic difference exists between the SHR strain and the WKY strain (Cowley *et al.*, 2004).

In addition to elevated blood pressure, the SHR exhibits many of the co-morbidities observed in human hypertension, such as insulin resistance, hypertriglyceridemia, and abdominal obesity (Friese *et al.*, 2005). The SHR model spontaneously develops hypertension without need of any dietary or surgical manipulation and has become the most used animal model for research on polygenic hypertension.

The worldwide availability of SHR has made possible not only to identify numerous cardiovascular abnormalities in this model, but also to estimate the degree of their

genetic determination, to study their ontogenetic aspects in detail and to evaluate their role in the pathogenesis of hypertension by means of various interventions (Zicha & Kunes, 1999). The SHR is not a strictly inbred strain and gives way to a wide variety of genes to cosegregate and may mimic a subtype of human primary hypertension that is inherited in a Mendelian fashion. The genetic mechanisms of hypertension in SHR have been attributed to both neural and vascular alterations (Lerman *et al.*, 2005).

As we can see in Figure 3.4, the blood pressure of SHR newborns was usually found to be significantly higher compared with that of WKY rats, characteristic acceleration of the blood pressure rise in SHR mainly occurs between the 3rd and 10th week of age when their blood pressure rapidly increases by ~ 30% above that of WKY rats. The blood pressure of WKY rats reaches adult levels by ~ 10 wk of age, but in SHR, it continues to rise at least until the age of 20 wk. There is no doubt that high blood pressure in hypertensive humans or animals is caused by an elevation of systemic resistance, the greater part of which is partially caused by the decrease of arteriolar lumen diameter due to media thickening or remodelling (Zicha & Kunes, 1999).

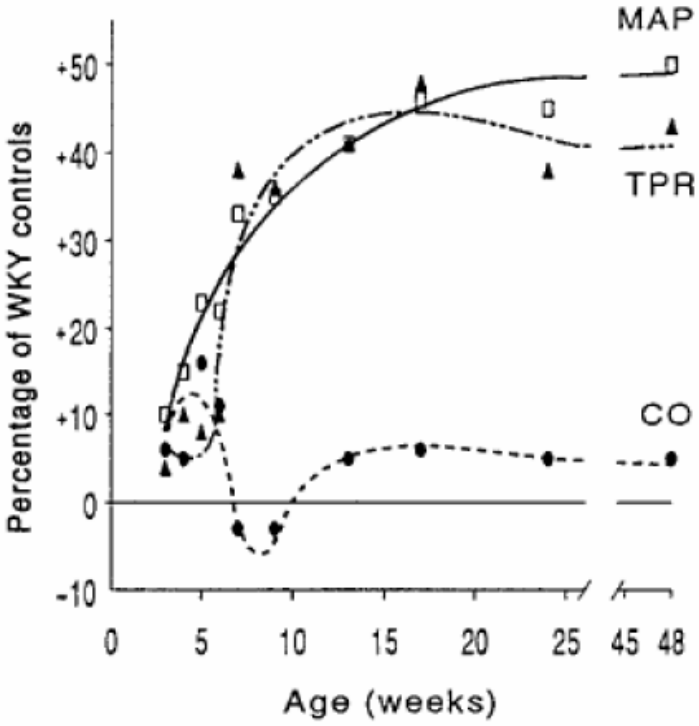

Figure 3.4. Ontogeny of mean arterial blood pressure (MAP), cardiac output (CO), and total peripheral resistance (TPR) in spontaneously hypertensive rats (SHR) expressed as percentage of Wistar-Kyoto (WKY) control rats. Data adapted from (Zicha & Kunes, 1999).

In SHR, the clinical course of the hypertensive disease and the pathological findings, such as cardiac hypertrophy and arterial sclerosis, are similar to those observed in human patients. Indeed, as in human primary hypertension, the blood pressure is relatively normal in the young SHR, increases with age and terminates in heart failure, cerebrovascular accidents, renal insufficiency or in the malignant phase of the disease (Grollman, 1972).

3.7 Hypertension and sleep

Epidemiologic evidence has provided important links between sleep-related disorders and primary hypertension. Since sleep ultimately consumes approximately one third of a human life, studying the relationship between sleep disorders and cardiovascular diseases has gained popularity. In particular, systemic hypertension is associated with sleep-related autonomic deregulation (Heald *et al.*, 1989; Kuo *et al.*, 2004a) and breathing disorders (Carley *et al.*, 2000) as well as with alterations in the sleep pattern (Kuo *et al.*, 2004b).

Up to 60% of patients with sleep apnea may have hypertension, but the mechanisms underlying the link between obstructive sleep apnea and hypertension are not completely established. Most studies show that treatment of sleep disordered breathing modestly improves nocturnal and diurnal blood pressure in patients with sleep apnea (Silverberg *et al.*, 2002) and often confers improvements in subjective symptoms, especially daytime somnolence.

Further evidences concerning the interaction between blood pressure and sleep come from non-dipper population studies. Normally during NREM sleep, systolic blood pressure is 10% to 20% lower than that during quiet wakefulness (Mancia *et al.*, 1971). Blood pressure is higher during REM sleep than during NREM sleep, but does not reach awake levels. Individuals with this 24-h blood pressure profile have been labelled ‘dippers’. This dipper pattern has been observed in most patients with essential hypertension. The flattened 24-h blood pressure pattern in ‘non-dippers’ occurs in a minority of patients with essential hypertension. It is not clear whether these individuals experience a reduction in the nocturnal decline in blood pressure or failure in appropriate blood pressure rise during the daytime (George, 2000). Dipping has been

postulated to be a restorative physiologic process (Rosansky *et al.*, 1996), with a potential impact also on the quality of sleep. A recent study reported that in a population of healthy subjects deeper sleep was associated with more blood pressure dipping; conversely, the same study could also suggest that lighter and more disturbed sleep may be associated with less dipping (Loredo *et al.*, 2004).

Whatever the specific role of sympathetic activity in the pathogenesis of hypertension, it appears to be involved in the increased cardiovascular morbidity and mortality that affect hypertensive patients during the early morning hours. Increased α-sympathetic activity occurs in the early morning in association with the preawakening increase in REM sleep and the assumption of upright posture after overnight recumbency (Panza *et al.*, 1991). As a consequence of the increased sympathetic activity, blood pressure rises abruptly and markedly. This rise must be at least partly responsible for the increase in cardiovascular catastrophes in the early morning hours (Muller, 1999).

4. Methods

Experiments were performed on 14 male Spontaneously Hypertensive Rats (SHR) and 7 male Wistar-Kyoto rats (WKY) (Charles River Italia, Calco, Italy). Animals were housed individually and were kept on a light/dark cycle of 12 hour period with light on at 9 a.m., ambient temperature at 23 ± 1 °C and free access to food (standard rodent diet, 4RF21, Mucedola, Milano, Italy) and tap water.
The study protocol was approved by the Bologna University ethical committee on animal experimentation.

4.1 Drug therapy of Spontaneously Hypertensive Rats

A group of 7 SHR, hereafter termed the SHRace group, was treated with Enalapril maleate from the age of 4 weeks to the end of the experiment. Enalapril (Enalapril maleate salt, Sigma-Aldrich, Milano, Italy), an ACE-inhibitor, was dissolved in tap water (25-30 mg/Kg/day) (Adams et al., 1990). The water consumption of the SHRace rats was monitored and the concentration of Enalapril was adjusted to maintain the ingested dose per unit body weight approximately constant across days.

4.2 Surgical procedures

At the age of 9 weeks, rats were implanted under general anaesthesia (1.2% halothane, 30% O_2, balance N_2O) and sterile conditions with electrodes for electroencephalographic (EEG) and electromyographic (EMG) recordings, a catheter in the abdominal aorta, and a thermistor in the nasal cavity to measure the breathing rate. Animals were placed on a heating pad throughout the experiment, and the rectal temperature was maintained at 37 ± 0.5°C.

Ketoprofen (Aventis, 1 mg / 100 g body weight) was administered subcutaneously for postoperative analgesia (Roughan & Flecknell, 2001). Benzilpenicillin benzatinic

(Fournier, 15000 IU / 100g body weight) and streptomycin sulphate (Bristol-Myers Squibb, 20000 IU / 100g body weight) were administered subcutaneously for antibiotic prophylaxis at the end of the surgery.

Catheter

A non-occlusive saline-filled catheter (0.30 mm in inner diameter, 0.64 mm in outer diameter) made of silicone rubber (Silastic™, Medical grade) was inserted in the abdominal aorta via the left femoral artery for blood pressure monitoring and blood sampling. The silicone rubber is suitable for this application because inert and bio-compatible. In addition, it can be sterilized in autoclave while remaining extremely flexible and hence minimally traumatic for blood vessels. One week before the surgery, the catheter was heparinized (Waynforth & Flecknell, 1992). The purpose of this procedure was to let heparin molecules stick to catheter wall, so as to avoid the deposition of the platelet-fibrin complex (thrombus) in the tip of the catheter after its implantation. The catheter was soaked for 30 minutes in 5% tridodecylmethylammonium chloride (TDMAC, Sigma-Aldrich, Milano, Italy) in ethanol 95%. It was then air dried and washed five times with distilled water. Following this, it was filled with heparin (Eparina Vister, 5000 U.I. /ml, Pfizer) solution and incubated for 30 minutes. Finally, the catheter was again allowed to air dry, washed five times with distilled water, and sterilized in a Composite IMO autoclave. During surgery, the catheter was tunnelled subcutaneously from an incision in the neck to an incision in the inguinal left region. The left femoral artery was then isolated and catheterized.

Electrodes

Rats were mounted in a stereotaxic instrument to immobilize the skull. Three miniature stainless steel screws were soldered to copper insulated wire and implanted into the skull for bipolar EEG recordings: one electrode 1.0 mm anterior and 2.0 mm lateral to bregma, two electrodes bilaterally to the lambda (0.0 mm anterior and 2.0 mm lateral). Besides, two electrodes for unipolar EEG recordings were implanted for record the K Complex (1.0 mm posterior and 2.0 mm lateral to bregma, and 5.0 mm posterior and 0.0 mm lateral to lambda) (Marini *et al.*, 2004) (see Figure 4.1). We used two EEG derivations: the first is the bipolar fronto-parietal derivation for the traditional scoring of the wake-sleep cycle in rodents; the second one is a monopolar derivation in order to

improve the amount of information for sleep scoring. In fact, one electrode (the active one) was placed just behind the bregma on the parietal bone, while the second electrode (the reference) was implanted in the midline above the cerebellum.

Two Teflon-coated stranded wire silver (Coonerwire, Chatsworth, CA, USA) electrodes were also implanted bilaterally in the dorsal nuchal muscles to record EMG activity.

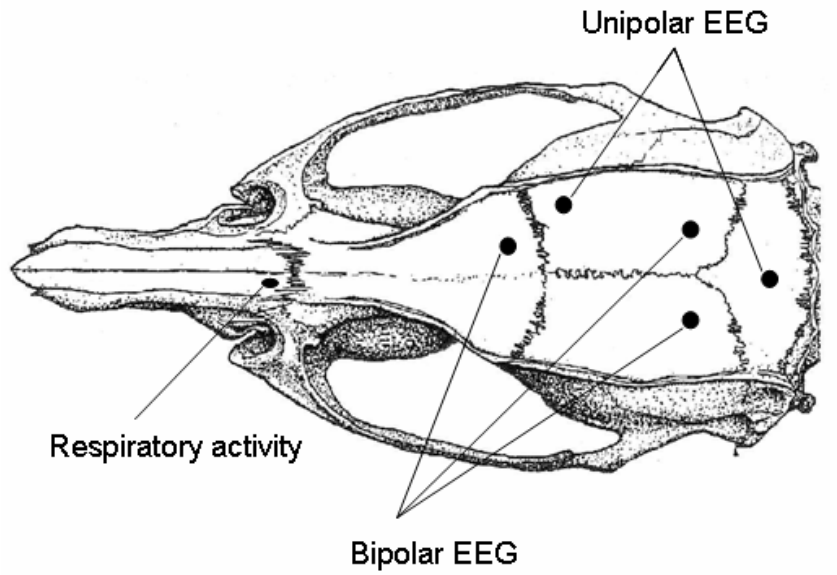

Figure 4.1. Position of electrodes on rat head.

Thermistor

A thermistor was inserted into left nasal cavity through the skull (2.0 mm anterior to the suture between the left frontal bone and the left nasal bone and 1.0 mm lateral to the suture between the nasal bones) to measure temperature variations resulting from ventilation (see Figure 4.1). There is a large temperature difference between air coming out of the respiratory system (body temperature) and air going into the respiratory system: during nasal expiration the temperature will increase; during nasal inspiration the temperature will decrease.

A thermistor is a thermally sensitive resistor that is supplied with a constant but low current. The use of a low current reduces the tendency of the thermistor to heat itself. It is desirable that small temperature changes produce large resistance changes. Expired airflow heats the sensor, increasing its resistance, and inspiratory airflow cools the sensor to ambient temperature, resulting in a relative decrease in the resistance that can

then be recorded. A thermistor ceases being an airflow sensor if it touches the skin, because it will remain at body temperature (Hirshkowitz & Kryger, 2005).

The electrodes and the thermistor were soldered to a connector and fixed to the skull together with the distal end of catheter by acrylic dental cement.

The surgery lasted approximately three hours. After the surgery, rats were housed individually in cages for recovery. Each animal was allowed 6 days for postoperative recovery, during which the catheter was flushed with heparinized saline solution (50 U/ml, 150 µl) every day, to avoid occlusion because of clot formation. By the end of the recovery period, rats did not show abnormalities in gaiting and feeding and regained weight normally.

4.3 Experimental procedures

After recovery, each animal was placed for recordings in a thermoregulated (minimum temperature: 22±1°C; maximum temperature: 24±1°C), sound-attenuated box. The catheter was connected via an external polyethylene tube to a transducer (P23, Statham, Hato Rey, Puerto Rico). Food and water were available ad libitum. The signals were continuously recorded for 4 consecutive days from 10.00 a.m. to 6.00 p.m., the light-period that represents the restorative period in rodents, with the animals undisturbed and freely moving in their own cages.

On the fifth recording day, the arterial catheter was connected to a thinner polyethylene tube (20 µl in volume) for blood withdrawal. The small calibre of this polyethylene catheter allowed a minimal dead space, but at the same time caused an elevate resistance, making it unsuited to record the blood pressure. For this reason, the arterial pressure was not recorded the fifth day. Blood was withdrawn to flush the dead space of the catheter before each blood sampling, and subsequently re-infused to minimize the animal's blood loss.

Blood samples (150 µl) were obtained in undisturbed rats and immediately analyzed by a blood-gas analyzer (Gem 3000, Instrumentation Laboratory, Milano, Italy) to measure pH, partial pressures of oxygen and carbon dioxyde, hematocrit and electrolyte concentrations in the arterial blood.

Once the blood sampling was completed, the animal was sacrificed with an overdose of anaesthetic and the autopsy was executed to check for inflammatory processes, particularly in the abdominal aorta and at the cranial level.

4.4 Data acquisition and storage

Electrophysiological signals were amplified and filtered with the signal conditioner. The bipolar EEG and unipolar EEG signals were band-pass filtered from 0.3 to 60 Hz; the EMG signal was band-pass filtered from 100 to 1000 Hz; the ventilatory activity signal was band-pass filtered from 0.3 to 15 Hz. The arterial pressure signal was low-pass filtered at 100 Hz. The gain of the amplification of EEG and EMG signals was adjusted by the operator for each rat to compensate for variable resistance of the electrodes.

Dedicated software developed in C was used to visualize and acquire data. The bioelectric signals were relayed to a 12-bit analog-digital converter connected to a personal computer. The sample rate for EEG, EMG and ventilatory activity signals were 128 Hz, while the arterial pressure signal was sampled at 1024 Hz.

All signals were visualized on-line so that the operator could make a preliminary discrimination of the sleep stages; this operation was also facilitated by a Fast Fourier Transform (FFT) analysis performed on-line that visualised the power spectrum of the EEG signal (δ band: 0.5-4.5Hz; θ band: 6-9Hz; σ band: 12-16Hz).

4.5 Data analysis

Polygraphic signals were visualized offline by means of software written ad hoc in MATLAB language (The MathWorks, Inc., U.S.A.).

4.5.1 Visual scoring

The sleep-wake state was scored visually according to electroencephalographic criteria, muscle tonus and twitches, and behavioural criteria; the analysis was performed with a temporal resolution of 2 seconds because of the polyphasic wake-sleep cycle of the rodents, which consists of several short-lasting stages alternating rapidly.

REM sleep was identified when EEG showed a prominent hippocampal theta rhythm and EMG tone was absent except for occasional muscle twitches (see Figure 4.2).

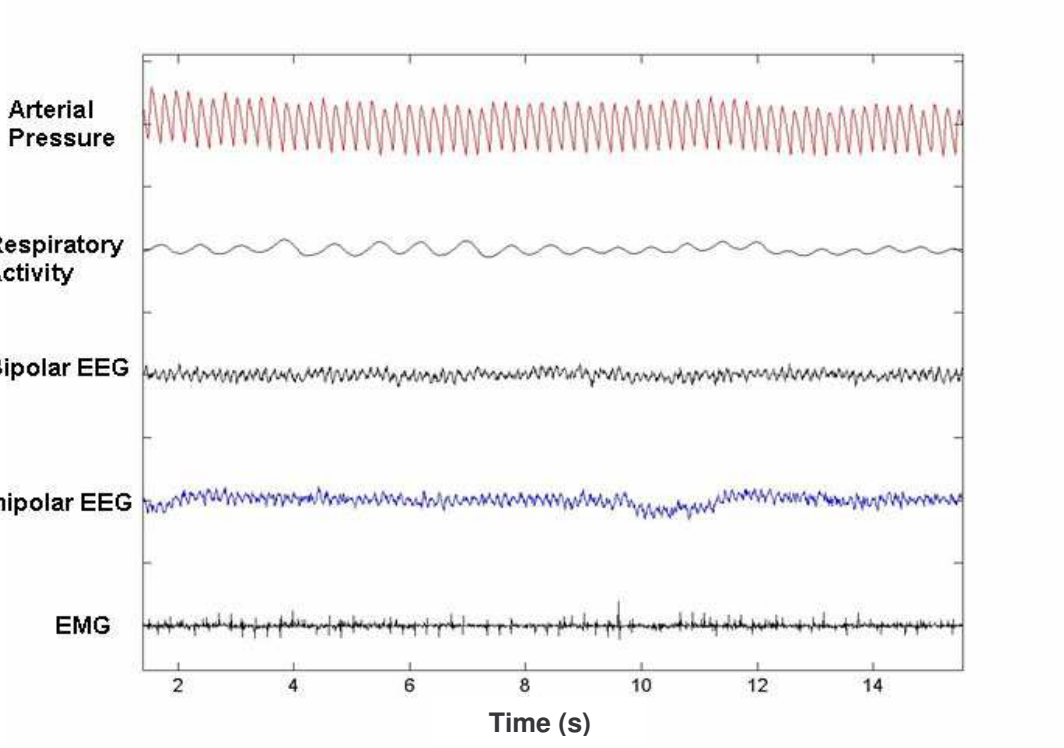

Figure 4.2. Representative image produced by the visualization software, showing 16 seconds of REM sleep.

Care was taken to exclude epochs of intermediate sleep (i.e., when EEG displayed prominent sleep spindles on a background of θ wave activity). With these criteria, REM sleep was discriminated from the states of wakefulness and NREM sleep. In fact, during wakefulness the EEG displayed a pattern of low-voltage, high-frequency activity and nuchal electromyographic tone was present. During NREM sleep, on the other hand, the EEG showed high-voltage, low-frequency activity (δ waves), with interspersed sleep

spindles and K complexes and EMG tone was present, although lower than that in wakefulness (see Figure 4.3).

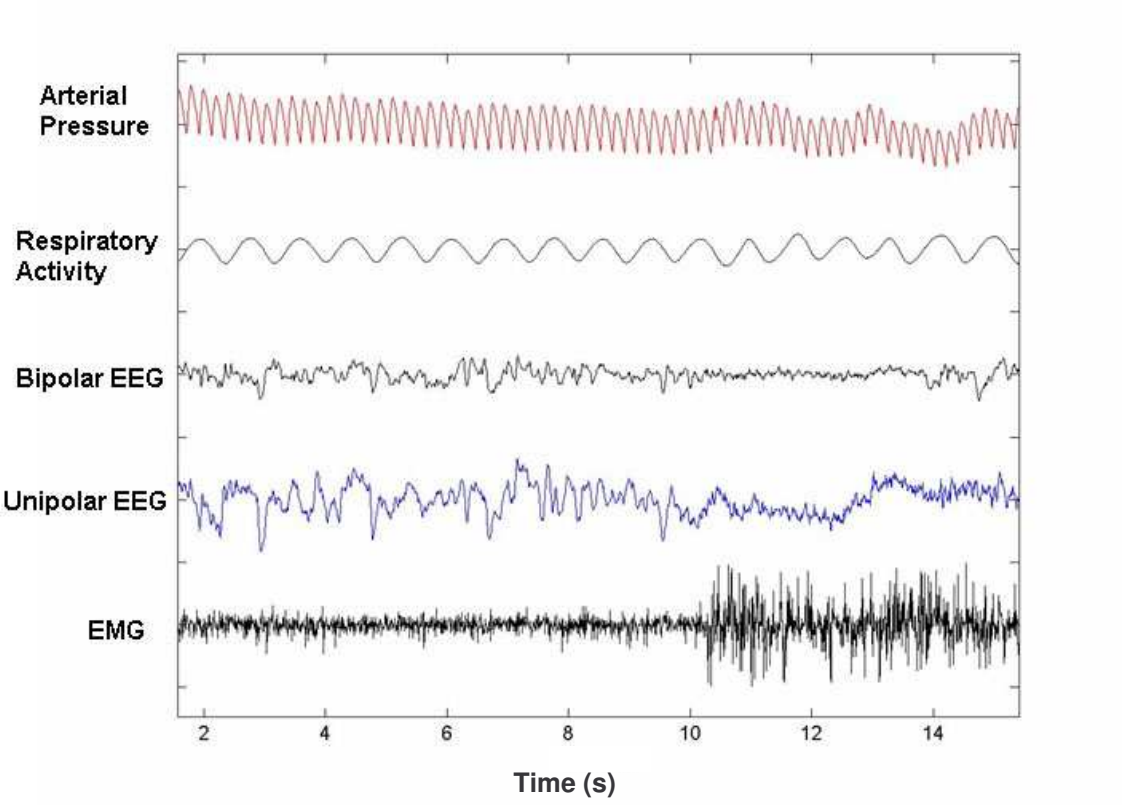

Figure 4.3. Representative image produced by the visualization software, showing 10 seconds of NREM sleep followed by an awakening.

4.5.2 Analysis of phasic hypertensive events

Data analysis was aimed at characterizing changes in MAP, HP, EEG and EMG during phasic hypertensive events in REM sleep. Analysis was performed on episodes of REM sleep of duration greater than 30 s.

Beat-to-beat time series of heart period (HP) and mean arterial pressure (MAP) were obtained from the arterial pressure signal.

HP was determined as the time interval between the onsets of successive systolic upstrokes (see Figure 4.4) and accuracy of the determination was ensured by manual editing of all the tracings. MAP was computed as the average arterial pressure during each HP. The analysis was performed on MAP because MAP is more reliable than systolic pressure in long-term pressure recordings in rats (Oosting *et al.*, 1997b).

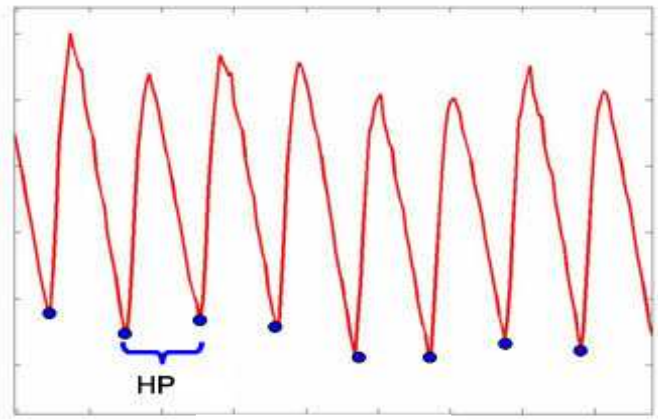

Figure 4.4. The drawing illustrates the determination of Heart Period (HP) as the time interval between the beginnings of successive systolic upstrokes (dots).

The bipolar EEG signal was analyzed to compute the frequency of the theta rhythm adapting the technique proposed by Sei and Morita (1996) (Sei & Morita, 1996). The analysis was performed on consecutive windows of 1 s duration and 0.75 s overlap. On each window, the EEG was band-pass filtered (Butterworth 1 pole filter) between 5 and 10 Hz. The theta frequency was determined as the reciprocal of the mean interval between successive points, at which the sign of the band-pass filtered EEG signal switched from negative to positive.

The EMG signal was analyzed to compute its root mean square (rms). The rms, also called the quadratic mean, is a statistical measure of the magnitude of a varying quantity. The rms is especially useful when the variable analyzed assumes both positive and negative values, as is the case for the EMG signal. Similarly to the analysis of the EEG, the analysis of the EMG was performed on consecutive windows of 1 s duration and 0.75 s overlap. On each window, the EMG values sampled at 128 Hz were squared, summed, and divided by their number (i.e., 128). The root mean square of this quantity was finally computed to determine the EMG rms in the window.

Time series of HP, MAP, EEG theta frequency and EMG rms were re-sampled at 16 Hz by linear interpolation (piecewise cubic Hermite polynomials) to yield data. Thus, all the signals were expressed with the same time axis.

The re-sampled time series of HP and MAP were low-pass filtered at 0.8 Hz (3 pole Butterworth filter), to eliminate fluctuations in cardiovascular variables around the

breathing rate, which would confound their changes associated with phasic hypertensive events.

Phasic hypertensive events in REM were automatically detected by applying quantitative criteria compatible with a definition of the phasic hypertensive events as phasic increases of arterial pressure with prominent amplitude. In particular, a phasic hypertensive event was defined as a MAP increase of at least 15 mmHg above baseline value for more than 1 s. The baseline MAP value was defined as the average MAP value in the REM sleep episode under study.

The variance of low-pass filtered time series of HP and MAP was computed within each episode of REM sleep, which comprised at least one phasic hypertensive event.

For each hypertensive event, the signals were then retained for analysis in the time interval starting 10 s before the MAP peak and ending 8 s after it. Such time interval had an extension appropriate to evidence all signal changes associated with hypertensive events, as assessed during preliminary analyses.

The signals in each time interval were then normalized to allow their comparison. The normalization procedure consisted of subtracting from each signal its mean value in the REM sleep episode under study. The EMG rms signal was further divided by its mean value in the REM sleep episode to compensate for confounding effects due to variable gain settings of the amplifier in different recordings.

After this normalization procedure, a coherent averaging of the signals during phasic hypertensive events was performed within each rat by aligning the data sequences temporally with the peak of the MAP surge. (Challis & Kitney, 1990).

At each time point in the interval comprising the phasic hypertensive events, the median values of the signals were computed within rat. Thus, for each rat, the analysis yielded four curves, describing the dynamics of HP, MAP, EEG theta frequency and EMG rms, respectively, that are associated with phasic hypertensive events. The median was chosen instead of the mean because it is more robust to extreme values in the signals.

4.6 Statistical analysis

Statistical testing was performed using standard procedure (SPSS, http://www.spss.com). Independent sample t tests were applied to test the significance ($P < 0.05$) of pre-planned comparisons between SHR and WKY rats and between SHR and SHRace. The t-test relies upon the assumption of homogeneity of variance, which was tested with the test of Levene. In case of a significant Levene test, which indicates the rejection of such assumption, the degrees of freedom used in the t-test were reduced to adjust for the increased likelihood of committing a type I error.

Data were expressed as mean within rat group ± SEM with $n = 7$ for each group.

5. Results

5.1 Characteristics of the animals under study

5.1.1 Body Weight of the animals under study

Table 5.1 shows values of body weight the day of the surgery, when rats were 9 week old, and the average weight during the recordings, one week later. Although a small weight loss occurred shortly after surgery, it is evident from the data that animals in all groups regained weight completely by the time of the recordings. No significant differences in body weight occurred either between SHR and WKY rats or between SHR and SHRace during the recordings.

	WKY	**SHR**	**SHRace**
Body weight at surgery (g)	235 ± 9	239 ± 6	242 ± 4
Body weight during the recordings (g)	243 ± 9	244 ± 5	241 ± 5

Table 5.1. Mean values of body weight at surgery and during the recordings. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM.

5.1.2 Dose of Enalapril maleate received by SHRace rats

Figure 5.1 shows the estimated daily dose of Enalapril maleate received by rats of the SHRace group from the 4th week of age to the end of the recordings. Doses were estimated based on the concentration of Enalapril in the water and daily measurements of body weight and water consumption. The mean daily dose of Enalapril received was 26.8 ± 0.2 mg/Kg and was remarkably stable across days and animals.

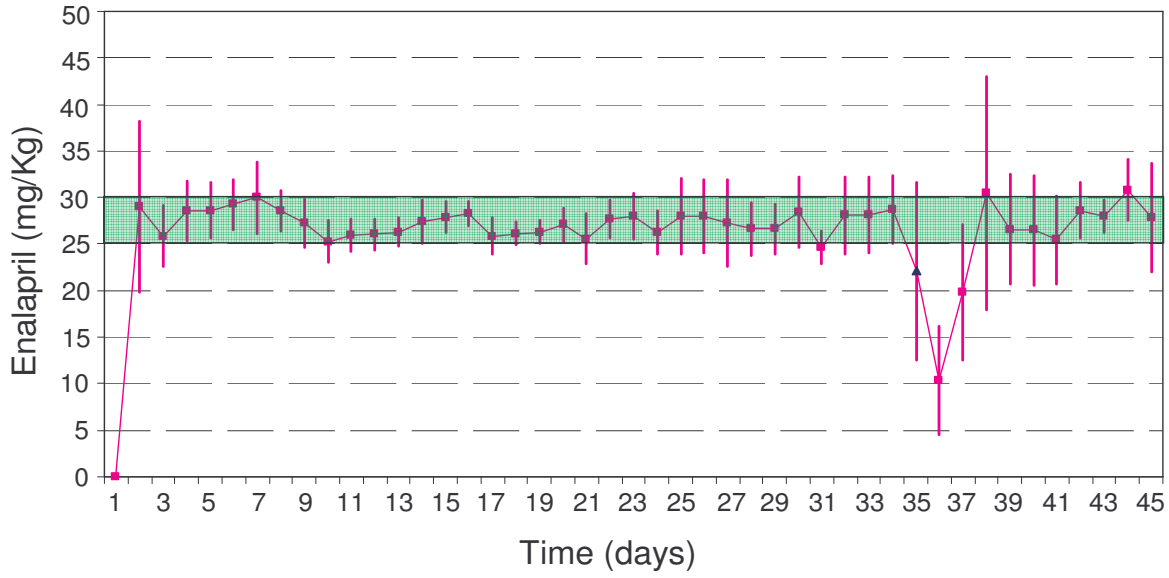

Figure 5.1. Estimated dose of Enalapril maleate received daily by SHRace rats from the 4th week of age to the end of the recordings. The green strip highlights the desired dose of the drug (25-30 mg/Kg) (Adams *et al.*, 1990). The 35th day of therapy, which is marked with a blue triangle, corresponds to the surgery. Post-operative recovery occurred between the 36th and the 40th day, while experimental sessions were scheduled between the 41st and the 45th day.

5.1.3 Arterial blood analysis

Table 5.2 shows the results of the arterial blood analysis. The arterial pH was significantly lower in SHR than in WKY rats, whereas it was similar and not significantly different between SHR and SHRace. No significant differences were observed in the concentrations of the partial pressure of oxygen and carbon dioxide, sodium ions, potassium ions, calcium ions, glucose, and lactate. Hematocrit was significantly lower in SHR than in SHRace.

	WKY	SHR	SHRace
pH	7.47 ± 0.01*	7.44 ± 0.01	7.44 ± 0.01
$PaCO_2$ (mmHg)	37 ± 2	39 ± 1	39 ± 1
PaO_2 (mmHg)	90 ± 2	92 ± 3	85 ± 2
$[Na^+]$ (mM)	142 ± 1	142 ± 1	143 ± 1
$[K^+]$ (mM)	3.4 ± 0.1	3.6 ± 0.1	3.7 ± 0.2
$[Ca^{2+}]$ (mM)	0.63 ± 0.05	0.57 ± 0.05	0.61 ± 0.09
[glu] (mM)	6.3 ± 0.3	6.3 ± 0.1	6.3 ± 0.1
[lat] (mM)	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Hct (%)	40 ± 1	41 ± 1	44 ± 1*

Table 5.2. Arterial blood analysis: pH, $PaCO_2$ and PaO_2 are arterial partial pressures of carbon dioxide and oxygen, respectively; [Na+], [K+], [Ca++], [glu], and [lat] are concentrations of sodium ions, potassium ions, calcium ions, glucose, and lactate, respectively; Hct, hematocrit. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

5.2 Phasic hypertensive events in REM sleep

A total of 1191 phasic hypertensive events were detected during REM sleep in the three groups of animals studied. In particular, 471 events were detected in SHR, 433 in WKY rats, and 287 in SHRace.

5.2.1 Mean values and variance of HP and MAP

The mean values of HP in episodes of REM sleep with phasic hypertensive events are reported in Figure 5.2. HP was significantly lower in SHR than in SHRace, while it did not differ significantly between SHR and WKY rats.

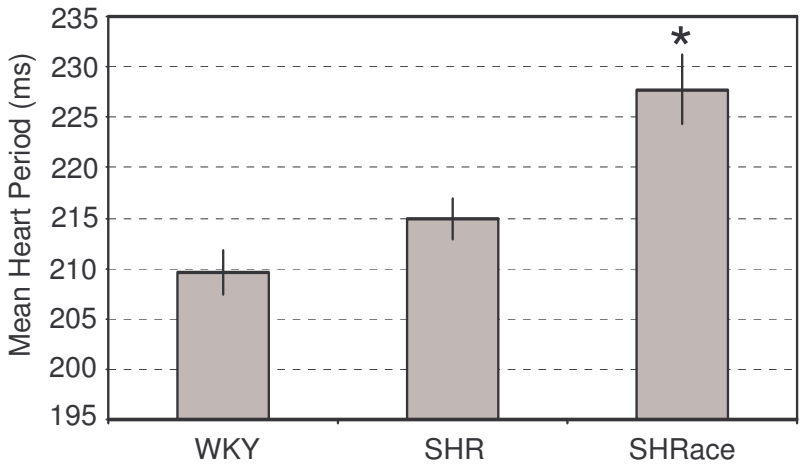

Figure 5.2. Mean values of heart period in episodes of rapid-eye-movement sleep with phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

The mean values of MAP in episodes of REM sleep with phasic hypertensive events are reported in Figure 5.3. MAP was significantly higher in SHR than either in WKY rats or SHRace.

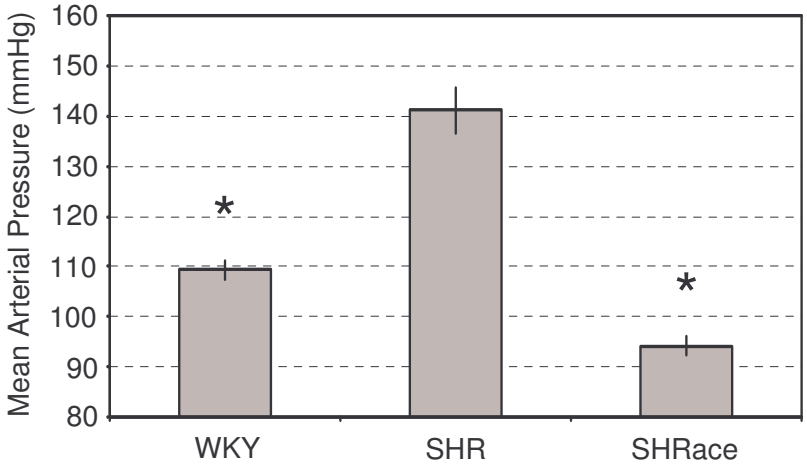

Figure 5.3. Mean values of mean arterial pressure in episodes of rapid-eye-movement sleep with phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7);

SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, P < 0.05 vs. SHR.

The variances of low-frequency (< 0.8 Hz) fluctuations of HP in the same episodes are reported in Figure 5.4. The variance of HP was significantly lower in SHR than in SHRace, whereas it did not differ significantly between SHR and WKY rats.

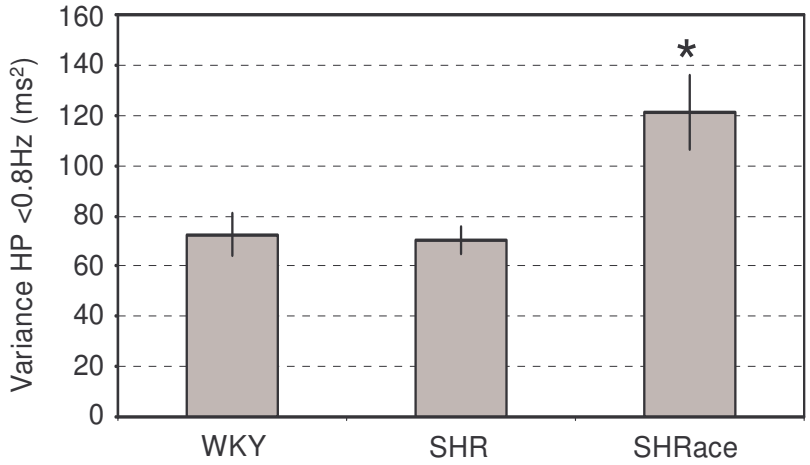

Figure 5.4. Variance of low frequency (<0.8 Hz) fluctuations of heart period (HP) in episodes of rapid-eye-movement sleep with phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, P < 0.05 vs. SHR.

The variances of low-frequency (< 0.8 Hz) fluctuations of MAP in the same episodes are reported in Figure 5.5. The variance of MAP was significantly higher in SHR than either in WKY rats or SHRace.

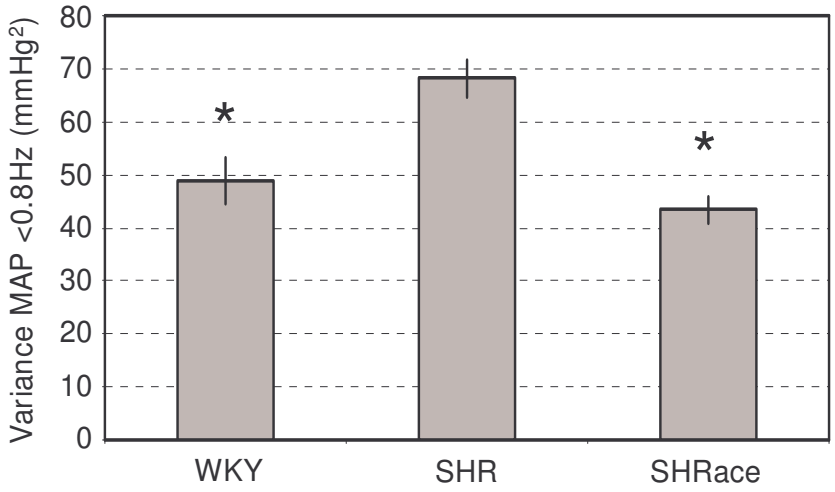

Figure 5.5. Variance of low frequency (<0.8 Hz) fluctuations of mean arterial pressure (MAP) in episodes of rapid-eye-movement sleep with phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

5.2.2 Mean values of THF and EMG rms

The mean values of THF in episodes of REM sleep with phasic hypertensive events are reported in Figure 5.6. THF was significantly higher in SHR than in WKY rats, while it did not differ significantly between SHR and SHRace.

The mean values of EMG rms were $3.95 \cdot 10^{-2} \pm 5.7 \cdot 10^{-3}$, $3.93 \cdot 10^{-2} \pm 4.9 \cdot 10^{-3}$, and $4.69 \cdot 10^{-2} \pm 6.8 \cdot 10^{-3}$ in WKY rats, SHR, and SHRace, respectively. The mean values of EMG rms did not differ significantly between SHR and either WKY rats or SHRace.

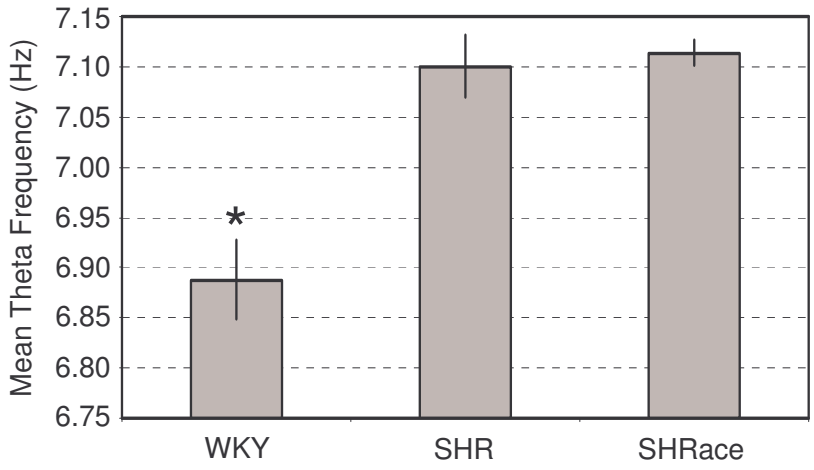

Figure 5.6. Mean values of theta frequency in episodes of rapid-eye-movement sleep with phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

5.2.3 Dynamics of HP, MAP, THF and EMG rms associated with phasic hypertensive events

Representative examples of phasic hypertensive events in WKY rats, SHR, and SHRace are reported in Figures 5.7, 5.8, and 5.9, respectively. In the three examples provided, phasic hypertensive events were associated with tachycardia, which was evident from the arterial pressure signal, an acceleration of the theta rhythm and a phasic increase in EMG activity.

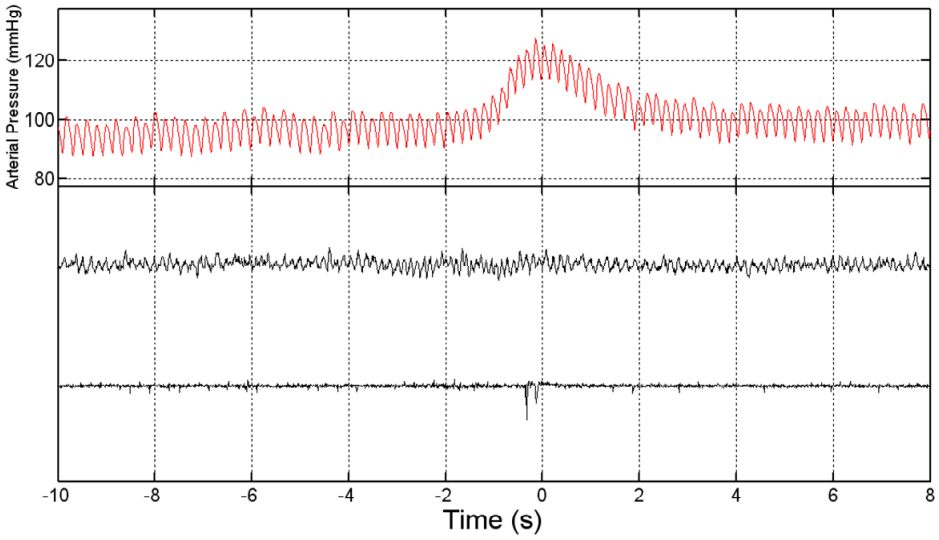

Figure 5.7. Representative example of a phasic hypertensive event during rapid-eye-movement sleep in a Wistar Kyoto rat. From the top: arterial pressure (mmHg, red line); bipolar electroencephalogram (black line); electromyogram (black line). Time 0 corresponds to the peak of the phasic hypertensive event.

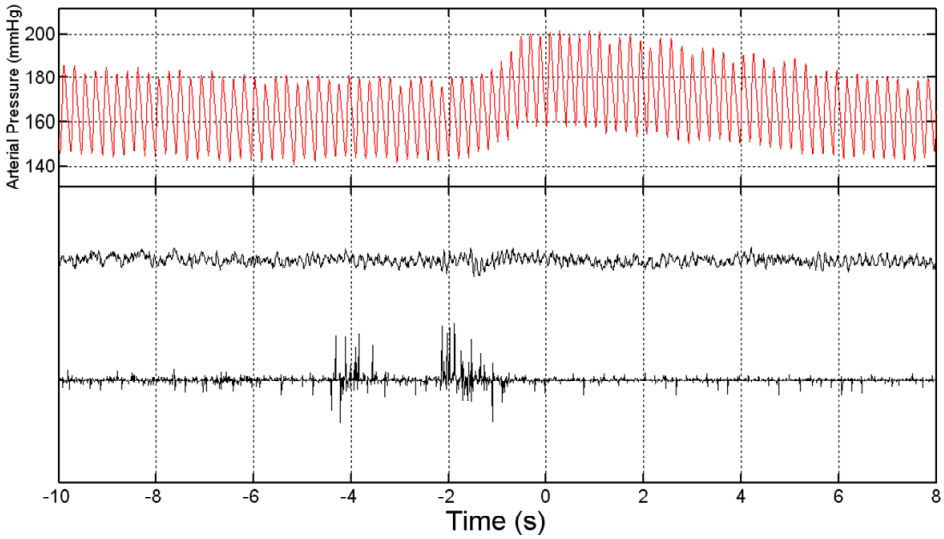

Figure 5.8. Representative example of a phasic hypertensive event during rapid-eye-movement sleep in a Spontaneously Hypertensive rat. From the top: arterial pressure (mmHg, red line); bipolar electroencephalogram (black line); electromyogram (black line). Time 0 corresponds to the peak of the phasic hypertensive event.

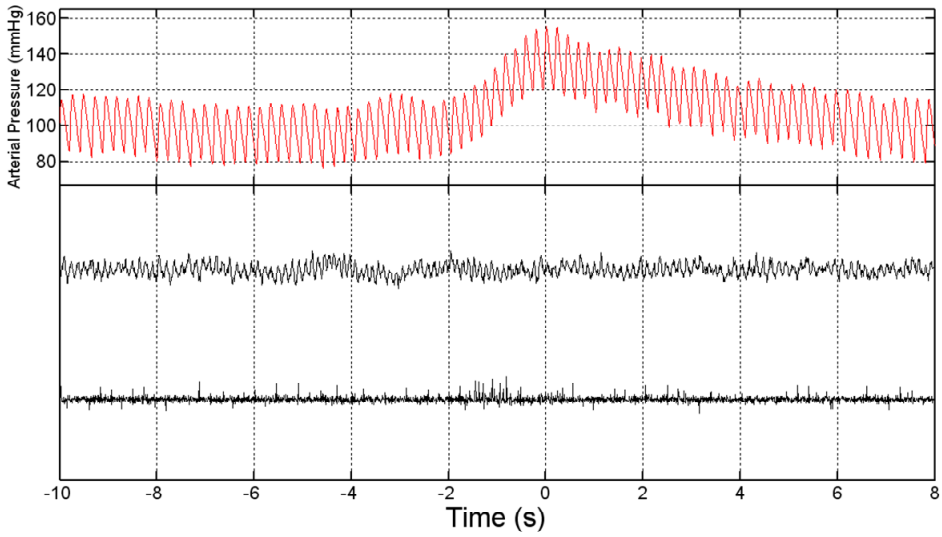

Figure 5.9. Representative example of a phasic hypertensive event during rapid-eye-movement sleep in a Spontaneously Hypertensive rat treated with Enalapril. From the top: arterial pressure (mmHg, red line); bipolar electroencephalogram (black line); electromyogram (black line). Time 0 corresponds to the peak of the phasic hypertensive event.

These associations were highly reproducible, as they were fully confirmed by the quantitative analysis performed with coherent averaging (see Methods). The results of this analysis are shown in Figures 5.10, 5.11, 5.12 and 5.13.

The coherent averaging procedure revealed that HP decreased for the whole duration of the phasic hypertensive events (Figure 5.10). The inspection of the curves described by the signals during phasic hypertensive events revealed in all groups a negative peak of the HP signal (Figure 5.10) and positive peaks of THF (Figure 5.12) and EMG rms signals (Figure 5.13), all peaks occurring from 4 s before the MAP peak to 2 s after it (Figure 5.11).

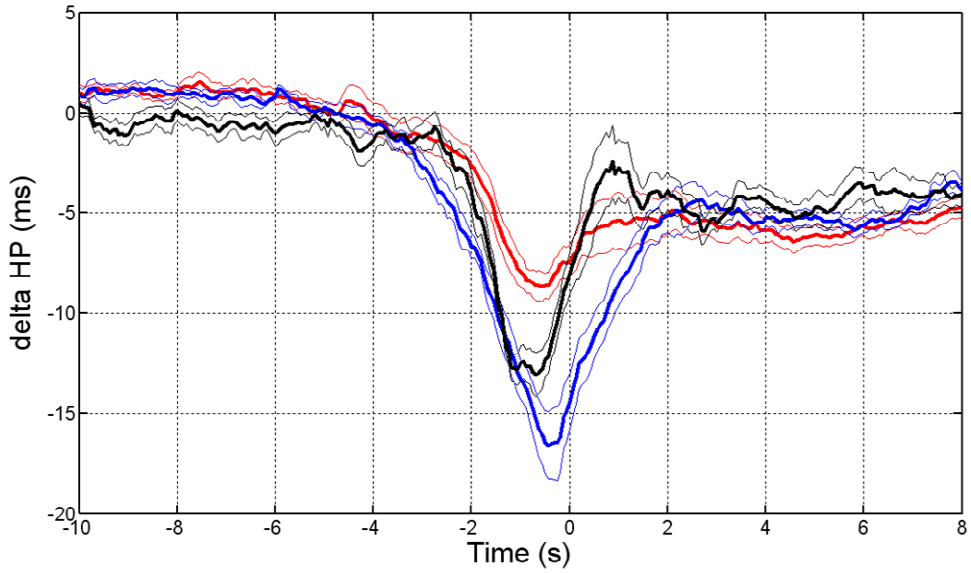

Figure 5.10. Result of the coherent averaging performed on heart period during phasic hypertensive events in rapid-eye-movement sleep. Data are mean ± SEM in Wistar Kyoto rats (blue, n = 7), Spontaneously Hypertensive Rats (red, n = 7), and Spontaneously Hypertensive Rats treated with Enalapril (black, n = 7). Delta HP, difference between heart period and its baseline value. Time 0 corresponds to the peak of the phasic hypertensive event.

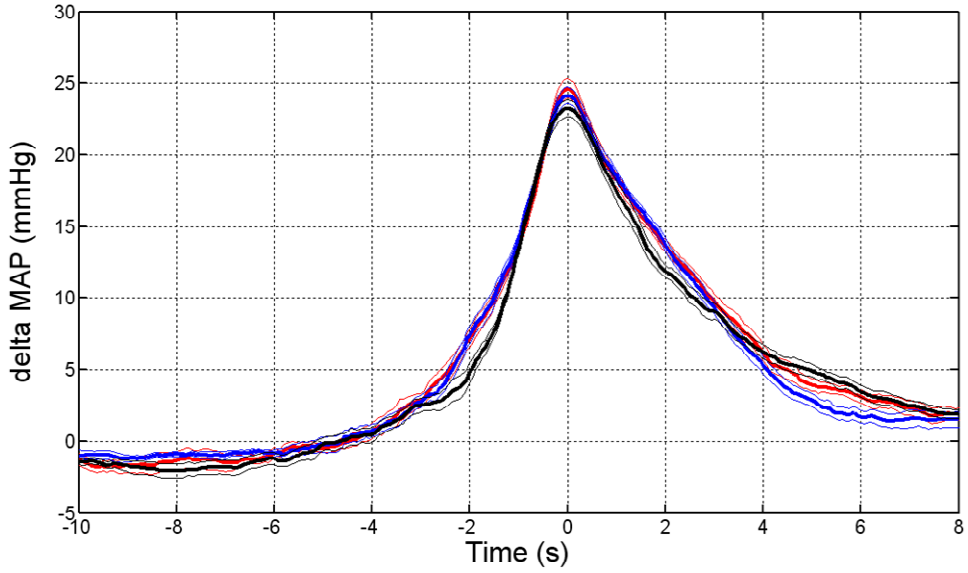

Figure 5.11. Result of the coherent averaging performed on mean arterial pressure during phasic hypertensive events in rapid-eye-movement sleep. Data are mean ± SEM in Wistar Kyoto rats (blue, n = 7), Spontaneously Hypertensive Rats (red, n = 7), and Spontaneously Hypertensive Rats treated with Enalapril (black, n = 7). Delta MAP, difference between mean arterial pressure and its baseline value. Time 0 corresponds to the peak of the phasic hypertensive event.

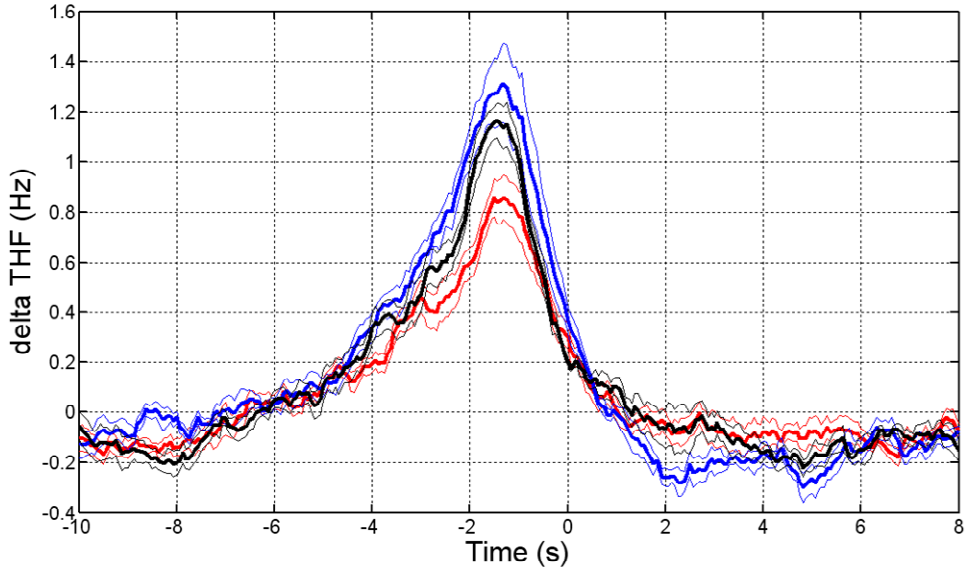

Figure 5.12. Result of the coherent averaging performed on the frequency of the electroencephalographic theta rhythm during phasic hypertensive events in rapid-eye-movement sleep. Data are mean ± SEM in Wistar Kyoto rats (blue, n = 7), Spontaneously Hypertensive Rats (red, n = 7), and Spontaneously Hypertensive Rats treated with Enalapril (black, n = 7). Delta THF, difference in the frequency of the theta rhythm and its baseline value. Time 0 corresponds to the peak of the phasic hypertensive event.

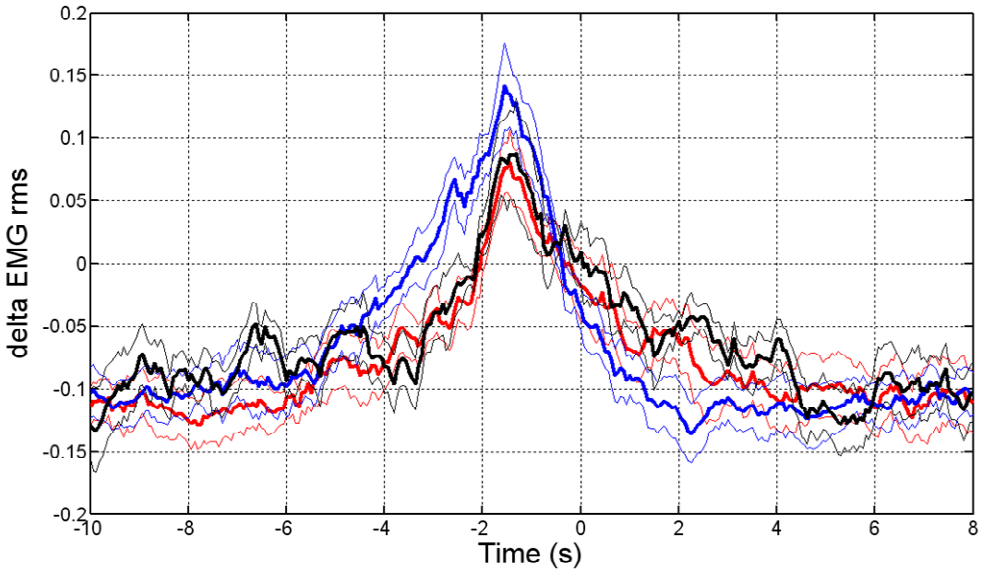

Figure 5.13. Result of the coherent averaging performed on the electromyographic activity during phasic hypertensive events in rapid-eye-movement sleep. Data are mean ± SEM in Wistar Kyoto rats (blue, n = 7), Spontaneously Hypertensive Rats (red, n = 7), and Spontaneously Hypertensive Rats treated with Enalapril (black, n = 7). Delta EMG rms, difference between the root mean square of electromyographic activity and its baseline value. Time 0 corresponds to the peak of the phasic hypertensive event.

Thus, for each rat, subsequent analysis was focused on: a) the positive peak value of MAP; b) the negative peak value of HP; c) the positive peak value of THF; and d) the positive peak value of the EMG rms.

The values of MAP at the peak of the phasic hypertensive events were 24.17 ± 0.58, 24.63 ± 0.69, 23.28 ± 0.57 in WKY rats, SHR and SHRace, respectively. The values of MAP at the peak did not differ significantly between SHR and either WKY rats or SHRace.

The magnitude of the negative peak value of HP was significantly lower in SHR than either in WKY rats or SHRace (Figure 5.14).

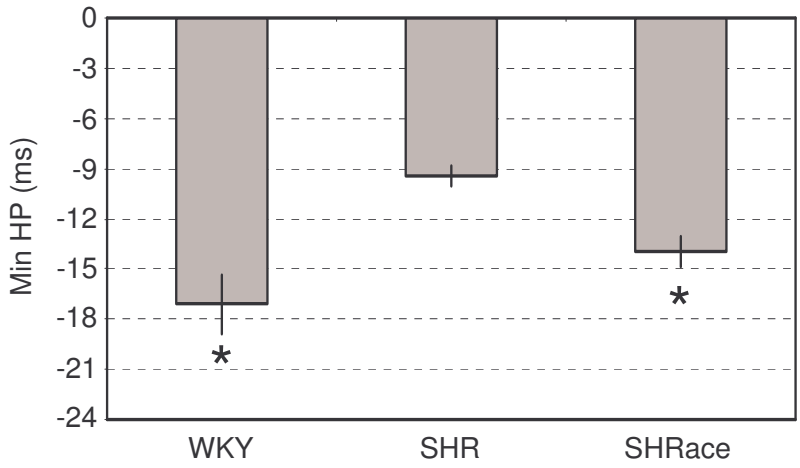

Figure 5.14. Mean values of heart period at the negative peak of phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

The time shift between the negative peak of HP and the MAP peak is reported in Table 5.3 and did not differ significantly between rat strains.

	WKY	SHR	SHRace
Time at min HP (s)	-0.42 ± 0.07	-0.25 ± 0.31	-0.82 ± 0.13
Time at max THF (s)	-1.34 ± 0.11	-1.27 ± 0.11	-1.46 ± 0.08
Time at max EMG rms (s)	-1.46 ± 0.08	-1.36 ± 0.13	-1.20 ± 0.29

Table 5.3. Time shifts between the peak of mean arterial pressure and the negative peak of heart period (min HP), the positive peak of theta frequency (max THF), and the positive peak of electromyographic activity (max EMG rms). WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM.

The positive peak value of THF was significantly lower in SHR than either in WKY rats or SHRace (Figure 5.15). The time shift between the THF peak and the MAP peak is reported in Table 5.3 and did not differ significantly between rat strains.

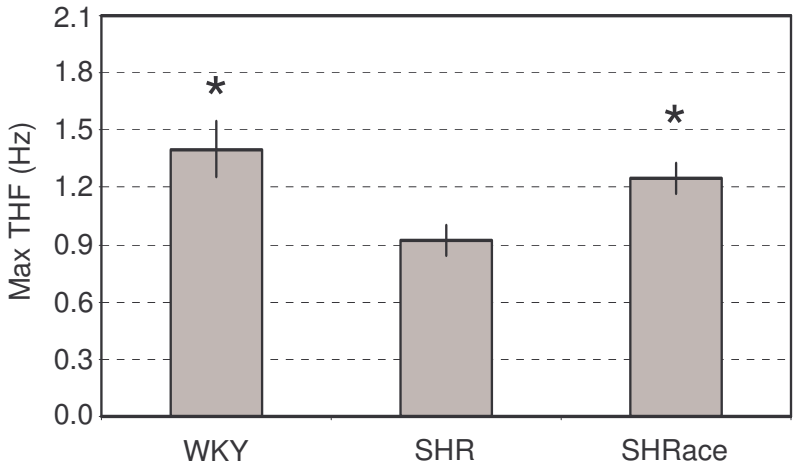

Figure 5.15. Mean values of theta frequency (THF) at the peak of phasic hypertensive events. WKY, Wistar Kyoto rats (n=7); SHR, Spontaneously Hypertensive Rats (n=7); SHRace, Spontaneously Hypertensive Rats treated with Enalapril (n=7). Values are mean ± SEM. *, $P < 0.05$ vs. SHR.

The values of EMG rms at the peak of the phasic hypertensive events were 0.16 ± 0.03, 0.10 ± 0.02, 0.13 ± 0.03 in WKY rats, SHR and SHRace, respectively. The values of EMG rms at the peak did not differ significantly between SHR and either WKY rats or SHRace.

Similarly, no significant differences were observed between groups in the time shift between the peak of EMG rms and the MAP peak Table 5.3.

6. Discussion

The aim of this study was to investigate whether SHR, which represent the most widely used model of essential hypertension, show an altered central autonomic control during phasic hypertensive events in REM sleep. The results of the present study indicate that the contribution of central autonomic commands to cardiovascular variability is reduced in SHR during REM sleep.

6.1 Spontaneously Hypertensive Rats

Experiments were performed on male SHR at the 10th week of age. The SHR strain is the most used animal model for research on polygenic hypertension and allows full control of genetic and environmental confounding factors. SHR were developed by Okamoto and colleagues in the early 1960 (Okamoto & Aoki, 1963) as a substrain of the WKY genetically-normotensive strain.

SHR spontaneously develop hypertension without the need of any dietary or surgical manipulation (Okamoto & Aoki, 1963). Hypertension develops typically by the third week and is maintained throughout adulthood in 100% of the population (Yamori *et al.*, 1974). In particular, blood pressure of WKY rats reaches adult levels by the 10th week of age, but in SHR it continues to rise at least until the age of 20 weeks (Zicha & Kunes, 1999) (Figure 3.4). SHR develop hypertensive complications similar to those in human patients, such as cerebral infarction or hemorrhage, myocardial infarction and nephrosclerosis (Yamori *et al.*, 1974). The average life span of SHR is approximately 18 months. In comparison, Wistar Kyoto rats typically live for at least 30–36 months. In the present study SHR at 10 weeks of age were considered, when full-blown hypertension is present, while confounding effects due to the long-term organ damage associated with the disease were avoided.

6.2 Experimental control groups

The SHR strain was developed by selective breeding of WKY animals that presented hypertension due to spontaneous random mutation(s) (Okamoto & Aoki, 1963). Thus, hypertension was the only phenotype used to control the breeding protocols, making it possible that other gene mutations were also captured by this breeding process. To summarize, SHR and WKY rats allow the reduction of genetic confounding factors within group, because both strains are fully inbred. However, genetic confounders possibly unrelated to arterial hypertension do exist between SHR and WKY rats (Carley *et al.*, 2000).

For the reasons mentioned above, the results obtained in SHR with those in two control groups were compared. One group consisted of 7 WKY rats, with age and sex matched to those of SHR. The other group (SHRace) consisted of 7 SHR, in which hypertension was prevented by continuous treatment with Enalapril maleate since the fourth week of age, as proposed by Adams et al. (1990). Enalapril is an inhibitor of the angiotensin converting enzyme (ACE). Angiotensin II plays a key role in the pathogenesis of hypertension in SHR, even though SHR have normal plasma renin activity (Bolterman *et al.*, 2005).

The treatment was well-tolerated by the rats, as shown by their normal body weight during the recordings and by the results of the blood gas analysis. During the course of the treatment, by adjusting the concentration of Enalapril in drinking water to the daily changes in body weight and water intake of the rats, a stable dose of Enalapril of 25-30 mg/Kg was administered daily (Figure 5.1). This dose was effective in preventing hypertension in the SHRace group.

To summarize, I compared SHR with WKY rats and SHRace. SHR are genetically and phenotypically hypertensive. WKY rats are genetically and phenotypically normotensive. SHRace are genetically hypertensive but phenotypically normotensive. Owing to this experimental design, I could attempt to dissect the contributions of hypertension and genetic determinants to the aspect of the cardiovascular phenotype under study, i.e., central autonomic control of the cardiovascular system.

6.3 Temporal pattern of the changes in cardiovascular variables during the phasic hypertensive events

There is ample experimental evidence that central autonomic commands underlie the phasic hypertensive events in REM sleep. Phasic hypertensive events occur in REM sleep in experimental animals: cat (Mancia *et al.*, 1971); rat (Sei *et al.*, 1999); mouse (Campen *et al.*, 2002); lamb (Fewell, 1993; Silvani *et al.*, 2005); and human subjects as well (Coccagna *et al.*, 1971). The pressure surges are driven by increases in peripheral vascular resistance (Fewell, 1993), particularly in the vascular bed of skeletal muscles (Mancia *et al.*, 1971). Such increase in muscle vascular resistance is due to central autonomic commands, as shown by its disappearance following sympathectomy but not following limb deafferentation (Baccelli *et al.*, 1974).

In all groups analyzed, the variables under study showed a remarkable variability in their pattern among different phasic hypertensive events. This remarkable variability of the vegetative phenomena is a well-known feature of REM sleep (Parmeggiani, 1980). However, owing to a quantitative analysis (coherent averaging) and to the high number of phasic hypertensive events analyzed, I could demonstrate that the variability conceals specific patterns of changes in different variables associated with phasic hypertensive events. In all groups, coherent averaging revealed a negative peak of the HP signal (Figure 5.10) and positive peaks of THF (Figure 5.12) and EMG rms signals (Figure 5.13), all peaks occurring from 4 s before the MAP peak to 2 s after it (Figure 5.11).

The coherent averaging procedure revealed that HP decreased for the whole duration of the phasic hypertensive events (Figure 5.10), this pattern being clearly evident in all groups studied. This finding agrees with previous results obtained in normotensive outbred rats of the Sprague-Dawley strain (Zoccoli *et al.*, 2001; Silvani *et al.*, 2003), indicating central autonomic commands prevail on the baroreflex at the cardiac level during the MAP surges in REM sleep. In fact, the baroreflex would be expected to cause bradycardia in response to the increase in MAP, a pattern opposite to the one observed. The coupling between hypertension and tachycardia is similar to the pattern observed during wakefulness in the course of physical exercise and defense reaction. In these conditions, central autonomic commands prevail temporally over negative feedback controls such as the baroreflex.

In rats, the only previous study on MAP surges reported that heart rate increased modestly and non significantly during the MAP surges, while it increased significantly after them (Sei & Morita, 1996). Methodological differences between the work of Sei and co-workers and my own work may partly underlie the differences in the reported results. Indeed, Sei chose bursts of rapid eye movements to synchronize cardiovascular variables in the coherent averaging procedure, with a resolution of only 1 s, whereas in the present work, data were synchronized at the peak of the MAP surge with a much higher resolution. In fact, data were recorded beat-to-beat and subsequently resampled at a rate of 16 Hz.

The prevalence of central autonomic commands at the cardiac level during the MAP surges, which is observed in rats, may be species-specific. In newborn lambs (Silvani *et al.*, 2005), heart period showed a biphasic fluctuation during MAP surges in REM sleep: HP decreased concomitantly with the onset of the phasic increases in MAP, whereas it increased over baseline later in the course of the surges. Thus, central autonomic commands prevail over the baroreflex on the heart as well as on blood vessels at surge onset both in newborn lambs and in adult rats. However, in lambs, but not in rats, the baroreflex effect on the heart prevails late in the course of the surges, in spite of enduring central control on blood vessel. This different pattern of cardiac response to arterial pressure surges may be due to differences between species or to differences in developmental age.

As mentioned above, the coherent averaging procedure evidenced distinct associations between the MAP surge and changes in THF and EMG rms. The association of cardiovascular, EEG, and EMG phenomena during phasic hypertensive events in REM sleep has been previously reported on a quantitative basis by Sei et al. (Sei & Morita, 1996). The results of the present study are in excellent agreement with their data in terms of the magnitude of the increase in THF preceding the MAP surge. In addition, the present study evidences quantitatively an association of the MAP surges with an increase in the EMG rms, reflecting muscle twitches on a background of muscle atonia. Taken together, these results fully support the view that phasic hypertensive events in REM sleep are produced by central autonomic commands. These commands manifest at the EEG level with an increase in theta frequency, at the muscle level with brief muscle twitches, at the cardiac level with tachycardia, and at the circulatory level with arterial hypertension.

6.4 Peak changes of cardiovascular, EEG, and EMG variables during the phasic hypertensive events

The value of MAP at the peak of the phasic hypertensive events was similar between groups and did not differ significantly between SHR and either WKY rats or SHRace (see Results). This is an interesting observation, because it is apparently in disagreement with the notion that vascular reactivity to central autonomic control is enhanced in SHR. This notion was introduced by means of an elegant experimental work by Kuo et al. (Kuo & Yang, 2000). These authors performed a broad-band electrical stimulation of the rostral ventrolateral medulla in anesthetized SHR and recorded the changes in arterial pressure that were produced as a result of the stimulation. The evoked variability of arterial pressure and the magnitude of the transfer function between the spike rate variability of the stimulus and the arterial pressure variability were higher in SHR than in WKY rats, these differences being abolished by combined α and β adrenoreceptor blockade. The increased reactivity of the cardiovascular system to sympathetic commands may underlie the increased variance of arterial pressure observed in SHR with respect to WKY rats (Figure 5.5). Given the increased reactivity of arterial pressure to rostral ventrolateral medulla in SHR, the peak MAP increase during the phasic hypertensive events would be expected to be higher in SHR than in WKY rats. However, this difference was actually negligible and not statistically significant. Thus, the analysis of the peak MAP value suggests that central autonomic control of peripheral resistance is reduced in SHR with respect to WKY rats, this reduction being compensated by a greater vascular reactivity. Similarly, the difference in the peak MAP value was modest and not statistically significant also between SHR and SHRace, although the MAP variance during the whole REM sleep episodes was significantly and substantially higher in SHR than in SHRace.

The hypothesis that in REM sleep, SHR show a reduction in the central autonomic control of the cardiovascular system is further supported by the analysis of the increase in THF. The positive peak value of THF was significantly lower in SHR than either in WKY rats or SHRace (Figure 5.15). The time shift between the THF peak and the MAP peak is reported in Table 5.3 and did not differ significantly between rat strains. The increase in THF is a more direct correlate of central autonomic commands in REMS

than the cardiovascular changes, because its magnitude does not depend on the reactivity of the cardiovascular effectors.

It must be noted that the peak increase in EMG rms did not significantly differ between SHR and either WKY rats or SHRace, although it tended to be lower in SHR. The control of muscle activity during REM sleep is complex, with bursts of activation and inhibition impinging on motorneurons (Chase & Morales, 2005), possibly explaining the disagreement between the data on THF and on EMG rms.

Finally, the analysis of changes in HP associated with phasic hypertensive events fully supports the hypothesis that central autonomic control of the cardiovascular system is reduced in SHR during REM sleep. The magnitude of the negative peak value of HP was significantly lower in SHR than either in WKY rats or SHRace. The time shift between the negative peak of HP and the MAP peak is reported in Table 5.3 and did not differ significantly between rat strains. As discussed above, a tachycardia in the presence of an increase in MAP cannot be ascribed to the baroreflex, but rather reflects central autonomic commands.

To summarize, the present study indicates that the peak MAP increase during the phasic hypertensive events in REM sleep did not differ significantly between SHR and WKY rats, while on the other hand SHR showed a reduced increase in THF and a reduced decrease in HP with respect to WKY rats. The same pattern of changes in MAP, HP, and THF was observed between SHR and SHRace. This is an important observation, because SHR do not differ from WKY rats only in terms of arterial hypertension, but also due to multiple unknown genetic differences. SHR were developed by selective breeding of WKY rats based only on the level of arterial pressure. However, in this process, multiple genes possibly unrelated to hypertension may have been selected together with the genetic determinants of hypertension. The fact that this represents more than a theoretical possibility has been convincingly demonstrated by Carley et al. (Carley *et al.*, 2000). These authors showed persistent sleep-related breathing disorders in SHR despite effective cardiovascular normalization due to long-term captopril treatment. Similarly, this study indicated that SHR differ from WKY rats, but not from SHRace, in terms of arterial pH and THF. The arterial pH is more acidic in SHR than in WKY rats despite similar levels of arterial pCO_2 (Table 5.2). This difference may be due to a reduced renal tubular reuptake of bicarbonate ions in SHR (Lucas *et al.*, 1988). Since no difference was observed between SHR and SHRace, this feature may be due to genetic determinants unrelated to hypertension. For what concerns THF, it is interesting

to note that lines of rats that differ in their voluntary alcohol drinking behaviour differ also in terms of the peak frequency of hippocampal theta activity during REM sleep (Morzorati *et al.*, 1994). This suggests that the THF is strongly under genetic control in rats. In sharp contrast, the persistence of differences in the peak HP decrease and the peak THF increase during MAP surges between SHR and SHRace demonstrates that the observed reduction in central autonomic control of the cardiovascular system in SHR is not an irreversible consequence of inherited genetic determinants in SHR. Rather, the comparison between SHR and SHRace indicates that the observed differences in central autonomic control are the result of the hypertension per se.

The present results support the hypothesis that surges, during REM sleep, may constitute a diagnostic stress test capable of disclosing pathological differences in cardiovascular regulation (Verrier *et al.*, 1996). This distinctiveness of REM sleep may be due to the fact that this state represents a stable behavioral condition, devoid of any external, voluntary or motivational influence. It must be noted that behavioral trait differences between SHR and WKY rats contribute to the increased cardiovascular responsiveness to environmental stress in SHR (Knardahl & Hendley, 1990). One phenomenon that is unique to REM sleep is the total loss of activity in the antigravitary musculature (Jouvet, 1967) and accordingly the stability in terms of metabolic demands. On the other hand, in spite of such stability, REM sleep involves prominent fluctuations of physiological variables without any evident adaptive function, but determined only by the sleep process.

In conclusion, I analyzed the cardiovascular, EEG, and EMG changes associated with phasic hypertensive events during REM sleep in the SHR model of essential hypertension. SHR were compared not only with their genetic WKY control strain, but also with a group of SHR made phenotypically normotensive by means of a chronic treatment with an ACE inhibitor. The results of this study indicate that central autonomic control of the cardiovascular system is reduced in SHR during REM sleep, and provide evidence that this reduction is due to hypertension per se rather than to genetic factors. This work supports the view that the study of cardiovascular regulation in sleep provides fundamental insight on the pathophysiology of hypertension. Systemic hypertension is associated with sleep-related autonomic dysregulation (Kuo *et al.*, 2004a) and breathing disorders (Carley *et al.*, 2000) as well as with alterations in the sleep pattern (Kuo *et al.*, 2004b). The study of the interaction between sleep and

hypertension may thus contribute to the understanding of this disease, which is a major health problem in European countries (Wolf-Maier *et al.*, 2003) with its burden of cardiac, vascular, and renal complications.

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