Published in final edited form as:

J Hypertens. 2013 September ; 31(9): 1837–1846. doi:10.1097/HJH.0b013e3283622198.

Role of angiotensin AT2 receptors and nitric oxide in the cardiopulmonary baroreflex control of renal sympathetic nerve activity in anaesthetised rats

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Abstract

This study investigated the role of AT2 receptor activation and the possible interaction with nitric oxide (NO) in low-pressure baroreceptor regulation of renal sympathetic nerve activity (RSNA). Renal sympatho-inhibition to a saline volume expansion (VEP, 0.25% bwt/min I.V. for 30 min) was studied following intracerebroventricular (i.c.v.) saline, CGP42112 (CGP, AT2 agonist), PD123319 (PD, AT2 antagonist) and losartan (AT1 antagonist), and then in combination with L-NAME (NO synthase inhibitor). i.c.v. saline, PD, CGP, and Losartan did not change baseline mean arterial pressure, heart rate or RSNA. VEP decreased RSNA in all groups by 58 to 60% ($P<0.05$). CGP enhanced the decrease in RSNA compared to saline (79% vs. 60%, $P<0.05$), whereas PD was without effect (58% vs. 57%). L-NAME only increased baseline RSNA when co-administered with PD ($P<0.05$). VEP-induced reduction in RSNA following L-NAME was less than during i.c.v. saline (39% vs. 53%, $P<0.05$). In the group where PD preceded L-NAME, the fall in RSNA was smaller than when PD was infused alone (40% vs. 63%, $P<0.05$) but not if PD followed L-NAME (52% vs. 44%). L-NAME did not change the magnitude of VEP-induced sympatho-inhibition following CGP (55% vs. 61%). Losartan, enhanced the renal sympatho-inhibition to VEP (65% vs. 58%, $P<0.05$) the magnitude of which was unchanged when L-NAME was present (67% vs. 62%). These findings suggest that NO is important in allowing the normal renal sympatho-inhibitory response to VEP which does not depend on AT2 receptors but AT2 receptor activation independently enhances the VEP induced reduction in RSNA.

Keywords

AT2 receptors; nitric oxide; cardiopulmonary baroreflex; Sympatho-inhibition; L-NAME; PD123319

Introduction

Angiotensin II (Ang II) AT1 and AT2 receptors have been reported to exist in many areas of the central nervous system (CNS) including the nucleus tractus solitaries (NTS), caudal and

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Author contributions

The authors have equally contributed to the design of the experiments, data collection, interpretation, and manuscript revision.

rostral ventrolateral medulla (RVLM), and paraventricular nucleus (PVN) (Mendelsohn *et al.* 1988, Downie *et al.* 2009). Although the contribution of central AT1 receptors in the control of cardiovascular function has been extensively examined, the specific role of AT2 receptors centrally and its importance to baroreflex control mechanisms is still enigmatic. The expression of AT2 receptors in brain regions involved with sympathetic function emphasizes the potential contribution of AT2 receptors to the regulation of sympathetic outflow (Lenkei *et al.* 1997, Gao *et al.* 2008). Brain AT2 receptors are hypothesized to antagonize the AT1 receptor-mediated pressor response by their action on the sympathetic outflow (Li *et al.* 2003b). Moreover, activation of AT2 receptors in the RVLM using its selective ligand, CGP42112 resulted in a sympatho-inhibitory response suggesting a potential role of AT2 receptors in blood pressure control (Gao *et al.* 2008). It has been proposed that AT2 receptors exert their actions through an increase in nitric oxide (NO) or bradykinin release (Sosa-Canache *et al.* 2000, Tsutsumi *et al.* 1999). Moreover, AT2 receptors possess a facilitatory effect on membrane potassium currents in cultured neurons (Kang *et al.* 1993). The latter effect was abolished by the pretreatment with the AT2 antagonist, PD123319, and the NO synthase (NOS) inhibitor *N*^ω-nitro-L-arginine methyl ester (L-NAME) (Gao & Zucker, 2010). A recent study revealed that AT2 receptors are functionally related to neuronal NOS in somata and dendrites of NTS neurons (Wang *et al.* 2012). According to this study, centrally produced NO antagonizes AT1 receptor-mediated generation of reactive oxygen species (ROS) and inhibits L-type Ca²⁺ currents. Nevertheless, there is a lack of supporting evidence for a contribution of centrally generated NO on AT2 receptor functions in *in vivo* whole animal studies.

The renal sympatho-inhibitory mechanisms mediated by the cardiopulmonary baroreceptors play a major role in determining sodium and water balance via the neural control of the kidney, in the maintenance of cardiovascular homeostasis. The afferent components of the reflex are necessary for the vascular adjustments to volume expansion (Colombari *et al.* 2000) as these mechanoreceptors are sensitive to an increase or decrease in circulatory volume (Ludbrook, 1990) and thereby result in reflex blunting or enhancing of renal sympathetic nerve activity (RSNA) (Shepherd, 1982).

The aim of this study was to investigate whether within the central nervous system NO contributed to the impact of AT2 receptor activation on low-pressure baroreceptor regulation of RSNA. This was accomplished by provoking cardiopulmonary receptors, using an acute saline volume expansion to cause a renal sympatho-inhibition, before and following sequential blockade of brain AT1 and AT2 receptors or following AT2 receptor activation either before or following NOS inhibition with L-NAME.

Methods

Male Wistar rats, 300–350 g, were purchased from commercial suppliers and maintained in the Biological Service Unit at University College Cork, Cork, Ireland. The rats were fed a regular laboratory diet and tap water *ad libitum* and were under a 12:12-h dark-light regime at 20±3°C and 35% humidity. All procedures were performed in accordance with national guidelines and the European Community Directive 86/609/EC and with the approval of the local Animal Experimentation Ethical Committee at University College Cork.

Surgical procedure

The overnight fasted rats were anaesthetized with an intraperitoneal injection of a chloralose/urethane mixture (Sigma-Aldrich, UK) (16.5 and 250 mg/ml, respectively) of 1-1.2 ml initially and maintained with supplemental doses of 0.05 ml *i.v.* being given approximately every 30 min. Body temperature was kept between 34-36 °C throughout the surgery and experiment. The trachea was cannulated to allow free air passage. A cannula (PE 50, Portex, UK) was inserted into the right femoral artery, for mean arterial pressure (MAP) and heart rate (HR) measurement and the femoral vein for infusion of saline (150 mM NaCl; 3 ml/h), the saline volume load (0.25% bwt/min *i.v.* saline for 30 min) and supplementary anaesthetic as required. The bladder was catheterized through a small abdominal incision to allow free flow of urine. The rat head was placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) and a hole was drilled into the skull and a stainless steel cannula was inserted into the right lateral cerebral ventricle at 1.0 mm posterior to the bregma, 2.5 mm lateral to the midline, and 2.55 mm ventral to the surface of the dura (Huang & Johns, 2001, Paxinos & Watson, 2005, Houghton *et al.* 2010). The correct positioning of the cannula was verified by observing slow extrusion of cerebrospinal fluid from the end of the cannula (Kapusta & Kenigs, 1999, Wainford & Kapusta, 2009) and by injection of Brilliant Blue dye at the end of the experimental protocol (Houghton *et al.* 2010) to confirm distribution of dye through the ventricles. The *i.c.v.* cannula was connected to a 25 µl Hamilton microsyringe (Hamilton, USA) fitted to a microinfusion pump (KD Scientific, Linton Instruments, UK). The left kidney was exposed retroperitoneally, by a flank incision and a renal sympathetic nerve bundle dissected out, with the use of an operating microscope, and cleared of connective tissue and sealed in place onto stainless-steel recording electrodes using silicon dental glue (Klasse4Dental, Augsburg, Germany).

Blood pressure was monitored using a pressure transducer attached to a quad bridge amplifier (ADInstruments, Hastings, UK). RSNA was recorded using a high impedance head stage mounted on a micromanipulator and attached to a low noise and high gain amplifier (NeuroAmp EX®, ADInstruments, Hastings, UK). The renal sympathetic nerve signals were distributed into an audio amplifier and a PowerLab data acquisition system connected to a computer. RSNA was amplified and filtered (gain 100x; high- and low pass filters set at 100 Hz to 2 KHz, respectively), digitized with a sampling rate of 1000 Hz/s, and stored in the computer for later off-line analysis. LabChart 7 software (ADInstruments, Hastings, UK) was used to process and analyze the data. Raw signals for pulsatile blood pressure were utilized to generate MAP and HR while the original RSNA signal was rectified and integrated. The integrated signal was used to study the baroreceptor controlled renal sympatho-inhibition.

Protocols

The animals were allowed at least 2 h after the surgical procedure before baseline MAP, HR and RSNA were recorded for 5 min. The basal value of RSNA was taken as 100% and the percentage reduction from this value was calculated at 5 min intervals. *i.c.v.* administration of saline or drugs was initiated for 10 min at 30 µl/h followed by a maintenance infusion of 7.5 µl/h for 20 min before the first acute saline volume expansion was carried out whereby

saline was infused via the femoral vein at a rate of 0.25% body weight per min for 30 min (Patel *et al.* 1995, Buckley & Johns, 2011), followed by a 30-min recovery period in order for variables to return to baseline levels. The *i.c.v.* maintenance infusion of saline or drug was continued throughout the 30 min volume expansion period. Data were continuously collected throughout the volume expansion and the recovery period. A second *i.c.v.* infusion was then initiated 2 h after the first volume expansion and a second acute saline volume expansion challenge was performed followed by a 30-min recovery period. A 5-min baseline recording of MAP, HR and RSNA was taken before and after the first and second *i.c.v.* infusion. Similarly, a 5-min baseline value of MAP and HR was recorded before and 30 min following the start of *i.v.* saline volume load. At the end of the experimental protocol, the animals were killed humanely using an intravenous overdose of anaesthetic and 30 min later the background noise was recorded. The noise value was subtracted from all original recordings of the integrated signal and used during the volume expansion and baseline data analysis.

Experimental groups

Nine groups of rats were used:

The first five groups received saline in the first *i.c.v.* infusion at 30 μ l/h as a loading dose for 10 min then the rate was reduced to 7.5 μ l/h as a maintenance dose. The second *i.c.v.* infusion was then switched to either saline (n=5); CGP42112 (CGP, 50 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=6) (Gao *et al.* 2008), a selective AT₂ receptor agonist; PD123319 (PD, 50 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7) (Gao *et al.* 2008), a selective AT₂ receptor antagonist; losartan (7.5 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7) (Huang & Johns, 2001), a selective AT₁ receptor antagonist, or nitro-L-arginine methyl ester (L-NAME, 150 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7) (Kadekaro *et al.* 1998, Moore *et al.* 1991).

Another three groups (n=7) of rats received CGP (50 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose), PD (50 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose) or losartan (7.5 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose) in the first *i.c.v.* infusion. Then the second *i.c.v.* infusion was switched to CGP plus L-NAME (50 μ g/kg+150 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7), PD plus L-NAME (50 μ g/kg+150 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7) or losartan plus L-NAME (7.5 μ g/kg+150 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose, n=7) respectively.

One group (n=7) received L-NAME (150 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose) in the first *i.c.v.* infusion followed by L-NAME plus PD (150 μ g/kg+50 μ g/kg at 30 μ l/h for 10 min loading then the rate was reduced to 7.5 μ l/h as a maintenance dose) in the second one.

Data analysis

Data were analysed offline and presented as mean $\pm$ SEM. The baseline values of MAP, HR and integrated RSNA, recorded for 5 min immediately prior to the start of the volume expansion were presented as the actual values and were compared using a paired Student's *t*-test. During the volume expansion and recovery periods 5 min bins of data were averaged. The comparisons of the reduction in RSNA due to volume expansion 25-30 min after starting i.v. saline infusion are presented as a percentage change from the baseline within the groups and were analysed using repeated-measures two-way ANOVA followed by Bonferroni *post hoc* test using GraphPad Prism® 5.0 for Windows (GraphPad Software, San Diego, California, USA) for specific means comparisons with reference to baseline values. Data for the time control group were compared by repeated-measures one-way ANOVA followed by Bonferroni *post hoc* test. A paired Student's *t*-test was utilized to compare responses between the first and second volume expansions. Values of $P < 0.05$ were considered statistically significant.

Results

Table 1 shows that the baseline levels of MAP, HR and RSNA were similar before and after saline infusion in the time control group. The haemodynamic and RSNA values in all saline i.v. infused rats were not significantly different from the values recorded before i.v. saline infusion. The i.v. infusion of PD, CGP, and Losartan had no effect on baseline MAP, HR and integrated RSNA. On the other hand, i.v. L-NAME infusion significantly increased ($P < 0.05$) baseline RSNA when administered together with i.v. PD in the PD/PD+L-NAME and L-NAME/L-NAME+PD groups but had no significant impact in the Saline/L-NAME, CGP/CGP+L-NAME or Losartan/Losartan+L-NAME groups.

The MAP and HR levels at baseline, following 30 min volume expansion, and after 30 min recovery period during the first and the second volume expansion are shown in Table 2. After 30 min volume expansion period, there was a significant decrease in MAP values from the baseline levels of 5 to 9 mmHg following i.v. saline infusion in saline/saline, saline/L-NAME and saline/CGP groups. In addition, the baseline HR values decreased significantly ($P < 0.05$) by 21 to 25 bpm after 30 min volume expansion except in the saline/losartan, losartan/losartan+L-NAME and CGP/CGP+L-NAME groups. During the second volume expansion period, the baseline MAP decreased significantly ($P < 0.05$) after 30 min volume expansion except in saline/losartan or losartan/losartan+L-NAME group. Similarly, baseline HR decreased significantly ($P < 0.05$) after 30 min volume expansion in all groups (Table 2). The magnitude of the decrease in MAP following saline volume expansion after i.v. administration of CGP+L-NAME was significantly greater ($P < 0.05$) than the decrease following i.v. CGP alone. Likewise, the decrease in HR following saline volume expansion after i.v. administration of CGP+L-NAME or losartan+L-NAME was significantly greater ($P < 0.05$) than the decrease following i.v. CGP or losartan alone respectively.

The time course of the reflex renal sympatho-inhibition due to the acute saline volume expansion is given in figure 1. The figure shows that the patterns and magnitudes of renal sympatho-inhibition to the first and second volume expansions had no time related

differences between them. The reduction in RSNA following the initiation of saline volume expansion during the first and the second challenges was evident 5 min after starting the saline volume load and became significantly ($P<0.05$) different from the baseline at 15 min in the first phase and remained significantly lower than the baseline 5 min after stopping the i.v. infusion. In both first and second volume expansions, the decrease in RSNA reached a maximum of 58% at the end of the 30 min of saline volume load i.v. infusion. Following cessation of volume challenge, the RSNA started to recover back to almost its initial baseline level following both challenges.

Figures 2 and 3 illustrate the RSNA response in all experimental groups which were subjected to two consecutive periods of acute volume expansion followed by a 30-min recovery period. The first and the second volume expansion periods elicited a similar maximum decrease in RSNA in response to the 30 min saline challenge (Fig. 2A). In the Saline/PD group, the first period of volume expansion, produced a maximum decrease in RSNA of about 58%, ($P<0.05$) from the baseline level (Fig. 2B) and in the second period when i.c.v. PD was infused, RSNA decreased by some 57% ($P<0.05$). There was no significant impact of PD on the reflex renal sympatho-inhibition induced by volume expansion. On another hand, in the saline/CGP group, there was a significant ($P<0.05$) decrease of nearly 60% during the i.c.v. saline infusion, but during the second saline load when i.c.v. CGP was infused, the decrease in RSNA was approximately 79% ($P<0.05$) (Fig. 2C) which was significantly larger ($P<0.05$) than that during the initial saline i.c.v. infusion. The effect of i.c.v. Losartan administration compared to vehicle is given in Fig. 2D. During the first volume expansion period, whereby i.c.v. saline was infused, RSNA decreased by about 58% ($P<0.05$) but when i.c.v. losartan was administered RSNA decreased approximately 65% ($P<0.05$) which was significantly greater ($P<0.05$) than that recorded during the initial i.c.v. saline challenge. The effect of L-NAME on the reflex sympatho-inhibition in response to the volume expansion is shown in Fig 2E. The magnitude of the response of RSNA to volume expansion when saline was given i.c.v. was a decrease by almost 61% ($P<0.05$) and following the i.c.v. L-NAME infusion, RSNA decreased by an approximate 48% ($P<0.05$) from the baseline level which was significantly smaller ($P<0.05$) than the response obtained when saline was infused i.c.v.

The effect of L-NAME administration on the reflex renal sympatho-inhibitory response to volume expansion in the presence of AT2 agonist and antagonist and AT1 antagonist is shown in Fig. 3. When PD was administered during the first saline overload period, the maximum decrease in RSNA following volume expansion was nearly 64% ($P<0.05$) (Fig. 3A) but during the second volume expansion whereby L-NAME was infused together with PD, the maximum decrease reached approximately 42% ($P<0.05$) which was significantly ($P<0.05$) smaller than when undertaken with i.c.v. PD alone (Fig. 3A). When L-NAME was administered prior to the first volume expansion period, RSNA was reduced by some 52% ($P<0.05$) (Fig. 3B) while in the second volume expansion period in which PD was given along with L-NAME i.c.v., RSNA decreased by approximately 44% ($P<0.05$) (Fig. 3B) which were responses that were not significantly different from each other. In the CGP plus L-NAME group, CGP was administered i.c.v. initially and the reflex sympatho-inhibition induced by volume expansion was an approximate 61% decrease ($P<0.05$) (Fig. 3C) but

during the second volume expansion period, when CGP was administered *i.c.v.* together with L-NAME, RSNA was reduced by almost 55% ($P<0.05$) which was not significantly different from that produced following *i.c.v.* CGP infusion (Fig. 3C). When losartan was administered in the first volume expansion period, RSNA decreased by 62% ($P<0.05$) (Fig. 3D) and in the second volume expansion challenge when L-NAME was co-infused with losartan, the RSNA decreased by about 67% ($P<0.05$), which was similar to the first volume expansion (Fig. 3D).

Discussion

The findings from the current study reveal significant independent roles for both central AT2 receptors and NO in the low-pressure baroreflex control mechanisms initiated by the acute saline volume expansion. The primary objective of the current study was to investigate whether there was a contribution of NO to the low-pressure baroreceptor regulation of RSNA and whether this was dependent on AT2 receptors. Rats were saline volume expanded and the reduction in RSNA measured before and after AT2 receptor stimulation or blockade, and AT1 receptor and NOS blockade. To our knowledge, this study showed for the first time that under normal conditions, the level of endogenous activation of AT2 receptors in the brain was such that it did not contribute significantly to the cardiopulmonary control of RSNA. By contrast, exogenous stimulation of AT2 receptors with its agonist produced an enhancement in the magnitude of the reflex inhibitory response but this effect was clearly not dependent on an intact NO system. These findings support the notion that the reflex renal sympatho-inhibitory mechanism to volume expansion is dependent on a functional NO system in the brain and may be independently modulated by activation of AT2 receptors.

In the present study, anaesthetized rats were subjected to an acute saline volume load of 0.25% body weight for 30 min which probably increased central venous pressure and activated cardiopulmonary baroreceptors which initiated a characteristic reflex renal sympatho-inhibition (Colombari *et al.* 2000, Patel *et al.* 1995, Morita & Vatner, 1985). This resulted in a substantial reduction in RSNA of between 58 to 60% by the end of the saline challenge period and quickly recovered back to its baseline level during the recovery period. This experimental challenge has been used previously and had been found to cause a similar 50-70% reduction in RSNA in a reproducible time related manner (Buckley & Johns, 2011).

The baseline values of RSNA in the group treated with PD plus L-NAME were significantly increased which accords with previous reports showing that NO acts as a sympatho-inhibitory substance within the central nervous system (Patel *et al.* 2001). In addition, the intracisternal injection of the NOS inhibitor, L-NMA was reported to produce an increase in RSNA (Togashi *et al.* 1992). The data from the sympatho-inhibitory response to saline volume expansion supports the increase in RSNA following *i.c.v.* infusion of PD plus L-NAME. It can be seen that PD plus L-NAME *i.c.v.* blunted the renal sympatho-inhibition in response to volume expansion compared to PD phase, which could be interpreted as an underlying renal sympatho-excitation. It should be noted that *i.c.v.* L-NAME produced no significant change in arterial pressure or heart rate which is in agreement with a previous study in the mouse using a similar dose of L-NAME *i.c.v.* (Moore *et al.* 1991). Losartan

injection into the lateral ventricle in the present study had no impact on baseline levels of MAP, HR or RSNA which is consistent with that reported in conscious rabbits using a dose comparable to that utilized in the present study (Badoer *et al.* 2000).

Data regarding the effect of saline volume expansion on baseline arterial pressure and heart rate revealed a decrease in these parameters compared to the baseline levels in most of the experimental groups. The decrease in arterial pressure and heart rate following the saline challenge is associated with an inhibition of efferent RSNA in all groups. Such pattern of results was shown previously to be attributed to the interaction between cardiopulmonary and arterial baroreflex (Wong & Johns, 1999, Persson, 1996).

As reviewed previously by Gao and Zucker (2010), the contribution of AT₂ receptors to the central nervous system function and the sympatho-inhibitory mechanism has been reported via studies involving gene deletion of AT₂ receptors in mice (Ichiki *et al.* 1995). In addition, there is clear evidence that central AT₂ receptors are involved in the regulation of sympathetic outflow in the rat (Gao *et al.* 2008). These investigators showed that microinjection of the AT₂ receptor agonist, CGP42112 into the RVLM, at a relatively similar dose to that utilized in the current study, produced hypotension, bradycardia, and sympatho-inhibition. The administration of PD into the lateral ventricle in the present study produced no significant impact on the reflex renal sympatho-inhibition elicited by saline challenge compared to the vehicle. This contrasts with the CGP infusion which increased the magnitude of the renal sympatho-inhibitory response to volume expansion compared to the control phase. This may indicate that under normal conditions endogenous stimulation of AT₂ receptors is minimally involved in initiating a change in the reflex regulation of RSNA in response to volume expansion. Moreover, it would suggest a more predominant role played by AT₁ compared to AT₂ receptors at these sites of the brain. Indeed, losartan administration was found to enhance the sensitivity of the cardiopulmonary baroreceptor mediated renal sympatho-inhibition to volume expansion.

A previous report from this lab demonstrated that blockade of brain Ang II receptors with losartan resulted in a heightened sensitivity of the high pressure baroreflex gain curve for RSNA (Huang *et al.* 2006). This would be compatible with a tonic inhibitory action by endogenous Ang II acting via AT₁ receptors. In addition, i.c.v. administered losartan has been shown to increase the increment of renal sympatho-inhibition due to volume loading in normal (DiBona *et al.* 1998) as well as heart failure rats (DiBona *et al.* 1995). These authors suggested that endogenous Ang II significantly impacts on the normal cardiac baroreflex regulation of RSNA during volume expansion. Interestingly, it has been shown in rabbits that the injection of losartan into the lateral ventricle produced no significant impact on the reflex renal sympatho-inhibition elicited by volume expansion (Badoer *et al.* 2000). One possible reason may be that the blockade of AT₁ receptors by losartan means that the agonist is available to bind to AT₂ rather than AT₁ receptors to initiate a sympatho-inhibitory response similar to that produced when CGP was administered. Alternatively, the enhanced renal sympatho-inhibitory response elicited by CGP to the volume expansion may be due to AT₂ receptor activation of central NO pathways. Furthermore, a recent *in vitro* study by Wang *et al.* (2012) suggested that both AT₂ receptors and neuronal NOS co-localise in somata and dendrites of the NTS and that AT₂ receptors activation enhances NO

production. However, the results from the current study have clearly shown that the volume expansion induced renal sympatho-inhibitory response was unaltered when CGP was co-administered with L-NAME.

Certain brain regions which contribute to cardiovascular functions, such as PVN, have high levels of NOS enzyme suggesting an important role for NO in the brain (Vincent & Kimura, 1992, Li *et al.* 2002). In agreement with this view, the present study showed that i.c.v. L-NAME significantly reduced the magnitude of renal sympatho-inhibition compared to vehicle. This would be consistent with the notion that inhibition of NO synthesis impairs the low-pressure baroreflex control of RSNA (Scrogin *et al.* 1994). Conversely, it has been shown that inhibition of NO by L-NAME in the PVN does not impact on the normal renal sympatho-inhibition elicited by volume expansion in rabbits (Ng *et al.* 2004). Acute saline volume expansion has been suggested to increase NO production within the PVN which leads to renal sympatho-inhibition which in turn results in diuresis and natriuresis (Li *et al.* 2003a). The administration of L-NAME into the lateral ventricle in the presence of losartan produced no impact on the increased cardiopulmonary baroreceptors sensitivity following losartan infusion alone. The absence of any response of NO blockade when losartan was co-infused was similar to the response to CGP infusion following L-NAME infusion.

In conclusion, the current study showed that a functional central NO system importantly contributes to the normal baroreflex mechanisms initiated by the volume expansion regardless of whether the central renin-angiotensin system was intact or blocked. The basal level of central AT2 receptors activation may not be necessary for a normal renal sympatho-inhibition due to volume expansion unless the counter regulatory AT1 receptors are blocked. Further, when AT2 receptors were exogenously activated by its selective agonist, an augmented sensitivity of the cardiopulmonary baroreceptors to acute saline volume expansion became evident which was NO independent. These observations may have significant relevance to clinical conditions for example in renal failure and hypertension whereby normal baroreflex mechanisms are impaired. However, the interaction between central AT2 receptors and NO in the control of arterial baroreflex mechanism and its clinical significance needs further investigation.

Acknowledgements

This project is supported by the Wellcome Trust.

Abbreviations

Ang II	angiotensin II
CNS	central nervous system
NTS	nucleus tractus solitaries
RVLM	rostral ventrolateral medulla
PVN	paraventricular nucleus
AT1 receptors	angiotensin II (type 1) receptors

AT2 receptors	angiotensin II (type 2) receptors
NO	nitric oxide
NOS	nitric oxide synthase
L-NAME	<i>N</i> ^ω -nitro-L-arginine methyl ester
ROS	reactive oxygen species
RSNA	renal sympathetic nerve activity
mM	mili molar
i.c.v.	intracerebroventricular
Hz	hertz
KHz	kilo Hertz
MAP	mean arterial pressure
HR	heart rate
CGP	CGP42112
PD	PD123319
VEP	volume expansion

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Fig. 1.

Time course of the reflex renal sympatho-inhibition due to volume expansion in the control group. After starting *i.v.* saline volume expansion, the percentage change in RSNA from the baseline was taken at 5 min intervals through the 30-min volume expansion period and the 30-min recovery period. *, # $P < 0.05$, compared with the baseline level for the first and second volume expansion respectively. RSNA, renal sympathetic nerve activity.

Fig. 2.

The renal sympathetic nerve activity responses to two sequential acute saline volume expansions in LCV. saline infusion followed by either saline (**A**), PD (**B**), CGP (**C**), Losartan (**D**) and L-NAME (**E**). The baseline value of RSNA was taken as 100% and the maximum sympatho-inhibition due to volume expansion was the average value over the 25-30 min while the recovery of RSNA was taken 30 min after stopping the saline challenge. * $P < 0.05$, compared with baseline. # $P < 0.05$, compared with the 25-30 min value of the first volume expansion. VEP, volume expansion.

Fig. 3.

The renal sympathetic nerve activity responses to two sequential acute saline volume expansions in PD/PD+L-NAME (**A**), L-NAME/L-NAME+PD (**B**), CGP/CGP+L-NAME (**C**) and Losartan/Losartan+L-NAME (**D**). The baseline value of RSNA was taken as 100% and the maximum sympatho-inhibition due to volume expansion achieved at 25-30 min following the start of the saline volume load while the recovery of RSNA was taken after 30 min of stopping the saline challenge. * $P < 0.05$, compared with baseline. # $P < 0.05$, compared with the first volume expansion. VEP, volume expansion.

Table 1

Mean arterial pressure, heart rate, and renal sympathetic nerve activity obtained before and following the first and the second I.C.V. infusions. Values are expressed as means $\pm$ SEM.

	Parameter	First I.C.V. infusion		Second I.C.V. infusion	
		Before	After	Before	After
Saline/Saline		<i>Saline</i>		<i>Saline</i>	
	MAP (mmHg)	74 $\pm$ 4	73 $\pm$ 4	75 $\pm$ 5	76 $\pm$ 5
	HR (bpm)	300 $\pm$ 12	298 $\pm$ 14	299 $\pm$ 10	302 $\pm$ 10
	RSNA (μ V.s)	0.99 $\pm$ 0.28	1.15 $\pm$ 0.29	0.90 $\pm$ 0.28	0.94 $\pm$ 0.27
Saline/PD		<i>Saline</i>		<i>PD</i>	
	MAP (mmHg)	70 $\pm$ 1	70 $\pm$ 1	77 $\pm$ 5	86 $\pm$ 3
	HR (bpm)	320 $\pm$ 14	315 $\pm$ 14*	321 $\pm$ 15	324 $\pm$ 17
	RSNA (μ V.s)	0.67 $\pm$ 0.14	0.74 $\pm$ 0.18	0.56 $\pm$ 0.14	0.61 $\pm$ 0.17
PD/PD+L-NAME		<i>PD</i>		<i>PD+L-NAME</i>	
	MAP (mmHg)	71 $\pm$ 3	73 $\pm$ 4	84 $\pm$ 6	84 $\pm$ 5
	HR (bpm)	314 $\pm$ 14	314 $\pm$ 16	321 $\pm$ 27	327 $\pm$ 24
	RSNA (μ V.s)	0.57 $\pm$ 0.16	0.58 $\pm$ 0.17	0.59 $\pm$ 0.21	0.69 $\pm$ 0.23*
Saline/L-NAME		<i>Saline</i>		<i>L-NAME</i>	
	MAP (mmHg)	70 $\pm$ 6	71 $\pm$ 7	78 $\pm$ 10	79 $\pm$ 9
	HR (bpm)	318 $\pm$ 16	312 $\pm$ 16	316 $\pm$ 19	315 $\pm$ 18
	RSNA (μ V.s)	1.18 $\pm$ 0.23	1.23 $\pm$ 0.20	1.42 $\pm$ 0.40	1.56 $\pm$ 0.45
L-NAME/L-NAME+PD		<i>L-NAME</i>		<i>L-NAME+PD</i>	
	MAP (mmHg)	71 $\pm$ 6	73 $\pm$ 6	86 $\pm$ 5	86 $\pm$ 5
	HR (bpm)	297 $\pm$ 11	289 $\pm$ 12	317 $\pm$ 14	318 $\pm$ 14
	RSNA (μ V.s)	0.77 $\pm$ 0.22	0.79 $\pm$ 0.25	0.78 $\pm$ 0.24	0.82 $\pm$ 0.24*
Saline/CGP		<i>Saline</i>		<i>CGP</i>	
	MAP (mmHg)	72 $\pm$ 5	70 $\pm$ 4	79 $\pm$ 5	78 $\pm$ 5
	HR (bpm)	312 $\pm$ 15	310 $\pm$ 16	300 $\pm$ 18	312 $\pm$ 15
	RSNA (μ V.s)	0.59 $\pm$ 0.14	0.59 $\pm$ 0.14	0.51 $\pm$ 0.10	0.55 $\pm$ 0.10
CGP/CGP+L-NAME		<i>CGP</i>		<i>CGP+L-NAME</i>	
	MAP (mmHg)	67 $\pm$ 4	68 $\pm$ 5	85 $\pm$ 4	83 $\pm$ 4
	HR (bpm)	288 $\pm$ 13	280 $\pm$ 14*	311 $\pm$ 17	321 $\pm$ 19
	RSNA (μ V.s)	0.75 $\pm$ 0.16	0.87 $\pm$ 0.22	0.71 $\pm$ 0.21	0.75 $\pm$ 0.22
Saline/Losartan		<i>Saline</i>		<i>Losartan</i>	
	MAP (mmHg)	70 $\pm$ 1	71 $\pm$ 2	78 $\pm$ 4	79 $\pm$ 2
	HR (bpm)	297 $\pm$ 13	292 $\pm$ 16	291 $\pm$ 13	304 $\pm$ 13
	RSNA (μ V.s)	0.65 $\pm$ 0.22	0.68 $\pm$ 0.22	0.75 $\pm$ 0.24	0.78 $\pm$ 0.26
Losartan/losartan+L-NAME		<i>Losartan</i>		<i>Losartan+L-NAME</i>	
	MAP (mmHg)	68 $\pm$ 3	68 $\pm$ 3	79 $\pm$ 6	77 $\pm$ 6
	HR (bpm)	321 $\pm$ 20	321 $\pm$ 21	354 $\pm$ 23	356 $\pm$ 24
	RSNA (μ V.s)	0.68 $\pm$ 0.11	0.70 $\pm$ 0.12	0.74 $\pm$ 0.16	0.80 $\pm$ 0.19

* $P < 0.05$ after compared to before I.C.V. infusion. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity.

Table 2

Mean arterial pressure and heart rate obtained at baseline and following 30 min volume expansion. Values are expressed as means $\pm$ SEM.

	Parameter	First VEP		Second VEP	
		Baseline	30 min VEP	Baseline	30 min VEP
Saline/Saline	<i>Saline</i>			<i>Saline</i>	
	MAP (mmHg)	73 $\pm$ 2	66 $\pm$ 3*	81 $\pm$ 3	71 $\pm$ 3*
	HR (bpm)	298 $\pm$ 15	275 $\pm$ 13*	331 $\pm$ 20	309 $\pm$ 20*
Saline/PD	<i>Saline</i>			<i>PD</i>	
	MAP (mmHg)	76 $\pm$ 4	71 $\pm$ 6	81 $\pm$ 5	65 $\pm$ 3*
	HR (bpm)	312 $\pm$ 16	289 $\pm$ 16*	322 $\pm$ 16	296 $\pm$ 15*
PD/PD+L-NAME	<i>PD</i>			<i>PD+L-NAME</i>	
	MAP (mmHg)	67 $\pm$ 8	64 $\pm$ 8	78 $\pm$ 5	71 $\pm$ 7*
	HR (bpm)	311 $\pm$ 22	283 $\pm$ 22*	315 $\pm$ 20	290 $\pm$ 18*
Saline/L-NAME	<i>Saline</i>			<i>L-NAME</i>	
	MAP (mmHg)	76 $\pm$ 7	67 $\pm$ 8*	79 $\pm$ 7	67 $\pm$ 6*
	HR (bpm)	308 $\pm$ 20	278 $\pm$ 23*	292 $\pm$ 15	260 $\pm$ 15*
L-NAME/L-NAME+PD	<i>L-NAME</i>			<i>L-NAME+PD</i>	
	MAP (mmHg)	75 $\pm$ 6	71 $\pm$ 6	90 $\pm$ 6	81 $\pm$ 5*
	HR (bpm)	294 $\pm$ 14	271 $\pm$ 11*	320 $\pm$ 17	294 $\pm$ 17*
Saline/CGP	<i>Saline</i>			<i>CGP</i>	
	MAP (mmHg)	74 $\pm$ 6	65 $\pm$ 6*	77 $\pm$ 6	67 $\pm$ 6*
	HR (bpm)	297 $\pm$ 22	271 $\pm$ 20*	300 $\pm$ 15	272 $\pm$ 13*
CGP/CGP+L-NAME	<i>CGP</i>			<i>CGP+L-NAME</i>	
	MAP (mmHg)	66 $\pm$ 5	63 $\pm$ 5	85 $\pm$ 6	76 $\pm$ 6*#
	HR (bpm)	281 $\pm$ 16	267 $\pm$ 16	325 $\pm$ 15	302 $\pm$ 17*#
Saline/Losartan	<i>Saline</i>			<i>Losartan</i>	
	MAP (mmHg)	74 $\pm$ 1	71 $\pm$ 3	72 $\pm$ 4	71 $\pm$ 5
	HR (bpm)	300 $\pm$ 18	284 $\pm$ 14	336 $\pm$ 19	308 $\pm$ 16*
Losartan/losartan +L-NAME	<i>Losartan</i>			<i>Losartan+L-NAME</i>	
	MAP (mmHg)	76 $\pm$ 5	79 $\pm$ 5	87 $\pm$ 7	80 $\pm$ 7
	HR (bpm)	322 $\pm$ 20	313 $\pm$ 17	362 $\pm$ 23	346 $\pm$ 21*#

* $P < 0.05$ compared with baseline.

$P < 0.05$ compared with first volume expansion. VEP, volume expansion; MAP, mean arterial pressure; HR, heart rate.