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Research Report

Cardiovascular responses evoked by activation or blockade of $GABA_A$ receptors in the hypothalamic PVN are attenuated in transgenic rats with low brain angiotensinogen

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ABSTRACT

Previous evidence indicates that a balance between inhibitory gabaergic and excitatory angiotensinergic factors in the PVN is important for cardiovascular control. We investigated the cardiovascular response evoked from activation or blockade of $GABA_A$ receptors in the paraventricular nucleus (PVN), in transgenic rats with low brain angiotensinogen [TGR(ASrAOGEN)]. Brain Ang II and Ang-(1–7) levels were also determined. In functional experiments, TGR(ASrAOGEN) and Sprague–Dawley rats (SD, control) were anesthetized with urethane and blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) were recorded. Brain Ang II and Ang-(1–7) levels were largely reduced in TGR(ASrAOGEN) compared with SD rats. Inhibition of PVN neurons with the $GABA_A$ agonist, muscimol (1 nmol/100 nL), resulted in an attenuated fall in all cardiovascular variables in TGR(ASrAOGEN) compared with SD rats. This difference was particularly pronounced in HR (TGR Mus −23±6 bpm vs. −77±9 bpm SD Mus; $P<0.05$) and RSNA (TGR −3±10% vs.−29±8% SD; $P<0.05$). Furthermore, the sympathetic response evoked by blockade of $GABA_A$ receptors in the PVN of TGR(ASrAOGEN) was also largely suppressed. The present data indicate that the sympathetic outflow mediated by PVN neurons under basal conditions is suppressed in TGR(ASrAOGEN) rats corroborating the functional significance of brain angiotensin production in the central regulation of sympathetic output to the cardiovascular system.

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1. Introduction

Brain renin-angiotensin system (RAS) is implicated in cardiovascular system control by interfering with the activity of sympathetic premotor neurons (Bader et al., 2001; Veerasingham and Raizada, 2003). Evidence indicates that RAS peptides might influence baseline sympathetic output by exciting premotor neurons of hypothalamic paraventricular nucleus (PVN) (Silva et al., 2005; Zucker et al., 2004). A high density of angiotensin II (Ang II) receptors is found in the PVN neurons (McKinley et al., 2003) and their stimulation or blockade may influence the sympathetic activity in physiological (Silva et al., 2005) or altered conditions (Chen and Toney, 2001; da Silva et al., 2011; Zhu et al., 2004).

The central GABA system plays an important role in cardiovascular control. PVN is a key site where GABA elicits an inhibitory effect on cardiovascular functions and sympathetic activity (Haywood et al., 2001; Martin et al., 1991). Blockade of gabaergic input in the PVN profoundly increases sympathetic outflow (Tagawa and Dampney, 1999), whereas activation of $GABA_A$ receptors in the PVN reduces sympathetic activity and blood pressure (Allen, 2002; Silva et al., 2005). It has been postulated that the overall increase in basal sympathetic tone in cardiovascular diseases such as in heart failure is caused, at least in part, by an unbalance between the inhibitory gabaergic and excitatory angiotensinergic factors within the PVN (Li and Patel, 2003; Zucker et al., 2004).

To study the functional relevance of the brain RAS we developed a transgenic model with low levels of brain angiotensinogen (AOGEN), named TGR(ASrAOGEN). This rat model expresses antisense RNA against AOGEN to suppress its production by the astrocytes (Schinke et al., 1999), the major source of brain AOGEN (Milsted et al., 1990). Indirect evidence suggests that TGR(ASrAOGEN) has a diminished sympathetic tone (Baltatu et al., 2004b; Wang et al., 2004). We have previously reported that injections of Ang II into the rostral ventrolateral medulla of TGR(ASrAOGEN) evoke a larger pressor response when compared to that observed in control SD rats (Baltatu et al., 2001). This finding leads us to conclude that a permanent reduction of AOGEN in the brain results in an increased sensitivity of Ang II receptors. In this study we utilized this transgenic model to test the hypothesis that the reduction in brain Ang II may affect the cardiovascular effects mediated by activation or blockade of $GABA_A$ receptors in the PVN. Therefore we (i) determined the levels of Ang peptides in the brain of TGR(ASrAOGEN), (ii) evaluated the renal sympathetic tone in TGR(ASrAOGEN), (iii) tested the cardiovascular reactivity to activation or blockade of $GABA_A$ receptors in the PVN of TGR(ASrAOGEN).

2. Results

Radioimmunoassay measurements revealed that transgene expression leads to a marked reduction in Ang II and Ang-(1–7) formation in the brain of TGR(ASrAOGEN) when compared with control rats. On the other hand, plasma levels of Ang I, Ang II and Ang-(1–7) and plasma renin activity were not different between TGR(ASrAOGEN) and SD rats (Fig. 1).

Table 1 shows the baseline MAP and HR just before injections of muscimol or bicuculline into the PVN of TGR(ASrAOGEN) or SD rats. No significant difference among groups was observed for MAP and HR. Fig. 2 shows grouped data illustrating the cardiovascular effects evoked by microinjections of muscimol (1 nmol/100 nL) into the PVN of TGR(ASrAOGEN) and SD rats. When compared to the effects evoked by muscimol into the PVN of SD rats, microinjections of muscimol into PVN of TGR(ASrAOGEN) resulted in an attenuated fall in MAP (TGR mus: −17±5 mm Hg vs SD mus: −32±3 mm Hg) and HR (TGR mus: −23±6 bpm vs SD mus: −77±9 bpm). Microinjections of muscimol into the PVN of TGR(ASrAOGEN) did not significantly alter baseline RSNA (−3±10%), in contrast to the large decrease in RSNA produced by muscimol injections into PVN of SD rats (SD mus:−29±8%). This is more clearly illustrated in Fig. 3, which shows examples of filtered renal neurograms and the average RSNA, calculated as spikes/s. As described for MAP and HR, no significant difference was observed in the basal levels of RSNA between TGR(ASrAOGEN) and SD rats (Figs. 3A–C). Muscimol microinjections into PVN, caused a smaller reduction in baseline sympathetic activity in TGR(ASrAOGEN) when compared to SD rats (Fig. 3D). In an additional series of experiments we observed that intravenous injection of hexamethonium also produced an attenuated fall in MAP, HR and RSNA in TGR(ASrAOGEN) when compared to SD rats (Fig. 4).

Finally, we compared the cardiovascular response produced by blockade of $GABA_A$ receptors into the PVN of SD and TGR(ASrAOGEN). We found that the increases in HR and RSNA evoked by bicuculline injections into the PVN of TGR(ASrAOGEN) were also greatly attenuated by 42% and 68%, respectively, when compared to those evoked in SD rats (Fig. 5).

The results reported above included only animals for which histological analysis showed injection sites located within or immediately on the borders of the PVN, from of 1.4 to 2.1 caudal to the bregma according to the atlas of Paxinos & Watson (Paxinos and Watson, 1986). Examples of the injection sites included in the analysis are illustrated in Fig. 6.

3. Discussion

The results of the present study confirm and extend previous findings indicating that the brain levels of Ang II and Ang-(1–7) are markedly reduced in the brain of TGR(ASrAOGEN) while plasma levels are unchanged (Caligiorne et al., 2008; Huang et al., 2001). We also found that the cardiovascular and sympathetic responses evoked by interference with $GABA_A$ receptors in the PVN are attenuated in TGR(ASrAOGEN). The current experiments provide further support for the hypothesis of an excitatory role for angiotensin peptides in the hypothalamic paraventricular nucleus in the regulation of sympathetic outflow.

It was previously reported that TGR(ASrAOGEN) exhibits more than a 90% reduction in AOGEN levels in the brain while plasma levels are unchanged (Schinke et al., 1999). This reduction is pronounced in the hypothalamus and medulla (Schinke et al., 1999). These brain regions are known as sites of action for Ang II and Ang-(1–7) within specific nuclei involved in the maintenance of sympathetic output

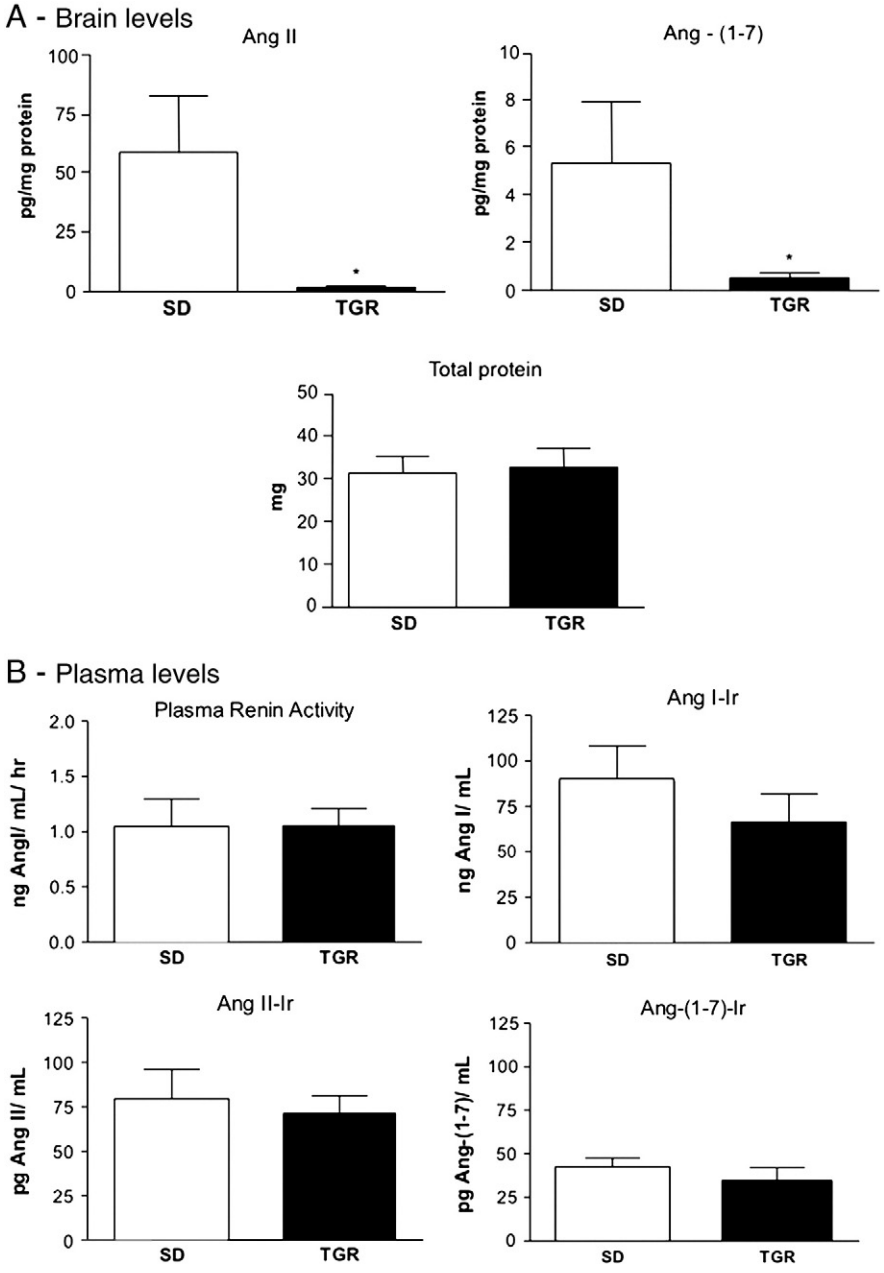

Fig. 1 – Brain and plasma profile of renin-angiotensin system components measured via radioimmunoassay. SD, Sprague Dawley rats; TGR, TGR(ASrAOGEN) (see text for details). *P < 0.05 compared with control SD rats (Student's t test).

Table 1 – Basal values for MAP and HR before muscimol microinjections into the PVN.

Experimental group	*n*	MAP (mm Hg)	HR (bpm)
SD Mus (1 nmol/100 nL)	5	101 ± 3	299 ± 3
TGR Mus (1 nmol/100 nL)	6	89 ± 4	290 ± 20
SD Bic (1 nmol/100 nL)	4	96 ± 2	356 ± 17
TGR Bic (1 nmol/100 nL)	4	94 ± 3	323 ± 13

All values are expressed as mean ± SEM. Data analyzed by ANOVA followed by Newman Keuls. MAP, mean arterial pressure; HR, Heart Rate.

and blood pressure, including the PVN and rostral ventrolateral medulla (RVLM) (Chen and Toney, 2003; Dampney et al., 2002a, 2002b; Fontes et al., 1994; Silva et al., 2005). While brain Ang II and Ang-(1–7) are reduced in the TGR(ASrAOGEN), we observed that plasma levels of Ang II, Ang-(1–7), Ang I and renin were unaltered in TGR(ASrAOGEN). The data are consistent with the hypothesis that the different functional findings observed in this model are due, at least in part, to a reduction in the activity of brain Ang II and Ang-(1–7).

Strikingly, we found that injection of muscimol into the PVN of TGR(ASrAOGEN) resulted in an attenuated fall in blood pressure, heart rate and RSNA when compared to the effect observed in SD rats. It is of interest to mention that muscimol is a powerful neuronal inhibitor acting as an

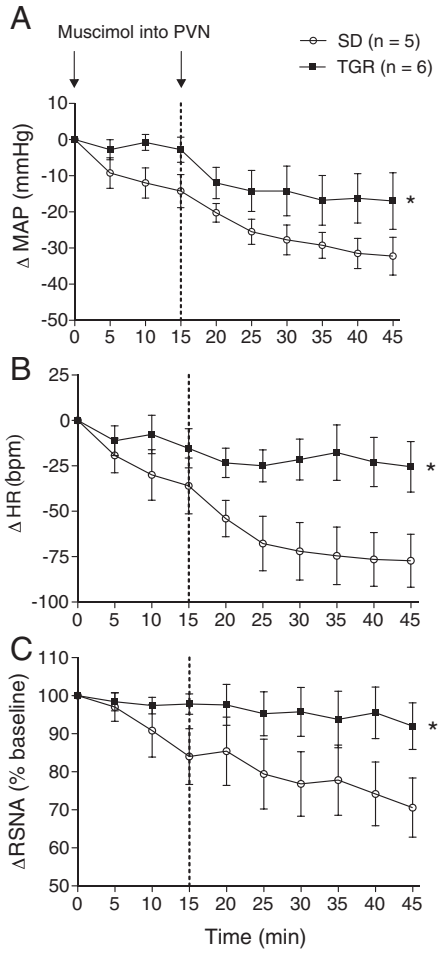

Fig. 2 – Grouped data showing changes in mean arterial pressure (A; MAP, mm Hg), heart rate (B; HR, bpm) and (C) renal sympathetic nerve activity (C; RSNA, % of baseline) evoked by unilateral (at 0 min) and contralateral (at 15 min) nanoinjections of muscimol (1 nmol/100 nL) into the PVN of TGR(ASrAOGEN) (TGR) or SD rats. * $P<0.05$ compared to SD group (Two-way ANOVA).

agonist on $GABA_A$ receptors, which virtually all neurons possess (Brown et al., 1981). Microinjections of muscimol into the PVN decreases blood pressure, heart rate and sympathetic activity, as observed in the control experiments in this and other previous studies (Allen, 2002; Ito et al., 2002; Silva et al., 2005). This attenuated effect leads us to conclude that the sympathetic output generated by PVN neurons in TGR(ASrAOGEN) is suppressed. In addition, the fall in MAP, HR and RSNA produced by intravenous hexamethonium was significantly reduced in TGR(ASrAOGEN) suggesting that overall sympathetic activity is reduced in this transgenic rat lineage. Our current results are in agreement with previous findings showing that the stress induced increases in blood pressure and plasma renin activity is greatly attenuated in this TGR model (Baltatu et al., 2004b). Since these effects are mediated by sympathetic reactivity, it further supports our conclusions.

Therefore, together with our current data showing a large reduction in brain levels of Ang II and Ang-(1–7) in this transgenic rat model, it is reasonable to suggest that the suppressed sympathetic outflow mediated by PVN neurons in TGR(ASrAOGEN) might be a consequence of the reduced excitatory activity exerted by angiotensin peptides in this premotor neuronal group. In this regard, the participation of RAS peptides in the PVN maintaining sympathetic outflow and blood pressure has been extensively investigated. All components of the RAS exist in the PVN (Ferguson and Washburn, 1998). Ang II and Ang-(1–7) can influence the excitability of PVN neurons (Ambuhl et al., 1992, 1994; Cato and Toney, 2005; Felix et al., 1991; Zhang et al., 2002b) and modulate sympathetic activity (Silva et al., 2005; Tagawa and Dampney, 1999). Taking into account that the deficiency of AOGEN synthesis is particularly evident in the hypothalamus of TGR(ASrAOGEN) (Schinke et al., 1999), it is likely that the formation of Ang II or Ang-(1–7) is also diminished in the PVN of this rat lineage. Assuming this possibility, the angiotensinergic excitatory drive over PVN neurons (Ambuhl et al., 1992; Cato and Toney, 2005; Zhang et al., 2002b) would also be reduced. At least for Ang II, a previous report showing that the AT_1 receptor binding is significantly higher in the PVN of TGR(ASrAOGEN) corroborates this hypothesis (Monti et al., 2001).

This hypothesis is also supported by additional experiments where we found that the increases in HR and RSNA evoked by removal of gabaergic tone produced by injection of bicuculline into the PVN of TGR(ASrAOGEN) was largely suppressed when compared to that observed in control rats. The increased neuronal activity through disinhibition must include a mechanism responsible for exciting a specific neuronal population. In the case of PVN, it was previously demonstrated in Sprague–Dawley rats that pressor, tachycardic and renal sympathoexcitatory responses to bicuculline injection into this nucleus were significantly attenuated by prior blockade of local AT_1 receptors (Chen and Toney, 2003). This finding provided evidence that at least part of the source of excitation that contributes to the autonomic responses produced by $GABA_A$ receptor blockade in the PVN depend on activation of local AT_1 receptors (Chen and Toney, 2003). Therefore, it is reasonable to conclude that the suppressed cardiac and renal sympathoexcitatory responses observed after injection of bicuculline into the PVN of TGR(ASrAOGEN) is due to a reduction in the excitatory input mediated by endogenous Ang II, and possibly Ang-(1–7) into this nucleus.

It could be argued that part of the response attributed to muscimol in this study is a consequence of the diffusion of this agent to the dorsomedial hypothalamus (DMH). Since the spreading of muscimol into the hypothalamus was not strictly defined, we cannot discard such possibility. However, as shown in the histological analysis our injections were all targeted to the PVN, which anatomical landmarks are easily identifiable. Second, the role of PVN in the maintenance of blood pressure and sympathetic activity is well known (Coote, 2005), as well as the effects of muscimol in the PVN, including using similar volume and dose (Allen, 2002; Silva et al., 2005; Zhang et al., 2002a). In spite of this, the cardiovascular reactivity to stress in TGR(ASrAOGEN) is largely suppressed when compared to SD rats (Baltatu et al., 2004a). Since the DMH is a key nucleus for the cardiovascular response to emotional stress (DiMicco et al., 2002) it is quite possible that the reactivity of DMH neurons in this transgenic rat can also be altered as a result of reduced

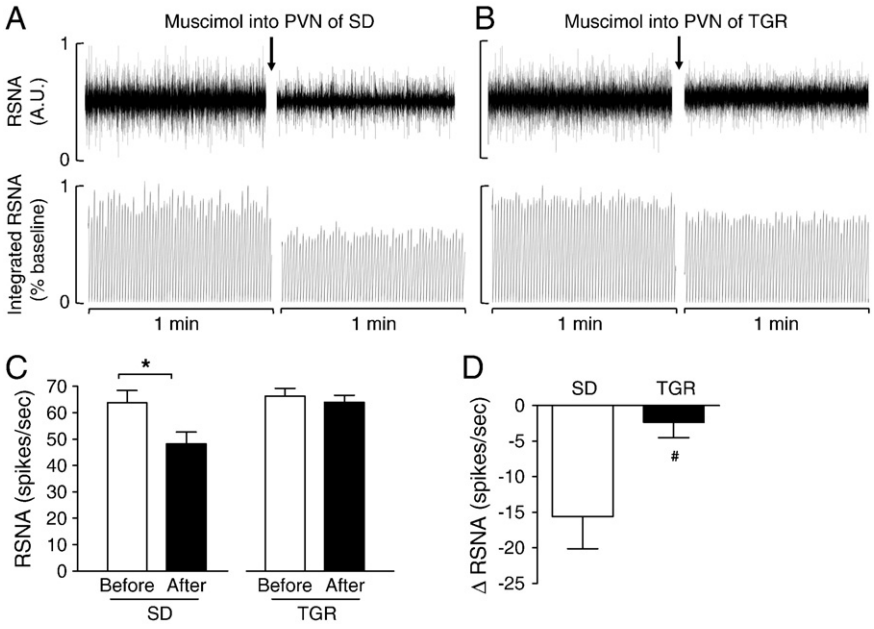

Fig. 3 – Panels A and B — Original recordings of renal sympathetic nerve activity (RSNA, V) illustrating the baseline period and changes induced by nanoinjections of muscimol 15 min after the bilateral injection (1 nmol/100 nL) into the PVN of SD (panel A) or TGR(ASrAOGEN) (TGR) (panel B). Panel C — Baseline and mean maximum renal sympathetic nerve activity changes (RSNA, spikes/s) measured after injections of muscimol (1 nmol/100 nL) into the PVN of SD rats or TGR. The significance between groups is shown in panel D after taking the difference of the two repeated measures (before and after as shown in Fig. 3C) for each group.* $P<0.05$ for TGR vs SD (unpaired t test).

angiotensin peptides in the brain. However, other experiments are necessary to further confirm this hypothesis.

It is important to mention that, apart from hypothalamus, there are other brain regions where the reduction in the excitatory drive exerted by angiotensins could influence in the sympathetic activity and cardiovascular function. In this regard we have previously reported that microinjections of Ang II into the RVLM of TGR(ASrAOGEN) evoked a larger pressor effect when compared to control rats (Baltatu et al., 2001). As mentioned above, the reduction of AOGEN is pronounced in the medulla of TGR(ASrAOGEN) (Schinke et al., 1999). Altogether, these findings suggests that a reduction of angiotensinogen in the medulla of this rat lineage leads to an increased sensitivity of Ang II receptors in the RVLM due to the absence of endogenous formation of Ang II. Therefore, because the RVLM is a key brain region involved in the maintenance of sympathetic activity and blood pressure, it is possible that the reduction of the excitatory input exerted by Ang II in the RVLM (Dampney et al., 2002b) may additionally contribute to the reduced sympathetic outflow observed in TGR(ASrAOGEN).

The question then arises as to why the basal RSNA is similar in TGR(ASrAOGEN) and SD if the sympathetic outflow mediated by PVN neurons in TGR(ASrAOGEN) is largely suppressed. Based on current data we cannot account for the underlying mechanisms responsible for this paradox. The simplest explanation is that, as a result of reduced excitatory drive exerted by angiotensins in the PVN of TGR(ASrAOGEN), another sympathetic premotor group could maintain the sympathetic outflow to the kidney stabilizing the RSNA baseline levels. This hypothesis is plausible since other neuronal circuits responsible for maintaining normal levels of blood pressure and sympathetic tone in anesthetized conditions are particularly located caudal to midbrain (Koshiya and Guyenet, 1994).

In conclusion, the results of our study indicate that the contribution of PVN to the renal sympathetic outflow is reduced in rats with low synthesis of brain AOGEN. It is possible that, this reduction is due to a diminished excitatory drive resulting from lowered hypothalamic angiotensins production in this rat lineage. Finally, our findings reinforce the need for further investigation of the contribution of Ang peptides in the PVN during pathophysiological conditions, such as hypertension or chronic heart failure.

4. Experimental procedures

4.1. *General procedures*

Adult male TGR(ASrAOGEN) and Sprague–Dawley (SD) Hanover rats, weighing 400–450 g, were obtained from the animal breeding unit of the Hypertension Laboratory at the Biological Sciences Institute (UFMG, Belo Horizonte, MG, Brazil). All experiments conform to the regulation set forth by National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication 80–23, revised in 1996) and were also approved by our local Institutional Animal Welfare Committee (CETEA–UFMG, protocol number: 137/2006). Under urethane anesthesia (1.2 to 1.4 g/kg i.p.), catheters were placed in a femoral artery and vein, followed by tracheotomy. The adequate quantity of anesthesia was verified by the absence of a withdrawal

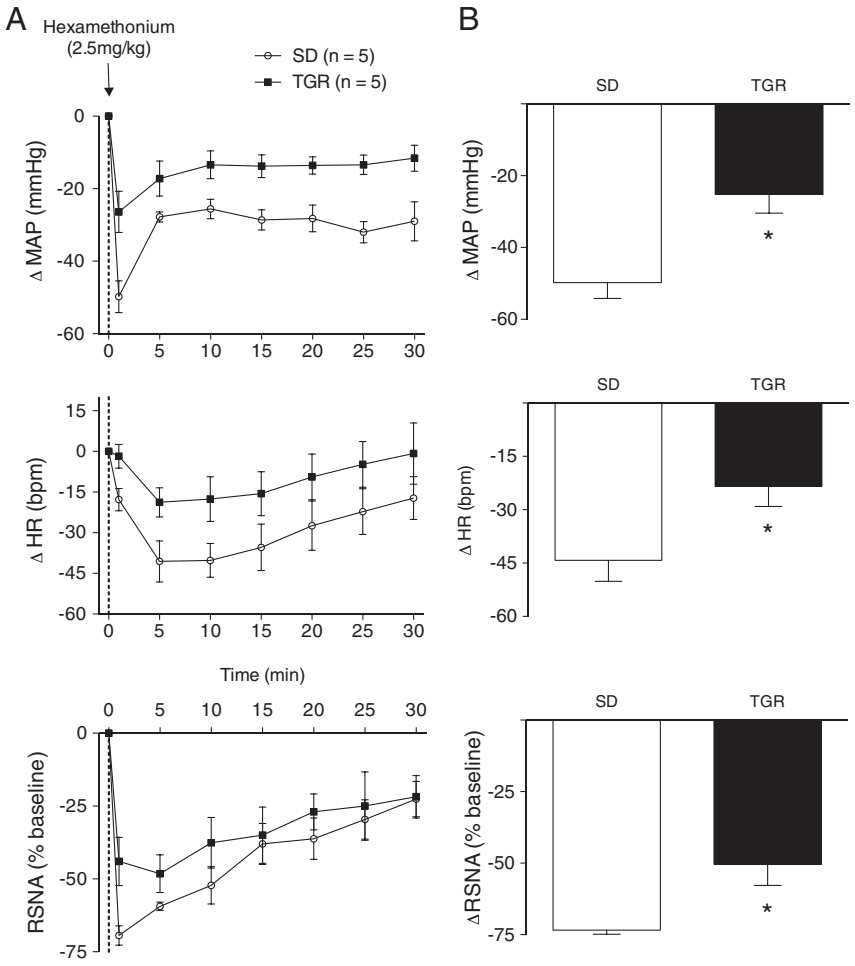

Fig. 4 – Cardiovascular effects produced by intravenous injection of the autonomic ganglion blocking agent hexamethonium (2.5 mg/kg, Sigma) in TGR(ASrAOGEN) and SD rats (n = 5 per group). (A) Time course and (B) maximum changes in cardiovascular variables during the period recorded. * $P < 0.05$ compared to SD group (unpaired t test).

response to nociceptive stimulation of the hindpaw. Supplemental doses of urethane (0.1 g/kg i.v.) were administered when necessary. Temperature was monitored and maintained in the range of 36.5 °C to 37.5 °C with a heating lamp. The head was placed in a stereotaxic frame with the tooth bar fixed −3.3 mm below the interaural line. A small craniotomy was made near the bregma to allow for a later insertion of a micropipette into the PVN.

4.2. *Renal SNA recording*

Renal nerve recording was performed as previously described (Silva et al., 2005). Briefly, the left kidney was exposed using a retroperitoneal approach. A nerve fascicle to the kidney was isolated, placed on a bipolar recording electrode and covered with mineral oil. The neural signals were amplified by 10 K, passed through a band pass filter (100–1000 Hz, Tektronix), displayed on a cathode ray oscilloscope (Tektronix) and monitored by means of an audio amplifier. The filtered nerve activity signal was rectified, integrated (resetting every 2 s) and collected for displaying and later analysis using a Power Lab data acquisition system (AD Instruments). All data were digitized at 1 kHz and stored in a PC.

4.3. *Experimental protocol*

For the duration of each experiment, MAP, HR and RSNA were recorded continuously (Power Lab, ADInstruments). After all surgical procedures were completed there was a waiting period of 20 min, to allow the measured cardiovascular variables to stabilize. The baseline MAP, HR and RSNA were then recorded for a 20 min period that was followed by microinjection procedures.

4.4. *Activation and blockade of $GABA_A$ receptors in the PVN, intravenous injection of hexamethonium*

Activation and blockade of $GABA_A$ receptors was performed by microinjecting the $GABA_A$ agonist muscimol (1 nmol/100 nL, Sigma) or the antagonist bicuculline methiodide (20 pmol/100 nL, Sigma), respectively, bilaterally into the PVN. Each compound was tested in separated group of rats. The effects were compared between TGR(ASrAOGEN) and SD rats (n = 5–6 per group). A 30-gauge injection needle connected to a Hamilton syringe (5 μL) was used as described previously (Silva et al., 2005). The coordinates for microinjections were determined by the atlas of Paxinos and Watson (Paxinos and

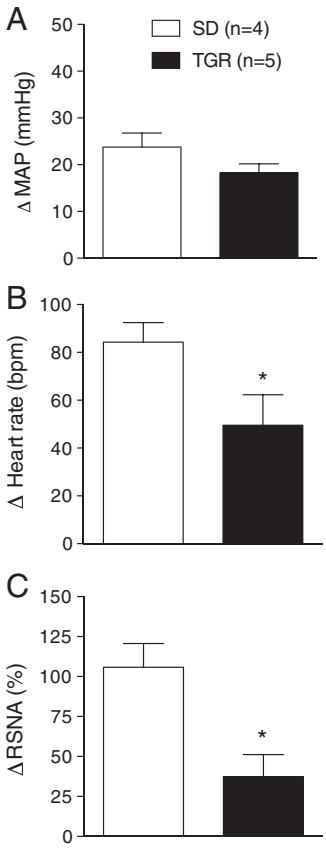

Fig. 5 – Average grouped results for the increases in mean arterial pressure (MAP) (panel A), heart rate (HR) (panel B) or renal sympathetic nerve activity (RSNA) (panel C) evoked by bilateral nanoinjection of bicuculline (BMI, 20 pmol/100 nL) into the PVN of TGR(ASrAOGEN) (TGR) (black bars) or SD rats (white bars). *P<0.05 compared with the effect produced by microinjection of BMI into the PVN of SD rats (unpaired *t* test).

Watson, 1986) in the track located 1.8 mm posterior, 0.5 mm lateral to the bregma and at a depth of 7.8 mm below the dura. At the end of each experiment, a bilateral microinjection of 2% alcian blue dye (100 nL) was made, locating the sites of injection within the PVN, which was confirmed histologically, as described previously (da Silva et al., 2011). In additional groups of experiments, the cardiovascular effects produced by intravenous injection of the autonomic ganglion blocking agent hexamethonium (2.5 mg/kg, Sigma) was compared between TGR(ASrAOGEN) and SD rats (n=5 per group).

4.5. *Peptides measurement*

Animals were killed by decapitation and the trunk blood was rapidly collected into chilled tubes containing EDTA (for plasma renin activity) or 1 mM p-hydroxymercuribenzoate, 30 mM of 1,10-phenanthroline, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM pepstatin A, and 7.5% EDTA (140 μL/mL of blood) for angiotensin measurements. In parallel, whole brain was quickly removed and frozen in liquid nitrogen and stored at −80 °C until assayed. After centrifugation, plasma samples were frozen in dry ice and stored at −20 °C. The brain was homogenized in 3 mL of HCl 0.045 N in ethanol containing 0.90 μM *p*-hydroxymercuribenzoate, 131.50 μM 1,10-o-phenanthroline, 0.90 μM PMSF, 1.75 μM pepstatin A, 0.032% EDTA and 5 μL of 5% protease-free bovine serum albumin (BSA). Next, plasma and brain homogenates were extracted using C18 Bond Elut cartridges (Analytichem International, Harbor City, CA) as previously described (Botelho et al., 1994). After extraction, samples were evaporated and resuspended in 500 μL of aqueous solution containing 0.1% BSA, 0.9% NaCl and 0.03% acetic acid. Angiotensin concentration was corrected for total protein determined by the Bradford method (Bradford, 1976).

4.6. *Data analysis*

To compare baseline RSNA between TGR(ASrAOGEN) and SD rats, the number of spikes of identified action potentials in the raw and filtered recordings was averaged using the Lab Chart/Spike histogram tool from (LabChart Pro 7.2 software — ADInstruments, Australia). For that, the background noise was determined from the post-mortem noise at the end of each experiment as previously reported for similar analysis (Hamza and Kaufman, 2007; Iwashita et al., 2002). A 5 min period of the background noise recording was selected, a 10 s bin was reselected by zooming (polarity turned positive), and the threshold was determined (mean maximum amplitude value averaged from noise level). Following, a 5 min period of the experimental recording was selected, a 10 s bin was reselected and the detected threshold was informed to the software. Starting from the threshold, the rate of spikes was determined as spikes/second (see details in methodological supplement). The RSNA responses evoked by injections into the PVN were also expressed as percent changes from baseline, calculated by the integrated voltage. The integrated voltage obtained during background noise was subtracted from the integrated voltage recorded during baseline and experimental recording periods.

The baseline values of MAP and HR were measured as the average values of these variables for the 1-minute period immediately preceding microinjections procedures. Experimental values were obtained from a mean across 1-minute, collected in 5 min intervals for the duration of the experiment.

Statistical comparisons between the baseline values of MAP and HR were assessed by ANOVA followed by Newman Keuls. Changes from baseline evoked by injection of drugs were assessed by paired *t*-test. Comparisons between responses evoked by injections of drugs into PVN of SD and TGR(ASrAOGEN) groups were determined by two-way ANOVA followed by Bonferroni post-hoc test or Student's *t*-test. Differences in peptide concentrations between SD and TGR(ASrAOGEN) were assessed by unpaired *t*-test. A value of $P<0.05$ was taken to indicate a statistically significant difference. All values are presented as mean±SEM.

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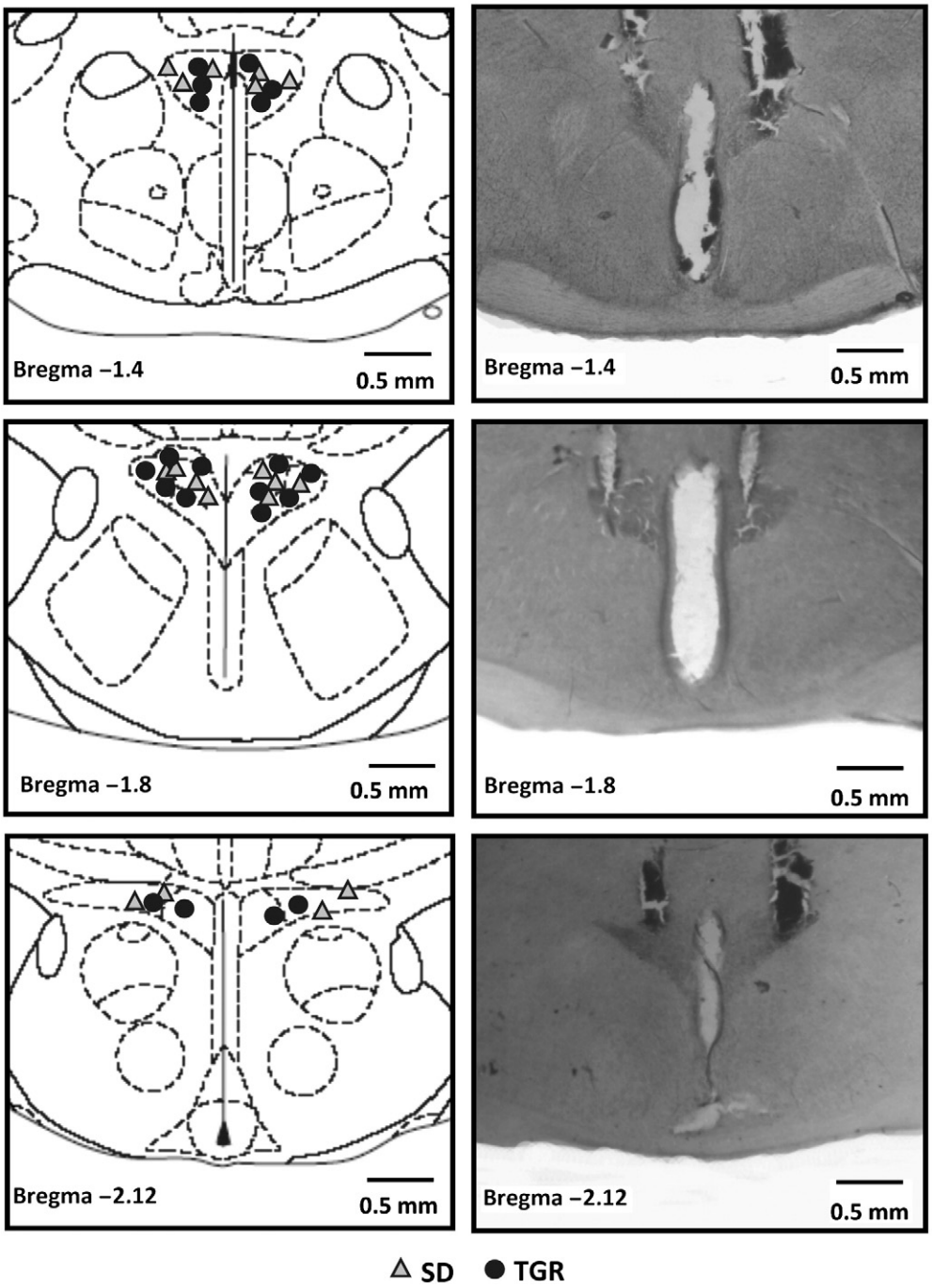

Fig. 6 – Schematic representations and histological images of serial sections from rostral (–1.4) to caudal (–2.1) extention of the PVN (Paxinos and Watson, 1986). Gray triangles represent nanoinjection sites into the PVN of Sprague Dawley (SD) rats while black circles represent nanoinjections sites into the PVN of TGR(ASrAOGEN) [TGR]; all representative symbols were considered within the PVN for a total of 19 rats.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.brainres.2012.02.021.

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