Angiotensin 1A receptors transfected into caudal ventrolateral medulla inhibit baroreflex gain and stress responses

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Aims

The caudal ventrolateral medulla (CVLM) is important for autonomic regulation and is rich in angiotensin II type 1A receptors (AT1AR). To determine their function, we examined whether the expression of AT 1AR in the CVLM of mice lacking AT1AR (AT1A2/2) alters baroreflex sensitivity and cardiovascular responses to stress.

Methods and results

Bilateral microinjections into the CVLM of AT1A2/2 mice of lentivirus with the phox-2 selective promoter (PRSx8) were made to express either AT 1AR (Lv-PRSx8-AT1A) or green fluorescent protein (Lv-PRSx8-GFP) as a control. Radiotelemetry was used to record mean arterial pressure (MAP), heart rate (HR), and locomotor activity. Following injection of Lv-PRSx8-GFP, robust neuronal expression of GFP was observed with ≏60% of the GFP-positive cells also expressing the catecholamine-synthetic enzyme tyrosine hydroxylase. After 5 weeks, there were no differences in MAP or HR between groups, but the Lv-PRSx8-AT1A-injected mice showed reduced baroreflex sensitivity (−25%, P = 0.003) and attenuated pressor responses to cage-switch and restraint stress compared with the Lv-PRSx8-GFP-injected mice. Reduced MAP mid-frequency power during cage-switch stress reflected attenuated sympathetic activation (Pgroup × stress = 0.04). Fos-immunohistochemistry indicated greater activation of forebrain and hypothalamic neurons in the Lv-PRSx8-AT1A mice compared with the control.

Conclusion

The expression of AT1AR in CVLM neurons, including A1 neurons, while having little influence on the basal blood pressure or HR, may play a tonic role in inhibiting cardiac vagal baroreflex sensitivity. However, they strongly facilitate the forebrain response to aversive stress, yet reduce the pressor response presumably through greater sympatho-inhibition. These findings outline novel and specific roles for angiotensin II in the CVLM in autonomic regulation.

Keywords

Angiotensin type 1A receptor • Caudal ventrolateral medulla • Cardiovascular stress response • Baroreflex gain

1. Introduction

Angiotensin in the central nervous system plays an important role in the regulation of thirst, fluid volume, autonomic reflexes, and blood pressure (BP). A major influence of angiotensin is to promote the increase in BP through vasoconstriction, particularly during aversive stress where angiotensin in the hypothalamus and the rostral ventrolateral medulla (RLVM) is a key neuromodulator. We recently found that transfection of angiotensin II (AngII) type 1 receptors (AT;R) into the RLVM of mice lacking AT1R (AT1A−/−) restored the pressor response to stress. Angiotensin can also induce sympa-tho-inhibition in other regions of the brainstem such as the caudal ventrolateral medulla (CVLM), which is a major site for the integration and regulation of neuronal information controlling autonomic and neuroendocrine activity. The CVLM is a source of inhibitory input to the sympathetic premotor neurons of the RLVM. The CVLM contains a diversified neuronal population, with different groups containing markers of γ-aminobutyric acid (GABA), glutamate, and...
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catecholamine synthesis. The noradrenergic A1 cells in the CVLM have efferent projections to several areas related to central regulation of cardiovascular homeostasis. The A1 neurons are activated by a wide variety of physiological stressors such as haemorrhage, physical exercise, pain, immune activation, hypoxaemia, and hypernatremia. Selective lesions of A1 neurons reduce the sympathetic-inhibition produced by hypernatremia, suggesting that A1 neurons may directly contribute to vasomotor-inhibition. They also project directly to anxiety-related sites localized in the forebrain and communicate via afferent and efferent projections with the hypothalamic paraventricular nucleus (PVN).

The CVLM is rich in AT1R which, when activated, inhibit the sympathetic nervous system (SNS) and increase vasopressin release. Cage-switch stress induces large, sustained increases in BP and HR, and neuronal activation in multiple brain regions, including the CVLM. Interestingly the pressor and HR responses to cage-switch stress are reduced in the AT1A−/− mice and this is accompanied by a greater Fos expression in CVLM neurons. The blockade of angiotensin receptors in the CVLM, by microinjection of non-selective antagonists, has no effect on the resting state, but increases baroreflex-dependent sympathetic activity. Taken together, it is likely that AT1R located on noradrenergic A1 neurons in the CVLM play an important sympathetic-inhibitory role in an activated state such as during aversive stress. The A1 neurons may do this by activating GABA projections from the CVLM to the RVLM, but it is also possible that the GABA neurons may also express AT1R.

The technique of using lentiviruses to target transgene expression to selected cells enables an investigation of the role of specific cells and receptor systems in behavioural paradigms. This study utilizes this approach to target phox2-expressing cells in the CVLM with the multimeric PRS domain promoter, PRSx8. We have used this promoter in lentiviruses to express AT1AR in AT1A−/− mice and thus examine the cardiovascular role of these receptors in the CVLM.

2. Methods

Experiments were performed on 19 male AT1A−/− mice at 10 weeks of age. The experiments were approved in advance by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice and the Directive 2010/63/EU of the European Parliament for Scientific Use of Laboratory Animals.

2.1 Telemetry surgery

BP telemetry transmitters (model TA11PA-C10; Data Sciences International, St Paul, MN, USA) were implanted under isoflurane open circuit anaesthesia (4% induction and 1.5–2% maintenance). The adequacy of the anaesthesia was determined by the respiration rate as well as the absence of the blink and paw pinch reflex. Analgesic carprofen (5 mg/kg) was administered subcutaneously pre-operatively and also after 24 h. The catheter of the telemetry device was inserted into the carotid artery and the transmitter probe was positioned subcutaneously along the right flank. The animals were housed individually at the Baker IDI in a 12:12 h light–dark cycle (1 h.m.—1 p.m. light) room with access ad libitum to water and mouse chow (Specialty Feeds, Glen Forrest, Western Australia, 19% protein, 5% fat, 5% fibre, 0.2% sodium).

2.2 Telemetric measurement of BP, HR, and locomotor activity

Continuous recordings of systolic, diastolic and calculated mean arterial pressure (MAP), HR, and locomotor activity were made in freely moving mice in their home cage. The recordings were sampled at 1000 Hz using an analog-to-digital data acquisition card (National Instruments 6024E) as described previously. The beat-to-beat arterial pressure and HR were detected online and analysed later using a program written in Labview. The animals were housed individually on top of the telemetric receivers for several days to allow environmental adaptation before telemetry recording began.

2.3 Lentiviral stereotaxic microinjection

Bilateral microinjections of lentivirus with the synthetic phox2 selective PRSx8 promoter driving expression of either green fluorescent protein (GFP) (Lv-PRSx8-GFP; GFPv mice, n = 9), used as a control, or AT1AR (Lv-PRSx8-AT1A; AT1A+ mice, n = 10) were made into the CVLM of the AT1A−/− mice. Viruses were generated and produced as described previously. The microinjections were made under anaesthesia with intraperitoneal (ip) injection of ketamine (75 mg/kg), xylazine (10 mg/kg), and atropine (1.2 mg/kg) using a computer-aided stereotaxic apparatus (Angle Two™, Leica, USA). Analgesia and the depth of anaesthesia were as described above. Two 100 nL microinjections per side were made (coordinates from bregma: anterior/posterior: −7.56 and −7.76 mm; medial/lateral ± 1.2 mm, dorsal/ventral −5.17 mm, Figure 1A).

2.4 Protocol and experimental procedures

Following a 10-day recovery period from telemetry surgery, baseline cardiovascular parameters, and locomotor activity were recorded over 48 h before the lentiviral microinjection (week 0). Twenty-four hour telemetry recordings were then performed once a week over 5 weeks. In the fourth and fifth week, behavioural stimuli were performed during the daytime when the animals were relatively inactive.

The animals were exposed to appetitive or aversive stimuli as described previously. Feeding, a positive stimulus, involved giving a piece of almond for 5 min. Averse stimuli included restraint stress, which involved guiding the mouse into a cylindrical plexiglass restrainer with a sliding back plate to confine the animal for 5 min. Dirty cage-switch stress involved removing the mouse from its home cage and placing it for 1 h in a cage previously occupied by another male mouse. To account for the contribution of locomotor activity to cage switch, a matching 1 h restraint stress was also included. Five min stresses were randomized with a recovery period of at least 60 min. One hour stresses were performed individually on two separate days.

2.5 Immunohistochemical analysis (Fos- and double-labelled cells)

Immediately following 1 h of dirty cage-switch stress, five mice from each group were anaesthetised with an ip injection of sodium pentobarbitone (100 mg/kg) and perfused as described previously. Each brainstem section was processed for immunohistochemical double staining of tyrosine hydroxylase (TH) and GFP or the macrophage marker CD68. The CVLM placement was identified using the landmarks of the area postrema, nucleus of the solitary tract, central canal, and pyramids (Figure 1A). The remaining sections were processed for Fos-immunohistochemistry and blind analysis of expression in the CVLM, RVLM, nucleus of the solitary tract, supraoptic nucleus, medial amygdala, PVN, and dorsomedial hypothalamus was carried out in up to four sections per animal. Total counts of black-stained nuclei within the known boundaries of the nucleus were recorded.

2.6 In vitro autoradiography

Separate mice receiving identical microinjections of Lv-PRSx8-GFP (n = 3) or Lv-PRSx8-AT1A (n = 3) were anaesthetised with pentobarbitone, decapitated and the brain was removed. AT1(A)-binding density within the CVLM was performed using [125I]-[Sar1, Ile8]Ang II as a radioligand as described previously. Losartan (10 mM) was added to displace binding
from AT1R and distinguish between AT1R and AngII type 2 receptors (AT2R).

2.7 Cardiovascular variability and cardiac baroreceptor sensitivity
Beat-to-beat data were analysed to calculate power spectra for low frequency (0.08–0.3 Hz), mid-frequency (0.3–0.5 Hz), and high frequency (0.5–3 Hz), cross spectra for calculating cardiac baroreflex sensitivity at the mid-frequency. The sequence technique was also used to calculate the spontaneous baroreceptor reflex during stress (see Supplementary material online).

2.8 Statistical analysis
Cardiovascular data were expressed as mean ± standard error of the mean (SEM). The data were analysed by multi-factor, nested split-plot analysis of variance (ANOVA), which allowed for within animal and between animal contrasts. In the case where control (pre-viral injections) measures were obtained, the between group sums of squares was partitioned into main effects of groups (GFPv vs. AT1Av), virus (week 0 vs. week 5) or stress (rest vs. stress) and their interaction (groups × virus or groups × stress). The latter term reveals the effect of the presence of AT1ARs. For stress responses performed at week 4 and 5, the between group main effect was used to indicate the effect of the AT1AR. Factors and interactions were deemed significant when \( p < 0.05 \).
3. Results

3.1 Immunohistochemical analysis

Microinjection of Lv-PRSx8-GFP resulted in strong expression of GFP in neurons of the CVLM of the GFPv mice (Figure 1A and B), where 57% ± 4 of all the GFP-positive cells were TH positive. Of all the TH-positive cells within the region injected, 62% ± 5 expressed GFP. The neurochemical phenotype of the remaining non-TH-expressing, GFP-positive cells was not determined. The expression of the macrophage marker CD68 was used to localize the injection site in the AT 1Av mice. In all cases CD68 expression co-localized with TH-immunoreactive cells indicating the injections were within the region of A1 noradrenergic cells in the CVLM (Figure 1C). The extent and spread of viral injections from all animals included in this study is shown in the schematic coronal sections of mouse medulla oblongata (Supplementary material online, Figure S1).

3.2 Autoradiography analysis

Using in vitro autoradiographic methods, it was not possible to detect AT1R expression in the CVLM of the GFPv mice (Figure 1D). Low-level expression of AT1R can be observed in the presence of losartan in the midline in the inferior olivary nucleus. In contrast the expression of AT1R was clearly observed in the CVLM of the AT1Av mice (Figure 1D, middle panel) and this binding was displaced by co-incubation with the AT1R antagonist, losartan. The control wild-type CVLM had 3.5 ± 0.3 dpm/mm² (4 observations), whereas the viral-injected mice density was 1.1 ± 0.4 dpm/mm² (10 observations) corresponding to 31.4%.

3.3 Cardiovascular measurements and locomotor activity

Before viral microinjection, the 24 h averages of MAP, HR, and body weight were 85 ± 3 mmHg, 513 ± 20 b.p.m., and 23.1 ± 0.4 g, respectively (n = 19, Figure 2, Supplementary material online, Table S1). Five weeks following injection with lentivirus, GFPv, and AT1Av mice showed similar increases in MAP (8.4 ± 3.0 vs. 5.4 ± 2.3 mmHg, respectively, Ptime < 0.001, Supplementary material online, Table S1) and HR (15 ± 15 vs. 30 ± 21 b.p.m., respectively, Ptime < 0.01, respectively, Supplementary material online, Table S1). Body weight did not change in either group (Supplementary material online, Table S1). In both groups, the day/night differences in MAP, HR, and activity were not affected by the viral expression in week 5 compared with week 0 (Figure 2).

3.4 Cardiovascular variability

MAP variability at all frequencies was not different between groups or between times and there was no group virus interaction during either the inactive (Supplementary material online, Table S2) or active phase (Supplementary material online, Table S3). The HR mid-frequency power (0.3–0.5 Hz) during the active phase had diminished after

![Figure 2](image-url)
5 weeks and was not specific to the expression of AT1AR (F<sub>1,88</sub> = 6.5, P = 0.01, Figure 3B, Supplementary material online, Table S3). There were no other differences in HR power observed during either phase at any frequency.

### 3.5 Cardiac baroreflex sensitivity

Cross spectral analysis of BP and HR during the inactive period showed a marked reduction in baroreflex gain 5 weeks after the expression of AT1AR (F<sub>1,42</sub> = 13, P = 0.001) compared with no change in the GFPv mice (F<sub>1,35</sub> = 1.3, P = 0.3). This was also statistically evident as a significant group × virus interaction (F<sub>1,88</sub> = 4.0, P = 0.048, Figure 3A). In contrast, there was no effect of AT1AR expression on baroreflex sensitivity during the active period although virus expression itself (or time) was associated with reduced baroreflex gain during the active period (P = 0.01), presumably due to a reduction in HR mid-frequency power in both groups.

### 3.6 Cardiovascular response to behavioural stress tests

#### 3.6.1 Feeding: 5 min

Animals had a rapid (over 30 s) and then sustained pressor and tachycardic response to the positive eating stimulus (Figure 4A). The changes in MAP and HR in response to feeding were not different between the AT<sub>1AV</sub> and GFPv mice. The locomotor response to presentation of the novel food was markedly greater in the AT<sub>1AV</sub> mice compared with the GFPv mice (P = 0.001).

#### 3.6.2 Restraint: 5 min

A 5 min restraint stress elicited sustained pressor and tachycardic responses which were similar in both groups (P > 0.05; Figure 4B).

#### 3.6.3 Restraint: 1 h

The response to 1 h of restraint was characterized by an increase in MAP and HR, which declined over the period of stress, but remained greater than resting values by the end of 1 h (Figure 5A). Mice expressing AT<sub>1A</sub>R in the CVLM had a 25% smaller increase in MAP (21.0 ± 2.2 mmHg) compared with control mice (27.9 ± 2.2 mmHg, F<sub>1,54</sub> = 6.7, P = 0.01). The tachycardia response to restraint stress was similar in both groups with a peak in the first 10 min (P = 0.5).

#### 3.6.4 Dirty cage switch: 1 h

Dirty cage-switch stress also induced an immediate increase in MAP, HR, and locomotor activity, which declined over the 1 h period, but as for restraint stress remained above baseline at the end of the exposure (Figure 5B). Animals expressing AT<sub>1A</sub>R in the CVLM had lower MAP over the 1 h test, resulting in a 34% smaller MAP increase (17.2 ± 2.4 mmHg) compared with the GFPv mice (26.2 ± 2.2 mmHg, F<sub>1,72</sub> = 9.9, P = 0.003). The change in HR was similar in both groups (P = 0.1) and the increase in locomotor activity in response to the stress was greater in the AT<sub>1AV</sub> mice compared with the GFPv mice (P = 0.04).

### 3.7 MAP variability and cardiac baroreflex sensitivity during stress

Spectral analysis was performed during rest and the first 20 min of stress, when the effects of dirty cage switch were most evident. Stress increased the MAP mid-frequency power (0.3–0.5 Hz) in both the AT<sub>1AV</sub> and GFPv mice compared with the resting period (F<sub>1,64</sub> stress = 194, P < 0.001, Supplementary material online, Figure S2A). This effect was reduced in AT<sub>1AV</sub> compared with the GFPv mice, reflected by a significant
group × stress interaction ($F_{1,44\text{group} \times \text{stress}} = 4.4, P = 0.04$, Supplementary material online, Figure S2A). Baroreflex gain was reduced similarly in both groups during the stress period compared with the resting period ($P_{\text{stress}}, 0.001$).

During the first 20 min of stress, the baroreflex-dependent sequences were increased in both groups ($P_{\text{stress}} < 0.001$), whereas baroreflex activation index and baroreflex gain were reduced ($P < 0.01$, Supplementary material online, Figure S2B) compared with the control period.

### 3.8 Fos-immunohistochemical analysis following dirty cage-switch stress

Following a 1 h period of cage switch, the AT1A mice had greater levels of neuronal activation in the PVN, dorsomedial hypothalamus, medial amygdala, suprachiasmatic nucleus, CVLM, and RVLM compared with the GFP mice ($P < 0.05$, Figure 6). Only the nucleus of the solitary tract had less Fos labelling in the AT1A mice compared with the GFP mice ($P < 0.05$).

### 4. Discussion

The present study used a novel method of viral expression to transduce phox2 neurons in the CVLM of the AT1A mice with AT1AR and examined changes in cardiovascular regulation. The major finding from this study was that while basal levels of MAP and HR were not different between control GFP- and AT1AR-transfected mice, the sustained pressor response to 1 h stress (restraint and cage-switch) was attenuated in the AT1A mice expressing the AT1AR in the CVLM.

This is likely mediated via less sympathetic activation during stress as there was less increase in mid-frequency MAP power (Figure 6A), which is thought to be related to sympathetic activity. The neuronal activation, as assessed by Fos-immunohistochemistry, was higher in the CVLM suggesting a novel role of AT1AR providing sympatho-inhibition during stress. Interestingly, the brain areas associated with the arousal showed much greater Fos activation suggesting that AT1AR transduction of neurons in the CVLM may have driven a higher degree of arousal-induced activation in the forebrain. This may explain the greater locomotor response to feeding and cage-switch stress but this did not result in a greater pressor response but rather the converse, a lesser response for restraint and cage switch. The increased locomotor activity is likely a result of the greater forebrain activation presumably from olfactory—motor cortex connections since locomotor activity in itself does not increase activity in the amygdala or hypothalamus. The other major finding related to cardiac baroreflex sensitivity, which was markedly less in the AT1A mice compared with the GFP mice during the inactive state (when baroreflex gain is maximum), whereas during the dark period (when baroreflex gain is lowest) baroreflex sensitivity was similar in both groups. Taken together, these findings indicate that expressing AT1ARi phox2-expressing neurons of the CVLM produces quite specific changes to cardiovascular regulation.

In the present study, we have assumed that differences between groups are due to the presence of the transduced AT1ARs compared with GFP and hence represent a physiological restoration of the functional properties of the receptor. Previously, the same lentivirus with the synthetic phox2-selective PRSx8 was used to induce AT1AR expression in C1 neurons of the AT1A mice. The transfected...
AT1ARs responded to AngII injected in the RVLM, inducing a pressor and sympathoexcitatory response.\textsuperscript{18} Although not directly tested, it is likely that the receptors in the present study are similarly functional. The current findings are distinct and mostly opposite from the effects of AT1ARs transfected into the RVLM,\textsuperscript{3} suggesting the findings are specific to the region injected.

An important question is whether the observed changes are consistent with these receptors being incorporated into normal physiological processes that regulate cardiovascular-related sympathetic and cardiac vagal activity. The decrease in the pressor response to restraint and cage-switch stress may be explained by a much greater sympatho-inhibitory response coming possibly from the CVLM, presumably due to the presence and activation of AT1AR. Indeed in the CVLM twice as many neurons were activated following cage-switch stress, but the possibility that this could have such a profound limiting effect on the expression of the vasomotor response to stress was somewhat unexpected. Recently, we found that the AT1A\textsuperscript{2-/-} mice had an attenuated pressor response to dirty cage-switch stress with greater Fos activation in the CVLM and nucleus of the solitary tract.\textsuperscript{12} The current study suggests that AT1AR transduction in CVLM neurons caused further activation inducing greater sympatho-inhibition, which is consistent with the lesser MAP power in the AT1Av mice exposed to 1 h dirty cage-switch stress. There are many studies which support the view that AngII in the CVLM reduces BP via sympatho-inhibition. Microinjection of AngII in the CVLM in anaesthetized rabbits decreases BP, HR, and renal sympathetic nerve activity (RSNA).\textsuperscript{11,25} Saralasin, a non-selective angiotensin receptor antagonist, injected into the rabbit CVLM had no effect on basal RSNA but increased baroreflex-dependent changes in activity.\textsuperscript{13} More recently, the AT1R selective antagonist losartan blocked the depressor effect of AngII in the CVLM of normotensive and 2K1C hypertensive rats suggesting that AT1Rs are involved in the sympatho-inhibition.\textsuperscript{26} These observations indicate that AT1Rs in the CVLM have a depressor and sympatho-inhibitory effect under certain conditions (anaesthesia or hypertension) but not in a basal state. Thus, it is not unexpected that 5 weeks following both Lv-PRSx8-GFP and Lv-PRSx8-AT1A lentivirus microinjection, the AT1A\textsuperscript{2-/-} mice had similar levels of the basal BP and HR. Central AngII is known for increasing sympathetic activity\textsuperscript{1,27} and the pressor response to stress at the level of the DMH and RVLM\textsuperscript{2,28}. However, this is the first study to suggest that AT1R in the CVLM may also modulate the response to stress by inhibiting the sympatho-excitation and reducing the pressor response. The precise quantification of the relative contribution of the CVLM compared with AT1R in other regions (RVLM, hypothalamus, amygdala) is beyond the scope of the current study due to the absence of all the other AT1R from all brain regions with the exception of the CVLM.

A large proportion of cells transduced also contained TH, so it is likely that at least some of the effects observed in this study are due to the transduction of A1 neurons. Previous studies have shown that noradrenergic A1 neurons contain moderately intense, diffuse cytoplasmic AT1R-like immunoreactivity, and also occasionally show receptors in the noradrenergic dendrites of these cells.\textsuperscript{29}
Thus, the transduction of AT1AR using the PSRx8 promotor approach targets cells that normally express these receptors. The forebrain activation during stress is consistent with the known role of A1 and other noradrenergic neurons in stress. The observed effects on baroreflex function could occur via the A1 neurons as the noradrenergic neurotoxin 6-hydroxydopamine administered to conscious rabbits via the fourth ventricle abolishes all the baroreflex effects of blocking AT1R with losartan given by the same route. Prior to the present study, evidence supporting the involvement of AT1R and A1 neurons in the cardiovascular responses to stress had come from studies examining Fos immunoreactivity. Noradrenergic projections from A1 neurons terminate in limbic nuclei (central amygdala and the bed nucleus of the stria terminals) and hypothalamic areas (lateral hypothalamus and PVN). Thus, AT1AR in A1 cells magnify the degree of brain activation induced by stress as suggested by greater locomotor activation during dirty cage-switch stress and also during a feeding stimulus. All brain regions except the nucleus of the solitary tract show markedly greater activation as indicated by Fos counts during cage-switch stress in AT1Av compared with the GFPv mice. Reduced activation of the nucleus of the solitary tract inhibition is not unexpected, as Fos activation in this region is mainly from baroreceptor activation and thus reflects the lesser BP elevation to stress in the AT1Av mice. Increased activation of regions such as the amygdala, PVN, and DMH, regions known to be involved in mediating the emotional, hormonal, and cardiovascular responses to stress, is more surprising.

Although the findings of the present study together with the supportive evidence from the literature suggest that differences between groups may be largely due to transfection of noradrenergic A1 neurons, an important consideration is that a large number of lentivirus-expressing cells were not A1 neurons. Card et al. who showed that while all TH-positive cells in the CVLM of adult rats expressed Phox2a, around 50% of all Phox2a positive were TH negative. Furthermore the evaluation of Phox2b expression in adult rats shows co-localization with both TH-positive and TH-negative neurons. Each of these Phox2-expressing, non-TH cell types would express transgene when transduced with the virus used in this study. The precise contribution of the non-TH AT1AR transduced cells to the cardiovascular changes observed in this study remains to be determined. Although their phenotype has not been characterized, it is possible that a proportion of these neurons are GABAergic interneurons in the baroreceptor reflex and could thus contribute to the observed changes in reflex function.

One of the important findings was that differences between the AT1Av and GFPv mice in the pressor responses to stress were only observed after several minutes delay. The pressor responses to 5 min of restraint stress and feeding were not affected, but responses to the more long-lasting 1 h dirty cage-switch stress and restraint stress were attenuated in the AT1AR transduced mice. A previous study with air jet stress directed at conscious rabbits showed that only the late response after several minutes was attenuated following administration of losartan into the DMH or RVLM with the early response being dependent on excitatory amino acid receptors. Further, we have shown that the expression of AT1AR in the sympathetic excitatory C1 cells induced changes in the late sustained phase of the stress response.

![Figure 6](image_url)
in this region.47 Additionally, glutamate injected into rat CVLM inhibits 
tonation by AngII since angiotensin immunoreactive terminals, AT 1AR 
Chen D, Bassi JK, Walther T, Thomas WG, Allen AM. Expression of angiotensin type 1A receptors in C1 neurons restores the sympatho- excitation to angiotensin in the rostral ventrolateral medulla of angiotensin type 1A knockout mice. Hypertension 2010;56:143–150.
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