Neuronal circuits involved in osmotic challenges

Marina Conceição dos Santos Moreira[1], Lara Marques Naves[1], Stefanne Madalena Marques[1], Elaine Fernanda Silva[1], Ana Cristina Silva Rebelo[1], Eduardo Colombari[2], Gustavo Rodrigues Pedrino[1].

[1]Center for Neuroscience and Cardiovascular Research, Universidade Federal de Goiás, Goiânia - GO – Brazil.

[2]Department of Physiology and Pathology, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara - SP – Brazil.

Running title: CNS and osmotic challenges

Corresponding author: Gustavo Rodrigues Pedrino

Department of Physiological Science

Federal University of Goiás

Estrada do Campus, s/n. Zip code: 74001-970. PO: 131. Goiânia – GO - Brazil

Phone/Fax: +55-62-3521-1774 e-mail: pedrino@pq.cnpq.br / gpedrino@gmail.com

Key words: Body fluid homeostase, osmossensory areas, acute hyperosmotic challenge, chronic hyperosmotic challenge.

Summary

The maintenance of plasma sodium concentration within a narrow limit is crucial to life. When it differs from normal physiological patterns, several mechanisms are activated in order to restore body fluid homeostasis. Such mechanisms may be vegetative and/or behavioral, and several regions of the central nervous system (CNS) are involved in their triggering. Some of these are responsible for sensory pathways that perceive a disturbance of the body fluid homeostasis and transmit information to other regions. These regions, in turn, initiate adequate adjustments in order to restore homeostasis. The main cardiovascular and autonomic responses to a change in plasma sodium concentration are: i) changes in arterial blood pressure and heart rate; ii) changes in sympathetic activity to the renal system in order to ensure adequate renal sodium excretion/absorption, and iii) the secretion of compounds involved in sodium ion homeostasis (ANP, Ang-II, and ADH, for example). Due to their cardiovascular effects, hypertonic saline solutions have been used to promote resuscitation in hemorrhagic patients, thereby increasing survival rates following trauma. In the present review, we expose and discuss the role of several CNS regions involved in body fluid homeostasis and the effects of acute and chronic hyperosmotic challenges.

Introduction

The maintenance of body homeostasis is crucial to life and is directly related to hydromineral balance. The sodium ion (Na^+) is, among all the inorganic ions of the organism, the one most often consumed (through dietary salt, NaCl), and is also the most important determinant of extracellular osmolarity. Variations in sodium plasma concentration cause osmotic flow between the intracellular and extracellular compartments, which can affect all perfused tissues and alter volume, metabolism, and cellular functioning. Left uncontrolled, these osmotic changes can harm the organism and contribute to the development of cardiovascular diseases and their related complications. The central nervous system (CNS) detects variations in the volume, tonicity, and composition of the extracellular compartment through peripheral and central receptors. Once detected, changes in the plasma sodium concentration will trigger a set of responses to re-establish normal physiological conditions. Studies have demonstrated that minimal elevations in plasma osmolarity and reductions in circulating volume (dehydration) are strong stimuli in the development of thirst behavior (Antunes-Rodrigues et al. 2004; Fitzsimons 1998). Regarding vegetative adjustments, hypernatremia triggers responses that modulate the renal excretion of water and sodium. Among these responses, we highlight changes in sympathetic nerve activity (Chen and Toney, 2003; Pedrino et al., 2008; Shi, Stocker et al., 2007; Stocker et al., 2013); renal vasodilation (Morita and Vatner 1985; Pedrino et al., 2005; Pedrino et al., 2006; Pedrino et al., 2009); renin, atrial natriuretic peptide (ANP), and oxytocin secretion (Antunes-Rodrigues et al. 1991, 1992; Godino et al., 2005; Haanwinckel et al., 1995; Huang et al. 2000; Huang et al., 1995; Morris and Alexander, 1988; Rauch et al., 1990); and natriuresis and diuresis (Bealer 1983; Bealer et al. 1983; Bie 1980; Bourque et al., 1994; Schoorlemmer et al., 2000; da

Silva et al. 2013).

Central circuitry involved in hyperosmotic activation

Central osmoreceptors localized in the forebrain lamina terminalis, the organum vasculosum of the lamina terminalis (OVLT), and the subfornical organ (SFO) have been described as participating in the autonomic adjustments activated by acute hyperosmotic challenges (Johnson et al., 1996; Shi et al., 2007). OVLT and SFO neurons are known as circumventricular organs (CVOs) because they lack a complete blood–brain barrier (McKinley and Johnson 2004; Stocker et al. 2013; Toney et al. 2003). These neurons can respond to blood-borne solutes that do not have free access to other regions of the brain (McKinley & Johnson, 2004). In fact, studies have reported that the OVLT and SFO contain neurons that exhibit intrinsic osmosensitivity (Bourque 2008; Ciura and Bourque 2006).

Experimental evidence has shown that either electrolytic lesion or inhibition of OVLT neurons by muscimol (GABAa receptor agonist) significantly reduced both lumbar and renal sympathoexcitatory responses promoted by the intracarotid infusion of hypertonic NaCl solutions (Shi et al., 2007). Moreover, Antunes *et al.* (2006) showed that a transection that disrupts the connections of the circumventricular organs (CVO) attenuated the sympathoexcitation induced by hyperosmotic perfusate in the arterially perfused decorticated *in situ* rat preparation. Indeed, raising the sodium concentration in the plasma or cerebrospinal fluid increases Fos expression, a marker of neuronal activation in the OVLT (Shi et al., 2008). Furthermore, SFO neurons contribute to the hypertension induced by chronic Ang II administration in rats on a high-salt diet (Ang II–salt model) (Osborn et al. 2014). In addition to initiating autonomic adjustments in response to hypernatremia, SFO

and OVLT also participate in triggering behavioral adjustments to hyperosmotic stimuli. Furthermore, combined electrolytic lesions of the OVLT and SFO attenuate the drinking of water induced by plasma hypernatremia (McKinley et al. 1992). These findings emphasize the role of the osmosensitive neurons of the lamina terminalis in the sympatoexcitatory and behavioral responses to hyperosmotic challenges.

Lamina terminalis neurons do not project directly onto sympathetic preganglionic neurons. Cerebrospinal fluid hypernatremia, capable of increasing renal sympathetic nerve activity (RSNA) and blood pressure, also induces increased Fos expression in OVLT neurons with axonal projections to the paraventricular nucleus of the hypothalamus (PVN) (Shi et al., 2008). OVLT and SFO osmosensitive neurons are responsible for triggering sympathetic reflex adjustments to hyperosmolality mainly through a descending pathway to PVN (McKinley et al., 1992; Shi et al., 2008; Swanson and Sawchenko, 1983). In turn, PVN neurons activate downstream sympathetic regulatory targets, including both presympathetic neurons localized in the rostral ventrolateral medulla (RVLM) (Brooks et al., 2004) and sympathetic preganglionic neurons of the spinal cord (Badoer and De Matteo, 2003).

The area postrema (AP) is another region known to participate in central osmosensory mechanisms. A study by Carlson et al. (1997) demonstrated that intragastric hyperosmolarity activates several regions of the CNS, such as the supraoptic nucleus (SON), PVN, the nucleus of the solitary tract (NTS), AP, and lateral parabrachial nucleus (LPBN), and that such activation is mediated by peripheral osmoreceptors throughout the splanchnic and vagal afferent projections. Following these observations, Carlson et al. (1998) demonstrated that PVN responses to intragastric hyperosmolarity are dependent on the connections with the AP, whereas the responses of other regions (NTS, SON, and LPBN) are independent of AP integrity. The authors have suggested, therefore, that peripheral osmosensitive projections to both the NTS and AP modulate the activity of the

SON and PVN. Moreover, both nuclei are necessary for a complete response; however, the NTS – but not the AP – may control hypothalamic function through projections to the LPBN.

Acute hyperosmotic challenges

In the past, the effects of acute changes in body fluid osmolality through hypertonic NaCl infusion have been studied and is well established. Weiss et al. (1996) showed that an intravenous infusion of hypertonic saline (HS) (2.5 M NaCl) produces a transient increase in arterial blood pressure (ABP) and that this response is related to increases in lumbar sympathetic activity and vasopressin release. By contrast, the authors reported that HS infusion is able to induce long-lasting renal vasodilation and decreases in RSNA.

Renal vasodilation is a significant factor in the regulation of water and sodium excretion, and it is one of the variables adjusted in response to acute variations in the osmolality of extracellular fluid (Pedrino et al. 2008). The mechanisms involved in the genesis and maintenance of renal vasodilation induced by increases in osmolality are complex and appear to involve neural and hormonal mechanisms (Amaral et al., 2014; Burnett et al., 1984; Haanwinckel et al. 1995; Huang et al. 1995; Shen et al., 1991; Wakitani et al., 1985). Regarding the neural mechanisms, studies have related reductions in RSNA to renal vasodilation and consequent natriuresis (Pedrino et al. 2008, 2012; da Silva et al. 2013; Weiss et al. 1996), showing that HS infusion causes a sustained increase in ABP and renal vascular conductance (RVC) but no changes in mesenteric or hindquarter vascular conductance. These findings indicate that vasodilation induced by HS infusion is specific to the renal vascular region.

Central areas and peripheral afferents known to contribute to body fluid homeostasis and cardiovascular regulation (Silva et al., 2013; Pedrino et al., 2009; Pedrino et al., 2005) have been described by their involvement in renal vasodilation and by

their sympathoinhibition to acute hypernatremia.

Acute/chronic electrolytic lesions of the lamina terminalis region eliminated HS-induced renal vasodilation (Pedrino et al., 2005). The chemical lesion of noradrenergic neurons located in the caudal ventrolateral medulla (CVLM; A1) and NTS (A2) prevented renal sympathoinhibition induced by intravenous HS infusion (Pedrino et al., 2012, 2008). More recently, we have shown that sinoaortic denervation (SAD) abolished renal vasodilation and sympathoinhibition induced by acute hypernatremia (Silva et al. 2015). These findings suggest the involvement of central regions and peripheral receptors in the renal hemodynamic and autonomic adjustments induced by acute NaCl overload.

In opposition to these results, experimental investigations have also demonstrated that hyperosmotic stimulation increases SNA in other regions (Shi et al., 2007). Specifically, acute intravascular infusion of hypertonic NaCl increases thoracic, lumbar, and splanchnic SNA (Holbein and Toney 2015). These changes in the sympathetic outflow generated by sodium overload have been described as altering cardiovascular functions and appear to serve an important homeostatic role. Furthermore, such SNA increases in several regions (excepting the renal region) seem to contribute to the increased BP observed after an acute osmotic challenge.

Hypertonic solutions and hemorrhagic shock

Hemorrhagic shock is defined as the decrease of ABP due to drastic blood loss. Such pathology is characterized by tissue hypoperfusion leading to decreases in nutrients and oxygen supply to the body's tissues (Krausz, 2006). Hemorrhagic shock can promote a series of disturbances in the physiological state, leading to multiple organ failure and causing high levels of mortality (Angele et al., 2008; Xu et al. 2013).

It has been shown that trauma is currently the main cause of death among younger

people (under 40 years old), and several studies have demonstrated that approximately 40% of such deaths result from uncontrolled blood loss (Curry and Davis, 2012). Therefore, treating hemorrhagic shock and promoting the cardiovascular recovery of hypovolemic patients are crucial to prevent death. Thus, the use of fluid resuscitation in patients in hemorrhagic shock has been studied in the past to determine its cardiovascular effects and therapeutic potential. Among several types of solutions used in hypovolemic patients, such as blood substitute and colloid, crystalloid, isotonic, and hypertonic solutions, hypertonic saline (HS) has stood out because it combines the cardiovascular benefits of the other solutions even at lower volumes (Tremblay et al., 2001

The treatment of hemorrhagic patients using hypertonic solutions has been practiced for more than 85 years. Pioneering studies by Velasco et al. (1980) have shown that the administration of HS (7.5% NaCl) in dogs submitted to hemorrhagic shock promoted the restoration of blood pressure and cardiac output and resulted in the survival of all the dogs in the study. The control group, which received the same volume of isotonic solution, did not show hemodynamic improvement or survival. In the same sense, de Felippe et al. (1980) demonstrated hemodynamic improvement in patients with refractory shock in intensive care units following hypertonic saline infusion.

Various studies have sought to determine the cardiovascular effects of intravenous HS infusions on hypovolemic hemorrhage (Lopes et al., 1981; Rocha-e-Silva et al., 1986; Younes et al., 1985). These studies demonstrated that hypertonic saline infusion promoted an instantaneous elevation in mean arterial blood pressure, increased pulse pressure, and re-established cardiac output and mesenteric, renal, and hind-limb circulation.

Although the hemodynamic responses to the infusion of HS in animals subjected to bleeding have already been established, the involvement of the CNS in these responses is still under investigation. Recent studies have linked peripheral afferents to cardiovascular recovery induced by HS. In 2009, de Almeida Costa et al. observed that, in the absence of

carotid afferents, cardiovascular recovery induced by HS after hemorrhagic shock is compromised, indicating that such afferents are crucial to HS cardiovascular effects, whereas it is independent of the integrity of baroreceptor afferents. In the same sense, Pedrino et al. (2011) demonstrated the participation of peripheral chemoreceptors in the detection of changes in plasma osmolarity. Such studies have demonstrated that the ligation of the artery responsible for irrigating the carotid corpuscle and its consequent inactivation interferes with the cardiovascular recovery induced by HS in hypovolemic rats. Taken together, such results support the hypothesis that increasing plasma sodium concentration activates several mechanisms that are dependent on peripheral afferents in order to restore physiological conditions in hypovolemic animals. In the absence of chemoreceptor afferents, such reflex mechanisms are compromised, indicating that these afferents play a key role in body fluid homeostasis.

The peripheral chemoreceptor afferents execute their first synapses in the NTS; from such nuclei, the information can be transmitted to various regions of the CNS, particularly to hypothalamic structures such as the lamina terminalis. The lamina terminalis includes structures such as the OVLT, the ventral portion of the median pre-optic nucleus (MnPO), the preoptic periventricular nucleus, and the more medial aspects of the medial preoptic nucleus (Andresen and Kunze, 1994; Bourque, 2008; Cunningham and Sawchenko, 1988; Saphier, 1993; Tanaka et al. 1997, 2002).

In past years, the lamina terminalis has been the focus of several studies that demonstrated that this region is an important integration center involved in the processing and correcting osmolarity and volume changes of extracellular compartments. Cardiovascular, endocrine, renal, and behavioral responses are recruited following the lamina terminalis's activation in an effort to restore plasma sodium concentration and blood volume. The lamina terminalis establishes reciprocal connections with CNS structures that are known to participate in cardiovascular regulation and hydroelectrolytic

balance, such as the vasomotor nuclei of the ventral surface of the medulla, RVLM and CVLM, SFO, and the limbic system (Tanaka et al., 1992; Tucker et al., 1987; Zardetto-Smith and Johnson, 1995).

Interestingly, studies have demonstrated that the lamina terminalis is also involved in cardiovascular recovery induced by hyperosmotic solution in rats submitted to hypovolemia. In 1992, Barbosa et al. observed that the integrity of the lamina terminalis is important to HS effects; lamina terminalis-lesioned rats submitted to hemorrhagic shock do not show cardiovascular recovery following HS infusion.

Moreover, a recent study from our laboratory demonstrated that MnPO integrity is crucial to cardiovascular recovery induced by HS infusion following hemorrhagic shock; following the pharmacological blockade of such nuclei, recovery is abolished (Amaral et al., 2014). Consistent with these results, unpublished observations from our laboratory demonstrate that inhibition of this region impairs the recovery of ABP and RSNA induced by HS in rats submitted to hemorrhagic shock (Figure 1; L.M.N and G.R.P., unpublished observations). Taken together, these results indicate that MnPO integrity is required for the development of cardiovascular and sympathetic responses induced by HS in hypovolemic conditions.

The MnPO is an integrative nucleus corresponding with body fluid homeostasis. Such nuclei receive projections from regions important to hydroelectrolytic balance, such as the A1 and A2 noradrenergic neurons, the SFO, and OVLT. Furthermore, the MnPO projections to the PVN promote the humoral and autonomic adjustments required to restore homeostasis. Thus, it is not unreasonable to suggest that the cardiovascular recovery induced by HS infusion after hemorrhagic shock could also depend on the integrity of others of these regions, such as the A1 and A2 noradrenergic groups.

Recently, we studied the participation of the A1 neuronal group in HS cardiovascular effects in hypovolemic rats. Our results demonstrate that A1-lesioned

animals present cardiovascular recovery induced by HS similar to sham rats after hemorrhagic shock (Figure 2; L.M.N. and G.R.P unpublished observations). Such observations allow us to conclude that the A1 noradrenergic neurons alone are not crucial to cardiovascular recovery induced by HS infusion in hypovolemic animals. However, the participation of the A2 noradrenergic neurons in this process is still under investigation.

Chronic hyperosmotic challenges

It has been well established that high salt intake results in harmful effects to the organism, especially to the cardiovascular system. He et al. (2008) investigated the relationship between high salt intake and blood pressure levels in children and adolescents. The results demonstrated that there was a significant association between high salt intake and raised levels of systolic blood pressure and pulsatile pressure. The authors observed that an increase of 1 g/day in salt intake was related to an increase of 0.4 mmHg in systolic and 0.6 mmHg in pulsatile blood pressure. In experimental models, the addition of dietary salt has been associated with the development of exacerbated arterial hypertension. Dahl et al. (1963) reported that two-thirds of the rats fed a high salt diet presented with increased blood pressure. In these animals, raised levels of ABP were maintained even after the re-establishment of normal NaCl intake. It is suggested that this effect could be etiological, indicating the existence of a group of salt-sensitive animals.

Several studies have demonstrated that salt intake enhances SNA and ABP, even in normotensive salt-resistant animals (Appel et al., 2011; Stocker et al., 2010). In this sense, Simmonds et al. (2014) found evidence that dietary salt intake modulates the responsiveness of central autonomic circuits promoting functional changes in the regulation of SNA and ABP. These results indicate that the level of dietary NaCl was directly correlated to the magnitude of the SNA and ABP responses during activation of sciatic afferents, stimulation of aortic depressor nerve, activation of vagal nerve afferents, volume expansion, and increased NaCl concentration in the cerebrospinal fluid.

Furthermore, consistent with a sensitization to the central autonomic pathways, a high NaCl diet promoted increased blood pressure variability (BPV) independent of changes in mean ABP (Simmonds et al., 2014). Brooks et al. (2004) studied the hyperosmolality effects on the excitatory drive of the RVLM. In this study, the authors observed that the excitatory amino acids (EAA) driving the RVLM blockade, using kynurenic acid (KYN), did not promote changes in the cardiovascular parameters of water-replete rats; however, such a blockade promotes a great decrease of ABP in dehydrated animals, indicating that the EAA drive is increased in this case. Furthermore, the EAA drive of the RVLM is partially influenced by plasma osmolality once the depressor response of KYN into the RVLM is diminished after the re-establishment of osmolality in previously hypovolemic rats. In normovolemic animals receiving HS, the KYN did not promote immediate hypotension; however, such a response developed gradually after three hours. These results demonstrate that acute and chronic increases in plasma osmolality promote an increase of the EAA drive of the RVLM.

Adams et al. (2007) showed that increased dietary salt exaggerates pressure responses induced by the RVLM. The study determined that the enhanced pressor responses were directly attributable to a greater increase in SNA, whereas these enhanced responses were balanced by a greater responsiveness of RVLM neurons to inhibitory input. In this study, the authors demonstrated that pharmacological stimulation or inhibition of the RVLM produced a significantly greater response (increase or decrease) in renal SNA, splanchnic SNA, and ABP in rats drinking NaCl for 14 days versus in rats drinking water. The enhanced SNA and ABP responses were not observed in rats drinking NaCl for 1 to 7 days but were present in animals under this treatment for 21 days, indicating its relationship to chronic salt intake. The withdrawal of a high NaCl diet re-established the baseline levels of these responses. These findings indicate that the potentiated ABP responses are dependent on a slowly developing and reversible form of

neuronal plasticity. In this sense, Simmonds et al. (2014) demonstrated that, in animals submitted to different levels of dietary salt, the magnitude of SNA and ABP responses to electrical stimulation of sciatic afferents or intracerebroventricular infusion of NaCl were increased according to the level of salt intake. The authors observed that there was a direct correlation between the level of dietary salt intake and the sympathoinhibitory responses produced by acute volume expansion, stimulation of the aortic depressor nerve, or cervical vagal afferents. By contrast, sympathetic and blood pressure responses to chemoreflex activation produced by hypoxia or hypercapnia were not affected by increased sodium intake.

In addition, the authors observed that the ability of dietary salt intake to modulate cardiovascular effects is negatively affected by chronic lesions of the lamina terminalis, indicating that the integrity of this area is crucial to salt-induced responses. In this study, animals submitted to a high-salt diet presented with elevated BPV but normal levels of MAP. These findings indicate that dietary salt intake works through the forebrain hypothalamus to modulate various centrally mediated cardiovascular reflexes and increase BPV.

The neuronal plasticity induced by salt overload was also the focus of a study by Kim et al. (2011), who observed that GABAa receptor-mediated transmission in the magnocellular neurosecretory cells of the PVN is inhibitory in almost 75% of the neurons under resting conditions. However, for animals submitted to NaCl 2% as a drinking supply for 7 days, the GABAa, post-synaptic potentials (PSPs), and currents (PSCs) of these cells were almost always excitatory. Such transmission becomes excitatory when there is a sustained demand for AVP and oxytocin secretion (Kim et al., 2011; De Koninck, 2007).

Several studies in animal models (Brown et al., 2009; Cook et al., 2007; He and MacGregor, 2009) demonstrated that, at present, high salt intake is directly related to increased morbidity of and mortality resulting from cardiovascular events. This increased

salt intake promotes elevation of ABP, increasing the possibility of myocardial infarction, stroke, coronary revascularization, or cardiovascular death. In an epidemiological study, Costa et al. (2012) demonstrated that increased sodium intake promotes higher oxidative stress, inflammatory response, myocardial stretching and remodeling, and higher total mortality after myocardial infarction in human patients. Taken together, the studies reinforce the understanding of the potentially harmful effects of a high-salt diet on cardiovascular parameters.

As previously described, the negative cardiovascular effects of high salt intake in adult animals are well established in the literature. Furthermore, it is known that the perinatal period (pregnancy and lactation) is crucial to the organism's development throughout adulthood (Bao et al., 1995; Barker, 1997; Li et al., 1995; Vidonho et al., 2004). However, little information exists regarding the post-natal period and its contributions to the organism's development. Recently, several researchers aimed to investigate the importance of hydromineral imbalance during the post-weaning period in the development of the organism to adulthood.

A study from Coelho et al. (2006) evaluated the effects of sodium restriction and sodium overload after rats were weaned. The authors observed that a high-salt diet promotes increased ABP; diminished body weight in adulthood; increased food intake, L-thyroxine concentration, and energy expenditure; lower non-fasting leptin; decreased plasma thyroid-stimulating hormone; and diminished plasma angiotensin II. In animals submitted to a low-salt diet, an increase in brown adipose tissue Ang II content was associated with decreased uncoupling protein 1 expression and energy expenditure. In this group, a low angiotensin II content in white adipose tissue was also found. These results were from rats that were still under a high-, normal-, or low-sodium intake regimen. The authors concluded that chronic alteration in salt intake is associated with changes in body weight, food intake, hormonal profile, energy expenditure, and tissue angiotensin II

content.

A recent study in our laboratory evaluated the effects of sodium overload during childhood on cardiovascular and behavioral parameters in adulthood. The study also sought to evaluate whether the sodium-overload effects were present even after withdrawing the high-sodium diet (Moreira et al. 2014). The authors observed that animals submitted to high salt intake presented increased MAP and HR, diminished baroreflex sensitivity, lower body weight, diminished sodium, increased water-induced intake, and compromised cardiovascular responses induced by acute hypernatremia. The results indicated that sodium-overload-induced modifications persist until adulthood, even after the withdrawal of a high-sodium diet.

Taken as a group, the studies demonstrated that chronic hyperosmolality or salt intake promotes increases in ABP and decreases in baroreflex sensitivity and alters the cardiovascular and autonomic responses to acute hyperosmolality. Such modifications can be transitory in adult animals but persist throughout adulthood when the hydromineral disturbance occurs during the early stages of life. Furthermore, sodium loading alters the sensitivity and/or activity of specific central nuclei involved in cardiovascular and autonomic control. Such effects can be determinants in the development of cardiovascular diseases. In fact, chronic high-salt intake has often been related to hypertension, as demonstrated by several studies.

Osmotic transduction signals

As previously noted, several regions of the CNS are responsible for the detection of changes in plasma osmolarity and angiotensin concentration. The central osmoreceptors send this information to other regions of the CNS, especially to the MnPO and the PVN (Saper and Levisohn, 1983; Saper et al.,1983; Tucker et al., 1987). Such regions promote necessary adjustments to sympathetic nerve activity and vasopressin/oxytocin secretion in order to restore the normal physiological state.

Several researchers have sought to determine how the peripheral and central osmoreceptors perceive changes in osmolarity (Bourque et al., 1994; Ciura & Bourque, 2006). Previous studies demonstrated that neurons from the SFO and OVLT are depolarized by increases in plasma sodium concentration and hyperpolarized by decreases in such parameters (Bourque et al., 1994). Other studies have observed that variations in hypothalamic neuronal activity are associated with the glutamatergic postsynaptic potentials from the OVLT neurons (Bourque et al., 1994). In fact, studies by Shi et al. (2008) demonstrated that the PVN-projecting OVLT neurons are activated following intracarotid hypertonic saline infusion. Such observations indicate that increased osmolarity stimulates both SFO and OVLT and that sending information to hypothalamic regions is dependent on glutamatergic neurotransmission, especially from the OVLT.

It has been demonstrated that the SON, another hypothalamic region, is also osmosensitive (Mason, 1980) and that hyperosmolarity of body fluids depolarizes the SON neurons independently of synaptic connections (Bourque, 1989; Oliet & Bourque, 1992). However, the mechanisms whereby such regions are stimulated are still under investigation. In 1993, Oliet and Bourque determined that changes in body fluid osmolarity induce changes in cell volume. Such volume change modulates the activity of membrane mechanosensitive cation channels in an adequate way to modulate the membrane voltage and action potential discharge. Therefore, the SON neurons are stimulated by deformations of their membranes, with changes in permeability enabling the action potential generation.

More recently, Ciura and Bourque (2006) demonstrated that the murine neurons of the OVLT are intrinsically sensitive to extracellular fluid osmolarity increases. The authors observed that hypertonicity increases the membrane conductance to cations, resulting in the depolarization of osmoreceptor potential and enhancing the action potential discharge. Moreover, such a cascade of osmosensory transduction is dependent on the

transient receptor potential vanilloid 1 (TRPV1), since in the absence of this receptor or after its blockage, the OVLT capacity to detect changes in osmolarity is lost. Thus, the OVLT neurons may act as first-order osmoreceptors, and a product of the TRPV1 genes may be crucial to the transduction of the osmosensory signals (Ciura & Bourque, 2006).

In summary, the central osmoreceptors are depolarized by changes in cell volume caused by plasma hyperosmolarity. Before today, it was widely known that the TRPV1 receptors are involved in the osmosensory processes. However, such processes are not completely understood.

Final considerations

The perception of changes in body fluid osmolarity and the adequate cardiovascular, autonomic, and behavioral adjustments are crucial to the maintenance of life. The sodium ion is one of the most important determinants of extracellular fluid osmolarity; therefore, the balance between its consumption and excretion is crucial to body fluid homeostasis. With its central importance to life, the osmosensory mechanism is a complex group of pathways that cooperate to produce hydroelectrolytic balance. However, the malfunction of even one of these mechanisms can be harmful to the organism and can trigger the development of cardiovascular diseases with several complications that, left untreated, can lead to death. Thus, understanding the functioning of the components involved in body fluid homeostasis is important in order to understand its malfunction and, it is hoped, to treat consequent disease processes and prevent death.

References

ADAMS JM, CHRISTOPHER JM, SVED AF AND STOCKER SD: Increased dietary salt enhances sympathoexcitatory and sympathoinhibitory responses from the rostral ventrolateral medulla. *Hypertension.* **50:**354–59, 2007.
DE ALMEIDA COSTA EF, PEDRINO GR, LOPES OU AND CRAVO SL: Afferent

pathways involved in cardiovascular adjustments induced by hypertonic saline resuscitation in rats submitted to hemorrhagic shock. *Shock.* **32**:190–93, 2009.

AMARAL NO, DE OLIVEIRA TS, NAVES LM, FILGUEIRA FP, FERREIRA-NETO ML, SCHOORLEMMER GHM, DE CASTRO CH, FREIRIA-OLIVEIRA AH, XAVIER CH, COLUGNATI DB, ROSA DA, BLANCH GT, BORGES CL, SOARES CMA, REIS AAS, CRAVO SL, PEDRINO GR: Efferent pathways in sodium overload-induced renal vasodilation in rats. *PloS one* **9**:e109620, 2014.

ANDRESEN MC AND KUNZE DL: Nucleus tractus solitarius-gateway to neural circulatory control. *Ann Rev Physiol.* **56**: 93–116, 1994.

ANGELE MK, SCHNEIDER CP AND CHAUDY IH: Bench-to-bedside review: latest results in hemorrhagic shock. *Crit Care.* **12**: 218, 2008.

ANTUNES-RODRIGUES J, RAMALHO MJ, REIS LC, MENANI JV, TURRIN MQ, GUTKOWSKA J, MCCANN SM: Lesions of the hypothalamus and pituitary inhibit volume-expansion-induced release of atrial natriuretic peptide. *Proc Natl Acad Sci USA.* **88**: 2956–60, 1991.

ANTUNES-RODRIGUES J, MACHADO BH, ANDRADE HA, MAUAD H, RAMALHO MJ, REIS LC, SILVA-NETTO CR, FAVARETTO AL, GUTKOWSKA J, MCCANN S: Carotid-aortic and renal baroreceptors mediate the atrial natriuretic peptide release induced by blood volume expansion. *Proc Natl Acad Sci USA.* **89**: 6828–31, 1992.

ANTUNES-RODRIGUES J, CASTRO M, ELIAS LLK, VALENÇA MM AND MCCANN SM: Neuroendocrine control of body fluid metabolism. *Physiol Rev.* **84**:169–208, 2004.

APPEL LJ, FROHLICH ED, HALL JE, PEARSON TA, SACCO RL, SEALS DR, SACKS FM, SMITH SC, VAFIADIS DK, VAN HORN LV: The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke: a call to action from the american heart association. *Circulation* **123**:1138–43, 2011.

BADOER E, CHI-WAI NG, DE MATTEO R: Glutamatergic input in the pvn is important in renal nerve response to elevations in osmolality. *Am J Physiol Renal Physiol.* **285**: F640–50, 2003.

BAO, W, THREEFOOR SA, SRINIVASAN SR, BERENSON GS: Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the bogalusa heart study. *Am J Hypertens.* **8**: 657–65, 1995.

BARBOSA SP, CAMARGO LAA, SAAD WA, RENZI A, DE LUCA LA, MENANI JV: Lesion

of the anteroventral third ventricle region impairs the recovery of arterial pressure induced by hypertonic saline in rats submitted to hemorrhagic shock. *Brain Res.* **587)**109–14, 1992.

BARKER DJ: Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition.* **13**: 807–13, 1997.

BEALER SL, HAYWOOD JR, GRUBER KA, BUCKALEW VM, FINK GD, BRODY MJ, JONHSON AK: Preoptic-hypothalamic periventricular lesions reduce natriuresis to volume expansion. *Am J Physiol.* **244**: R51–57, 1983.

BEALER SL: Sodium excretion following lesions of preoptic recess periventricular tissue in the rat. *Am J Physiol.* **244**: R815–22, 1983.

BIE P: Osmoreceptors, vasopressin, and control of renal water excretion. *Physiol Rev.* **60**: 961–1048, 1980.

BOURQUE CW: Central mechanisms of osmosensation and systemic osmoregulation. *Nat.Rev.Neurosci.* **9**: 519–31, 2008.

BOURQUE CW, OLLIET SH, RICHAR D: Osmoreceptors, osmoreception, and osmoregulation. *Front Neuroendocrinol.* **15**: 231–74, 1994.

BROOKS VL, FREEMAN KL, DONAUGHY TLO, VIRGINIA L, THERESA L: Acute and chronic increases in osmolality increase excitatory amino acid drive of the rostral ventrolateral medulla in rats. *Am J Physiol Regul Integr Comp Physiol.* **3098**: 1359–68, 2004.

BROWN IJ, TZOULAKI I, CANDEAS V, ELLIOTT P: Salt intakes around the world: implications for public health. *Int J Epidemiol.* **38**: 791–813, 2009.

BURNETT JC, GRANGER JP, OPGENORTH TJ: Effects of synthetic atrial natriuretic factor on renal function and renin release. *Am J Physiol.* **247**: F863–66, 1984.

CARLSON SH, BEITZ A, OSBORN JW: Intragastric hypertonic saline increases vasopressin and central fos immunoreactivity in conscious rats. *Am J Physiol.* **272**: R750–58, 1997.

CARLSON SH, COLLISTER JP, OSBORN JW: The area postrema modulates hypothalamic fos responses to intragastric hypertonic saline in conscious rats. *Am J Physiol Regul Integr Comp Physiol.* **275**: R1921–27, 1998.

CHEN QH, TONEY GM: Identification and characterization of two functionally distinct groups of spinal cord-projecting paraventricular nucleus neurons with sympathetic-related

activity. *Neuroscience* **118**: 797–807, 2003.

CIURA S, BOURQUE CW: Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. *J Neurosci.* **26**: 9069–75, 2006.

COELHO MS, PASSADORE MD, GASPARETTI AL, BIBANCOS T, PRADA PO, FURUKAWA LL, FURUKAWA LNS, FUKUI RT, CASARINI DE, SAAD MJA, LUZ J, CHIAVEGATTO S, DOLNIKOFF MS, HEIMANN JC: High- or low-salt diet from weaning to adulthood: effect on body weight, food intake and energy balance in rats. *Nutr Metab Cardiovasc Dis.* **16**: 148–55, 2006.

COOK NR, CUTLER JA, OBARZANEK E, BURING JE, REXRODE KM, KUMANYIKA SK, APPEL LJ, WHELTON PK: Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (tohp). *BMJ (Clinical research ed.)* **334**: 885–88, 2007.

COSTA APR, DE PAULA RCS, CARVALHO GF, ARAÚJO JP, ANDRADE JM, DE ALMEIDA OLR, DE FARIA EC, FREITAS WM, COELHO OR, RAMIRES JAF, QUINAGLIA E SILVA JC, SPOSITO AC: High sodium intake adversely affects oxidative-inflammatory response, cardiac remodelling and mortality after myocardial infarction. *Atherosclerosis* **222**: 284–91, 2012.

CUNNINGHAM ET, SAWCHENKO PE: Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J Comp Neurol.* **274**:60–76, 1998.

CURRY N, DAVIS PW: What's new in resuscitation strategies for the patient with multiple trauma? *Injury* **43**: 1021–28, 2012.

DAHL LK, HEINE M, TASSINARI L: Effects of chronic excess salt ingestion. Role of genetic factors in both DOCA-salt and renal hypertension. *J Exp Med* **118**: 605–17, 1963.

de FELIPPE J, TIMONER J, VELASCO IT, LOPES OU, ROCHA E SILVA M: Treatment of refractory hypovolaemic shock by 7.5% sodium chloride injections. *Lancet* **2**: 1002–4, 1980.

FITZSIMONS JT: Angiotensin, thirst, and sodium appetite. *Physiol Rev* **78**: 583–686, 1998.

GODINO A, GIUSTI-PAIVA A, ANTUNES-RODRIGUES J, VIVAS L: Neurochemical brain groups activated after an isotonic blood volume expansion in rats. *Neurosci* **133**: 493–505,

2005.

HAANWINCKEL MA, ELIAS LK, FAVARETTO AL, GUTKOWSKA J, MCCANN SM, ANTUNES-RODRIGUES J: Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. *Proc Natl Acad Sci USA.* **92**: 7902–6, 1995.

HE FJ, MACGREGOR GA: A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *J Hum Hypertens.* **23**: 363–84, 2009.

HE FJ, MARRERO NM, MACGREGOR GA: Salt and blood pressure in children and adolescents. *J Hum Hypertens.* **22**: 4–11, 2008.

HOLBEIN WW, TONEY GM: Activation of the hypothalamic paraventricular nucleus by forebrain hypertonicity selectively increases tonic vasomotor sympathetic nerve activity. *Am J Physiol.* **308**: R351–59, 2015.

HUANG W, LEE SL, SJOQUIST M: Natriuretic role of endogenous oxytocin in male rats infused with hypertonic NaCl. *Am J Physiol.* **268**: R634–40, 1995.

HUANG W, SJOQUIST M, SKOTT O, STRICKER EM, SVED AF: Oxytocin-induced renin secretion in conscious rats. *Am J Physiol.* **278**: R226–30, 2000.

JOHNSON AK, CUNNINGHAM JT, THUNHORST RL: Integrative role of the lamina terminalis in the regulation of cardiovascular and body fluid homeostasis. *Clin Exp Pharmacol Physiol.* **23**:183–91, 1996.

KIM JS, KIM WB, KIM YB, LEE Y, KIM YS, SHEN FY, LEE SW, PARK D, CHOI HJ, HUR J, PARK JJ, HAN HC, COLWELL CS, CHO YW, KIM YI: Chronic hyperosmotic stress converts gabaergic inhibition into excitation in vasopressin and oxytocin neurons in the rat. *J Neurosci.* **31**: 13312–22, 2011.

DE KONINCH Y: Altered chloride homeostasis in neurological disorders: a new target. *Curr Opin Pharmacol.* **7**: 93–99, 2007.

KRAUSZ MM: Initial resuscitation of hemorrhagic shock. *World J Emerg Surg.* **1**:14, 2006.

LI L, WANG L, CAO W, XU F, CAO J: Longitudinal studies of blood pressure in children. *Asia Pac J Public Health* **8**:130–33, 1995.

LOPES OW, PONTIERI V, ROCHA E SILVA M, VELASCO IT: Hyperosmotic NaCl and severe hemorrhagic shock: role of the innervated lung. *Am J Physiol.* **241**: H883–90, 1981.

MASON WT: Supraoptic neurones of rat hypothalamus are osmosensitive. *Nature* **287(5778)**:154–57, 1980.

MCKINLEY MJ, BICKNELL RJ, HARDS D, MCALLEN RM, VIVAS L, WISINGER RS, ODFIELD BJ: Efferent neural pathways of the lamina terminalis subserving osmoregulation. *Prog Brain Res.* **91**: 395–402, 1992.

MCKINLEY MJ, JOHNSON AK: The physiological regulation of thirst and fluid intake. *News Physiol Sci.* **19**:1–6, 2004.

MOREIRA MCS, DA SILVA EF, SILVEIRA LL, DE PAIVA YB, DE CASTRO CH, FREIRIA-OLIVEIRA AH, ROSA DA, FERREIRA PM, XAVIER CH, COLOMBARI E, PEDRINO GR: High sodium intake during postnatal phases induces an increase in arterial blood pressure in adult rats. *Br J Nutr.* **112**:1923–32, 2014.

MORITA H, VATNER SF: Effects of volume expansion on renal nerve activity, renal blood flow, and sodium and water excretion in conscious dogs. *Am J Physiol Renal Physiol* **249**: F680–87, 1985.

MORRIS M, ALEXANDER N: Baroreceptor influences on plasma atrial natriuretic peptide (ANP): Sinoaortic denervation reduces basal levels and the response to an osmotic challenge. *Endocrinol.* **122**: 373–75, 1988.

OLIET SH, BOURQUE CW: Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature.* **364**: 341–43, 1993.

OSBORN JW, OLSON DM, GUZMAN P, TONEY GM, FINK GD: The neurogenic phase of angiotensin ii-salt hypertension is prevented by chronic intracerebroventricular administration of benzamil. *Physiol Rep.* **2**:e00245, 2014.

PEDRINO GR, MONACO LR, CRAVO SL: Renal vasodilation induced by hypernatraemia: role of alpha-adrenoceptors in the median preoptic nucleus. *Clin Exp Pharmacol Physiol.* **36**: e83–89, 2009.

PEDRINO GR, ROSSI MC, SCHOORLEMMER GHM, LOPES OU, CRAVO SL: Cardiovascular adjustments induced by hypertonic saline in hemorrhagic rats: Involvement of carotid body chemoreceptors. *Auton Neurosci.* **160**: 37–41, 2011.

PEDRINO GR, FREIRIA-OLIVEIRA AH, COLOMBARI DAS, ROSA DA, CRAVO SL: A2 noradrenergic lesions prevent renal sympathoinhibition induced by hypernatremia in rats. *PLoS ONE* **7**: e37587, 2012.

PEDRINO GR, MAURINO I, COLOMBARI DSA, CRAVO SL: Role of catecholaminergic neurones of the caudal ventrolateral medulla in cardiovascular responses induced by acute changes in circulating volume in rats. *Exp Physiol.* **91**: 995–1005, 2006.

PEDRINO GR, SERA CTN, CRAVO SL, COLOMBARI DSA: Anteroventral third ventricle lesions impair cardiovascular responses to intravenous hypertonic saline infusion. *Auton Neurosci.* **117**: 9–16, 2005.

PEDRINO GR, ROSA DA, KORIM WS, CRAVO SL: Renal sympathoinhibition induced by hypernatremia: involvement of a1 noradrenergic neurons. *Auton Neurosci.* **142**: 55–63, 2008.

RAUCH AL, CALLAHAN MF, BUCKALEW VM, MORRIS M: Regulation of plasma atrial natriuretic peptide by the central nervous system. *Am J Physiol.* **258**: R531–35, 1990.

ROCHA E SILVA M, NEGRAES GA, SOARES AM, PONTIERI V, LOPPNOW L: Hypertonic resuscitation from severe hemorrhagic shock: patterns of regional circulation. *Circ Shock.* **19**: 165–75, 1986.

SAPER CB, REIS DJ, JOH T: Medullary catecholamine inputs to the anteroventral third ventricular cardiovascular regulatory region in the rat. *Neurosci Lett* **42**: 285–91, 1983.

SAPER CB, LEVISOHN D: Afferent connections of the median preoptic nucleus in the rat: anatomical evidence for a cardiovascular integrative mechanism in the anteroventral third ventricular (AV3V) region. *Brain Res.* **288**: 21–31, 1983.

SAPHIER D: Electrophysiology and neuropharmacology of noradrenergic projections to rat PVN magnocellular neurons. *Am J Physiol.* **264**: R891–902, 1993.

SCHOORLEMMER GH, JOHNSON AK, THUNHORST RL: Effect of hyperosmotic solutions on salt excretion and thirst in rats. *Am J Physiol Regul Integr Comp Physiol.* **278**: R917–23, 2000.

SHEN YT, GRAHAM RM, VATNER SF: Effects of atrial natriuretic factor on blood flow distribution and vascular resistance in conscious dogs. *Am J Physiol Heart Circ Physiol* **260**: H1893–1902, 1991.

SHI P, STOCKER SD, TONEY GM: Organum vasculosum laminae terminalis contributes to increased sympathetic nerve activity induced by central hyperosmolality. *Am J Physiol Regul Integr Comp Physiol.* **293**: R2279–89, 2007.

SHI P, MARTINEZ MA, CALDERON AS, CHEN W, CUNNINGHAM JT, TONEY GM: Intra-carotid hyperosmotic stimulation increases fos staining in forebrain organum vasculosum laminae terminalis neurones that project to the hypothalamic paraventricular nucleus. *J Physiol.* **586**: 5231–45, 2008.

SILVA EF, FREIRIA-OLIVEIRA AH, CUSTÓDIO CHX, GHEDINI PC, BATAUS LAM,

COLOMBARI E, DE CASTRO CH, COLUGNATI DB, ROSA DA, CRAVO SLD, PEDRINO GR: A1 noradrenergic neurons lesions reduce natriuresis and hypertensive responses to hypernatremia in rats.edited by nick ashton. *PLOS ONE* **8**: e73187, 2013.

SILVA EF, SERA CTN, MOURÃO AA, LOPES PR, MOREIRA MCS, FERREIRA-NETO ML, COLOMBARI DSA, CRAVO SLD, PEDRINO GR: Involvement of sinoaortic afferents in renal sympathoinhibition and vasodilation induced by acute hypernatremia. *Clin Exp Pharmacol Physiol.* **42**:1135–41, 2015.

SIMMONDS SS, LAY J , STOKER SD: Dietary salt intake exaggerates sympathetic reflexes and increases blood pressure variability in normotensive rats. *Hypertension* **64**:583–89, 2014.

STOCKER SD, MADDEN CJ, SVED AF: Excess dietary salt intake alters the excitability of central sympathetic networks. *Physiol Behav.* **100**: 519–24, 2010.

STOCKER SD, MONAHAN KD, BROWNING KN: Neurogenic and sympathoexcitatory actions of NaCl in hypertension. *Curr Hypertens Rep.* **15**: 538–46, 2013.

SWANSON LW, SAWCHENKO PE: Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci.* **6**: 269–324, 1983.

TANAKA J, HAYASHI Y, WATAI T, FUKAMI Y, JOHKOH R, SHIMAMUNE S: A1 noradrenergic modulation of av3v inputs to pvn neurosecretory cells. *Neuroreport* **8**: 3147–50, 1997.

TANAKA J, NISHIMIURA J, KIMURA F, NOMURA M: Noradrenergic excitatory inputs to median preoptic neurones in rats. *Neuroreport* **3**: 946–48, 1992.

TANAKA J, MASHIKO N, KAWAKAMI A, HATAKENAKA S, FUJISAWA S, NOMURA M: The action of the a1 noradrenergic region on the activity of subfornical organ neurons in the rat. *Neurosci Lett.* **332**: 41–44, 2002.

TONEY GM, CHEN QH, CATO MJ, STOCKER SD: Central osmotic regulation of sympathetic nerve activity. *Acta Physiol.* **177**: 43–55, 2003.

TREMBLAY LN, ROZOLI SB, BRENNEMAN FD: Advances in fluid resuscitation of hemorrhagic shock. *Can J Surg.* **44**: 172–79, 2001.

TUCKER DC, SAPER CB, RUGGIERO DA, REIS DJ: organization of central adrenergic pathways: i. relationships of ventrolateral medullary projections to the hypothalamus and spinal cord. *Journal Comp Neurol.* **259**: 591–603, 1987.

VELASCO IT, PONTIERI V, ROCHA E SILVA M, LOPES OU: Hyperosmotic NaCl and

severe hemorrhagic shock. *Am J Physiol.* **239**: H664–73, 1980.

VIDONHO AF, DA SILVA A, CATANOZI S, ROCHA JC, BEUTEL A, CARILLO B, FURUKAWA LNS, CAMPOS RR, BERGAMASCHI CMT, CARPINELLI AR, QUINTÃO ECR, DOLNIKOFF MS, HEIMANN JC: Perinatal salt restriction: a new pathway to programming insulin resistance and dyslipidemia in adult wistar rats. *Pediatr Res.* **56**: 842–48, 2004.

WAKITANI K, COLE BR, GELLER DM, CURRI MG, ADAMS SP, FOK KF, NEEDLEMAN D: Atriopeptins: correlation between renal vasodilation and natriuresis. *Am J Physiol.* **249**: F49–53, 1985.

WEISS ML, CLAASSEN DE, HIRAI T, KENNEY MJ: Nonuniform sympathetic nerve responses to intravenous hypertonic saline infusion. *J Auton Nerv Syst.* **57**:109–15, 1996.

XU P, WEN Z, SHI X, LI Y, FAN L, XIANG M, LI A, SCOTT MJ, XIAO G, LI S, BILLIAR TR, WILSON MA, FAN J: Hemorrhagic shock augments nlrp3 inflammasome ativation in the lung through impaired pyrin induction. *J Immunol. (Baltimore, Md. : 1950)* **190**: 5247–55, 2013.

YOUNES RN, AUN F, TOMIDA RM, BIROLINI D: The role of lung innervation in the hemodynamic response to hypertonic sodium chloride solutions in hemorrhagic shock. *Surgery* **98**: 900–906, 1985.

ZARDETTO-SMITH M, JOHNSON K: chemical topography of efferent projections from the median preoptic nucleus to pontine monoaminergic cell groups in the rat. *Neurosci Lett.* **199**: 215–19, 1995.
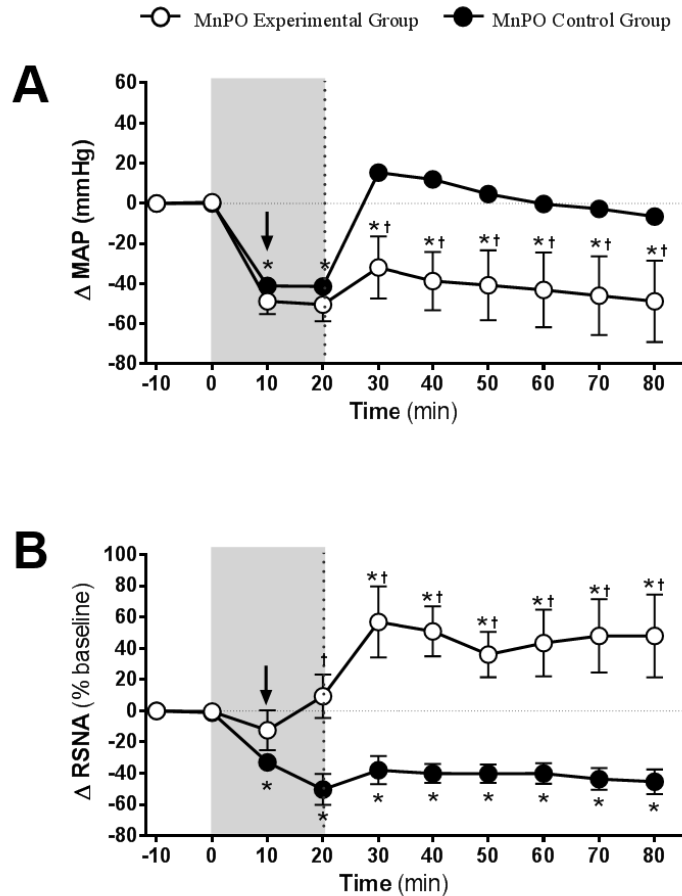
Figures

Figure 1: Cardiovascular and autonomic effects of HS infusion in rats submitted to hemorrhagic shock after saline or muscimol nanoinjections into the MnPO. Variations of mean arterial pressure (MAP; A) and renal sympathetic nerve activity (RSNA; B) following HS infusion in both control (saline) and experimental (muscimol) animals after hemorrhagic shock. The grey bar represents hemorrhagic shock; the dashed line represents HS infusion; the arrow represents MnPO nanoinjection of saline or muscimol. *Different from 0; † Different from control group; p <0.05. L.M.N and G.R.P., unpublished observations.
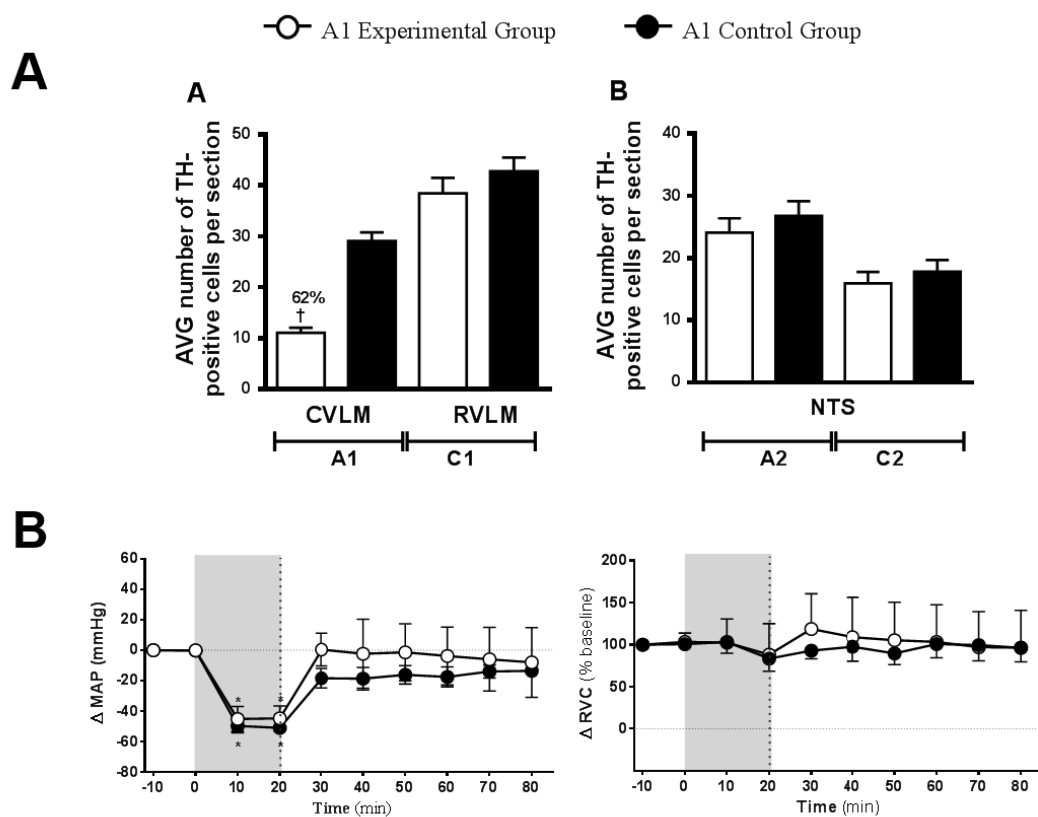
Figure 2: Cardiovascular effects of HS infusion in A1-lesioned rats and sham rats submitted to hemorrhagic shock. A) Neuronal count of the A2 and C2 (NTS), A1 (CVLM) and C1 (RVLM) neuronal groupments after immunocytochemistry to tyrosine hydroxilase, indicating a reduction in the neuronal number of the A1 groupments with no changes in the other groupment. B) Variations of mean arterial pressure (MAP) and renal vascular conductance (RVC) following HS infusion in both control and A1-lesioned animals after hemorrhagic shock. The grey bar represents hemorrhagic shock; the dashed line represents HS infusion. *Different from 0; † Different from control group; p <0.05. L.M.N and G.R.P., unpublished observations.