Blood pressure reactivity to emotional stress is reduced in AT1A-receptor knockout mice on normal, but not high salt intake

ARTICLE *in* HYPERTENSION RESEARCH · JUNE 2009

Impact Factor: 2.66 · DOI: 10.1038/hr.2009.59 · Source: PubMed

5 AUTHORS, INCLUDING:

Geoffrey A Head

Baker IDI Heart and Diabetes Institute

367 PUBLICATIONS **5,435** CITATIONS

SEE PROFILE

Hypertension Research (2009) 32, 559–564

www.nature.com/hr

ORIGINAL ARTICLE

Blood pressure reactivity to emotional stress is reduced in AT_{1A}-receptor knockout mice on normal, but not high salt intake

Daian Chen[1], Luisa La Greca[1], Geoffrey A Head[1], Thomas Walther[2] and Dmitry N Mayorov[3]

Pharmacological evidence suggests that angiotenisn II type 1 (AT_1) receptors are involved in the regulation of cardiovascular response to emotional stress and reinforcing effect of dietary salt on this response. In this study, we examined the effect of genetic deletion of AT_{1A} receptors on the cardiovascular effects of stress and salt in mice. AT_{1A} receptor knockout ($AT_{1A}^{-/-}$) and wild-type ($AT_{1A}^{+/+}$) mice were implanted with telemetry devices and placed on a normal (0.4%) or high (3.1%) salt diet (HSD). Resting blood pressure (BP) in $AT_{1A}^{-/-}$ mice (84 ± 3 mm Hg) was lower than in $AT_{1A}^{+/+}$ mice (107 ± 2 mm Hg). Negative emotional (restraint) stress increased BP by 33 ± 3 mm Hg in $AT_{1A}^{+/+}$ mice. This response was attenuated by 40% in $AT_{1A}^{-/-}$ mice (18 ± 3 mm Hg). Conversely, the BP increase caused by food presentation and feeding was similar in $AT_{1A}^{-/-}$ (25 ± 3 mm Hg) and $AT_{1A}^{+/+}$ mice (26 ± 3 mm Hg). HSD increased resting BP by 14 ± 4 mm Hg in $AT_{1A}^{-/-}$ mice without affecting it significantly in $AT_{1A}^{+/+}$ mice. Under these conditions, the pressor response to restraint stress in $AT_{1A}^{-/-}$ mice (30 ± 3 mm Hg) was no longer different from that in wild-type animals (28 ± 3 mm Hg). The BP response to feeding was not altered by HSD in either $AT_{1A}^{-/-}$ or $AT_{1A}^{+/+}$ mice (25 ± 2 and 27 ± 3 mm Hg, respectively). These results indicate that AT_{1A} receptor deficiency leads to a reduction in BP reactivity to negative emotional stress, but not feeding. HSD can selectively reinforce the cardiovascular response to negative stress in $AT_{1A}^{-/-}$ mice. However, there is little interaction between AT_{1A} receptors, excess dietary sodium and feeding-induced cardiovascular arousal.
Hypertension Research (2009) **32,** 559–564; doi:10.1038/hr.2009.59; published online 1 May 2009

Keywords: angiotensin II; blood pressure; knockout mouse; stress

INTRODUCTION

Increased cardiovascular reactivity to psycho-emotional stress is considered to be a risk factor for hypertension and heart disease.[1,2] The regulation of cardiovascular reactivity is a complex phenomenon involving several transmitters and pathways at different levels of the central and peripheral nervous system. Recent pharmacological studies, however, indicate that the activation of angiotensin II type 1 (AT_1) receptors, and specifically those in the dorsomedial hypothalamus (DMH) and rostral ventrolateral medulla (RVLM), is required for full expression of sympathetic cardiovascular responses to various psychoemotional stressors in rats and rabbits.[3–7] Nonetheless, the effect of genetic deficiency of AT_1 receptors on cardiovascular reactivity to stress is yet to be determined.

Apart from aversive events, appetitive stimuli are capable of inducing a distinct, sympathetically mediated rise in blood pressure and heart rate in animals.[8,9] Likewise, activated positive emotions, and in particular those initiated by personally relevant stimuli, are associated with increased blood pressure and heart rate in humans. In contrast to aversive events, these positive emotions are often linked to health protective responses.[10,11] Intriguingly, our initial studies showed that AT_1 receptors seem to be less essential in modulating cardiovascular arousal associated with appetitive events,[12] indicating that these receptors could be a selective therapeutic target for stress-related cardiovascular disorders.

It is well established that high dietary salt can facilitate the cardiovascular response to emotional stress in genetically predisposed animals and humans.[13–15] Central AT_1 receptors have been implicated in this reinforcing effect of dietary salt on the stress response in salt-sensitive rat models of hypertension.[16,17] Conversely, the effect of dietary salt loading on cardiovascular arousal associated with appetitive behavior remains to be determined, as is the role of AT_1 receptors in this effect.

In this study, we examined the cardiovascular response to aversive and appetitive stimuli in genetically engineered mice lacking one of the two main isoforms of murine AT_1 receptor (AT_{1A}). This receptor, which is pharmacologically indistinguishable from AT_{1B} receptor, has been shown to play a crucial role in the cardiovascular effects of angiotensin II.[18,19] We also determined the effect of dietary salt

[1]Baker Heart Research Institute, Melbourne, Victoria, Australia; [2]Centre for Biomedical Research, Hull York Medical School, University of Hull, Hull, UK and [3]Department of Pharmacology, University of Melbourne, Melbourne, Victoria, Australia
Correspondence: Dr DN Mayorov, Department of Pharmacology, University of Melbourne, Melbourne, Victoria 3010, Australia.
E-mail: dmayorov@unimelb.edu.au
Received 21 March 2009; accepted 23 March 2009; published online 1 May 2009

loading on cardiovascular arousal caused by aversive and appetitive stimuli in these animals. We used a telemetry blood-pressure monitoring system to take into account the confounding influence of locomotion on blood pressure,[20,21] which might mask or alter the effect of emotional arousal on cardiovascular function.

METHODS

General preparations

The experiments were carried out using 2–3-month-old male AT_{1A} receptor knockout ($AT_{1A}^{-/-}$) and wild-type ($AT_{1A}^{+/+}$) mice in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. $AT_{1A}^{-/-}$ mice and their control strain were bred at the animal facilities of the Baker Heart Research Institute. Mice were originally obtained from Prof. T Walther (animal facilities of Charité, Berlin, Germany). F2 generation mice were produced from crosses of (129×C57BL/6J) F1 $AT_{1A}^{-/+}$ parents. Mice were then maintained on a C57BL/6 background and genotyped using PCR analysis of DNA isolated from tail biopsies.

Under fluothane anesthesia, mice were implanted with TA11PA-C10 telemetry probes to measure blood pressure and locomotor activity (Data Sciences International, St Paul, MN, USA) as described elsewhere.[22] Each mouse was then housed individually. After a 1-week recovery period, mice were placed on a normal (0.4%) or high (3.1%) salt diet using the crossover design. Stress tests were conducted between 1100 and 1600 hours. Mice were maintained on a 12:12-h light–dark cycle (lights off at 1800 hours) with water and food *ad libitum*.

Measurement of BP, heart rate and locomotor activity

During the experiment, pulsatile arterial pressure and gross locomotor activity were monitored continuously, and were sampled at 1000 Hz using an analog-to-digital data acquisition card, and the beat-to-beat mean arterial pressure (MAP) and heart rate (HR) were detected online and analyzed using a program written in Labview (National Instruments, Austin, TX, USA), as described earlier.[21]

Emotional stress and feeding tests

The restraint and feeding tests were conducted as described earlier.[21] Briefly, the restraint test consisted of placing a mouse in a well-ventilated plexiglass restrainer for 5 min with a sliding back plate to confine the mouse without applying physical pressure on it. Feeding was initiated by placing a piece of almond ($\sim$0.5 g) in the mouse home cage. The tests were randomized and separated by at least 1-h recovery periods to ensure full recovery of cardiovascular parameters.

Cardiovascular reactivity to locomotor activity

To calculate the reactivity index, average MAP, HR and locomotor activity values were calculated over 30-s intervals, and the activity score was logarithmically transformed to correct for positive skew.[20,21,23] For each mouse, least-squares regression slopes for the relationships between MAP and activity, and HR and activity were calculated over 10-min periods (which included 5-min control and stress periods of 10 data points for each period) using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Statistical analysis

All values are expressed as mean ± s.e.m. Data were analyzed by two-way analysis of variance (ANOVA) to determine the effects of AT_{1A} receptor deficiency and stress on cardiovascular parameters and locomotion. The relationships between cardiovascular reactivity and physical activity (regression slopes) were compared by analysis of covariance (ANCOVA) using GraphPad Prism. Statistical significance was set at a value of $P<0.05$.

RESULTS

Restraint stress

Resting pre-stress levels of MAP in $AT_{1A}^{-/-}$ mice were lower by 25 ± 5 mm Hg than in $AT_{1A}^{+/+}$ mice ($P<0.001$), whereas basal HR and locomotor activity did not differ between groups (Table 1). Restraint stress caused prompt pressor (33 ± 3 mm Hg) and tachycardic (203 ± 19 b.p.m.) responses in $AT_{1A}^{+/+}$ mice (Figure 1). There was also a moderate increase in locomotor activity (245 ± 78 AU), as animals typically tried to escape from the restrainer. The pressor response to restraint stress was attenuated by 40% in $AT_{1A}^{-/-}$ mice (20 ± 3 mm Hg, P=0.01). The tachycardic and locomotor responses to restraint stress were similar between $AT_{1A}^{+/+}$ and $AT_{1A}^{-/-}$ mice (Figure 1).

There was a correlation between MAP and locomotion in both $AT_{1A}^{-/-}$ mice (r=0.35, $P<0.001$) and $AT_{1A}^{+/+}$ mice (r=0.51, $P<0.001$; Figure 1). Likewise, HR and locomotion were significantly correlated in both groups during the test (data not shown). MAP responsiveness to locomotion was not statistically different between $AT_{1A}^{-/-}$ mice (5.9 ± 1.0 mm Hg per $\log_{10}$ AU) and $AT_{1A}^{+/+}$ mice (8.2 ± 1.0 mm Hg per $\log_{10}$ AU). Similarly, HR responsiveness to locomotion did not differ between groups (data not shown).

Food presentation and feeding

Pre-feeding levels of MAP in $AT_{1A}^{-/-}$ mice were lower by 24 ± 4 mm Hg than in $AT_{1A}^{+/+}$ mice ($P<0.001$), whereas basal HR and locomotor activity did not differ between groups (Table 1). There was no difference between corresponding basal parameters before feeding and restraint tests (Table 1).

The presentation and eating of palatable food (almond) in $AT_{1A}^{+/+}$ mice elicited prompt behavioral arousal accompanied by pressor and tachycardic responses (26 ± 3 mm Hg and 113 ± 26 b.p.m.,

Table 1 Resting MAP, HR and locomotor activity before restraint and feeding in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice on normal salt intake

	Before restraint				*Before feeding*			
	Normal-salt diet		*High-salt diet*		*Normal-salt diet*		*High-salt diet*	
Strain	$AT_{1A}^{+/+}$	$AT_{1A}^{-/-}$	$AT_{1A}^{+/+}$	$AT_{1A}^{-/-}$	$AT_{1A}^{+/+}$	$AT_{1A}^{-/-}$	$AT_{1A}^{+/+}$	$AT_{1A}^{-/-}$
N	10	11	8	7	10	10	8	7
MAP, mm Hg	106 ± 2	81 ± 5*	114 ± 4	97 ± 2*#	107 ± 2	84 ± 4*	110 ± 4	95 ± 2*#
HR, b.p.m.	509 ± 16	515 ± 22	493 ± 17	491 ± 26	520 ± 17	524 ± 22	491 ± 17	500 ± 25
Activity, AU	7.9 ± 3.0	11.1 ± 3.2	11.7 ± 5.0	14.8 ± 8.0	8.4 ± 4.4	5.4 ± 2.9	9.2 ± 3.5	5.8 ± 1.8

Abbreviations: AU, arbitrary units; b.p.m., beats per minute; HR, heart rate; MAP, mean arterial pressure.
*$P<0.01$ *vs.* $AT_{1A}^{+/+}$ mice before the corresponding test.
#$P<0.05$ *vs.* normal-salt diet.
Values are mean ± s.e.m.
The mean values over 5 min immediately before stress in each animal were used to calculate resting values of MAP, HR and locomotor activity.

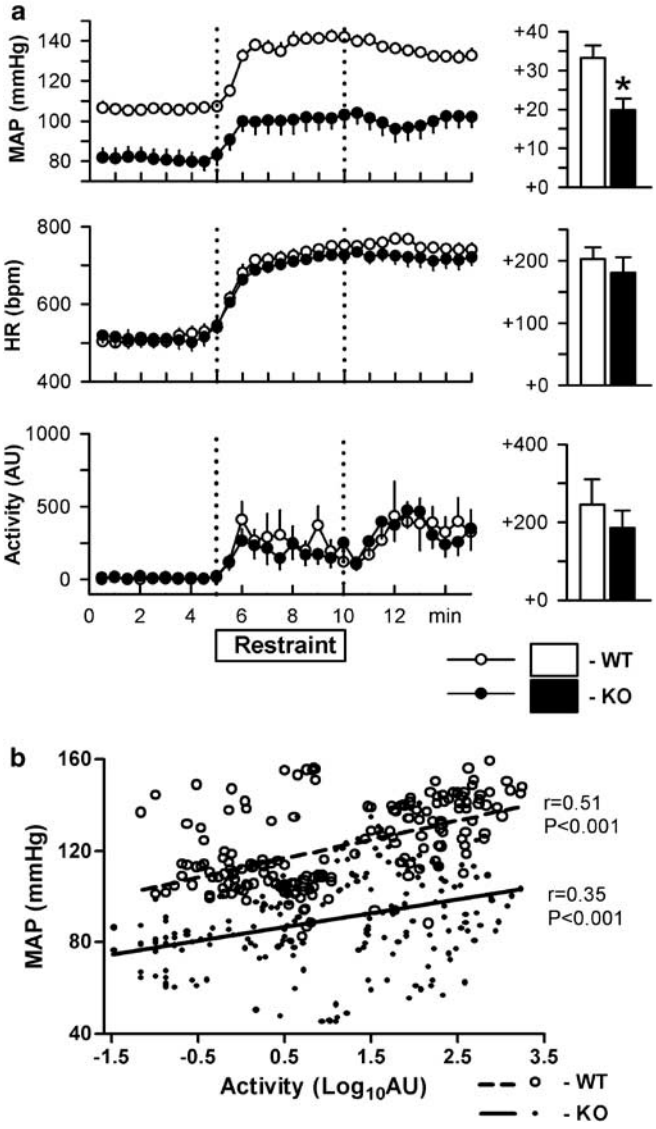

Figure 1 (**a**) Cardiovascular and locomotor response to restraint stress in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice. Each dot represents an average over a 30-s period. In the right panels, the mean values of mean arterial pressure (MAP), heart rate (HR) and activity over the last 4 min of stress exposure and 5 min immediately before stress in each animal were used to calculate the average responses. *P=0.01 *vs.* $AT_{1A}^{+/+}$ mice; AU, arbitrary units. (**b**) Relationship between MAP and locomotion during the restraint test in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice.

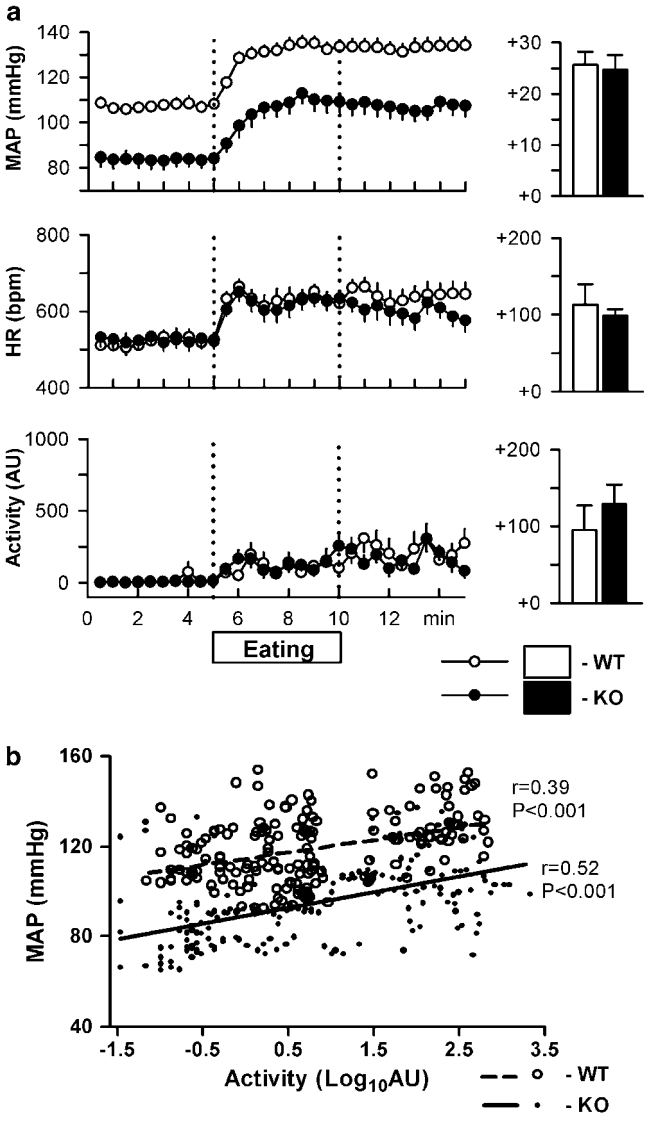

Figure 2 (**a**) Cardiovascular and locomotor response to feeding in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice. Abbreviations as in Figure 1. (**b**) Relationship between MAP and locomotion during feeding in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice.

respectively; Figure 2). These responses were not altered in $AT_{1A}^{-/-}$ mice (25 ± 3 mm Hg and 99 ± 9 b.p.m., respectively), neither were locomotor responses (Figure 2).

There was a significant correlation between MAP and locomotion in both $AT_{1A}^{-/-}$ mice (r=0.52, P<0.01) and $AT_{1A}^{+/+}$ mice (r=0.39, P<0.01) during the test (Figure 2). The responsiveness of MAP to locomotor activity was similar between $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice (7.0 ± 0.8 and 5.6 ± 1.0 mm Hg per $\log_{10}$AU, respectively), as was HR responsiveness to locomotion (data not shown).

Effect of high salt intake on restraint stress response

High salt intake increased resting pre-restraint values of MAP by 16 ± 6 mm Hg (P=0.02) in $AT_{1A}^{-/-}$ mice and also tended to increase MAP by 7 ± 4 mm Hg in $AT_{1A}^{+/+}$ mice (Table 1). Thus, on a high-salt diet, resting MAP in $AT_{1A}^{-/-}$ mice remained lower by 17 ± 5 mm Hg than that in $AT_{1A}^{+/+}$ mice (P<0.01). Conversely, high salt intake caused little changes in pre-stress values of HR and locomotor activity, which were thus not different between groups (Table 1).

Under conditions of high salt intake, the pressor response to restraint stress in $AT_{1A}^{-/-}$ mice (30 ± 3 mm Hg) was significantly increased (P=0.03) in comparison with that on normal salt intake (20 ± 3 mm Hg; Figure 1), and was no longer different from that in $AT_{1A}^{+/+}$ mice (28 ± 3 mm Hg; Figure 3). Likewise, the stress-induced tachycardia and locomotor activation did not differ between groups (Figure 3).

There was a significant correlation between MAP and locomotion in both $AT_{1A}^{-/-}$ mice (r=0.31, P<0.01) and $AT_{1A}^{+/+}$ mice (r=0.28, P=0.017) during the test. The $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice had a similar responsiveness of MAP to locomotor activity (4.2 ± 1.1 and 4.9 ± 2.0 mm Hg per $\log_{10}$ AU, respectively) during the test (Figure 3).

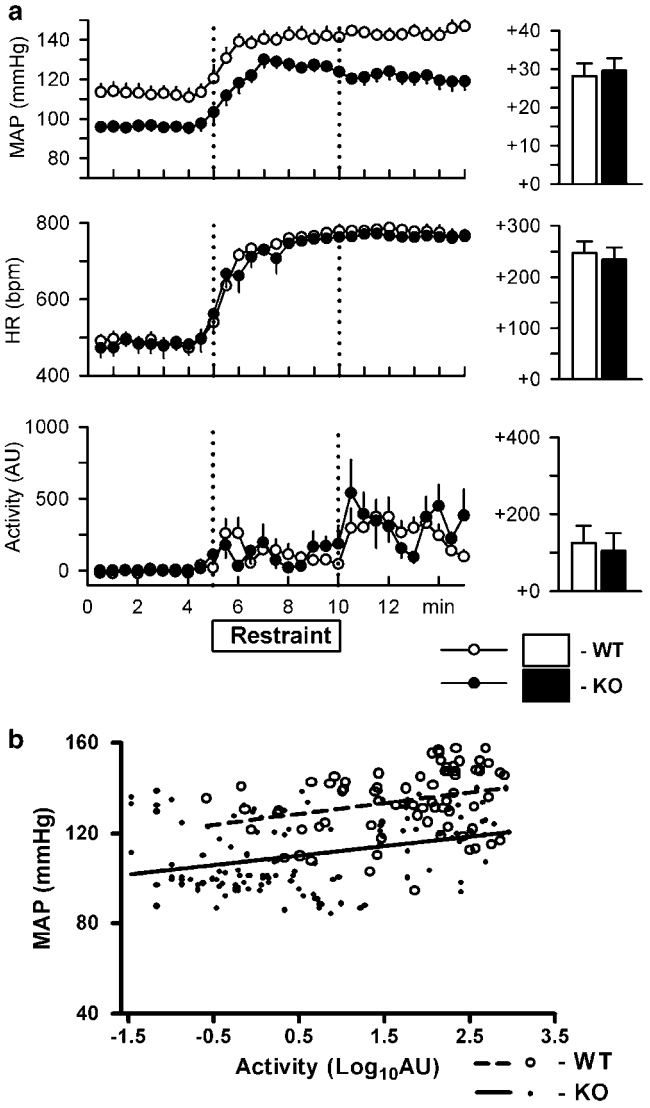

Figure 3 (**a**) Effect of high sodium intake on cardiovascular and locomotor response to restraint stress in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice. Abbreviations are as in Figure 1. (**b**) Relationship between MAP and locomotion during the restraint test in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice.

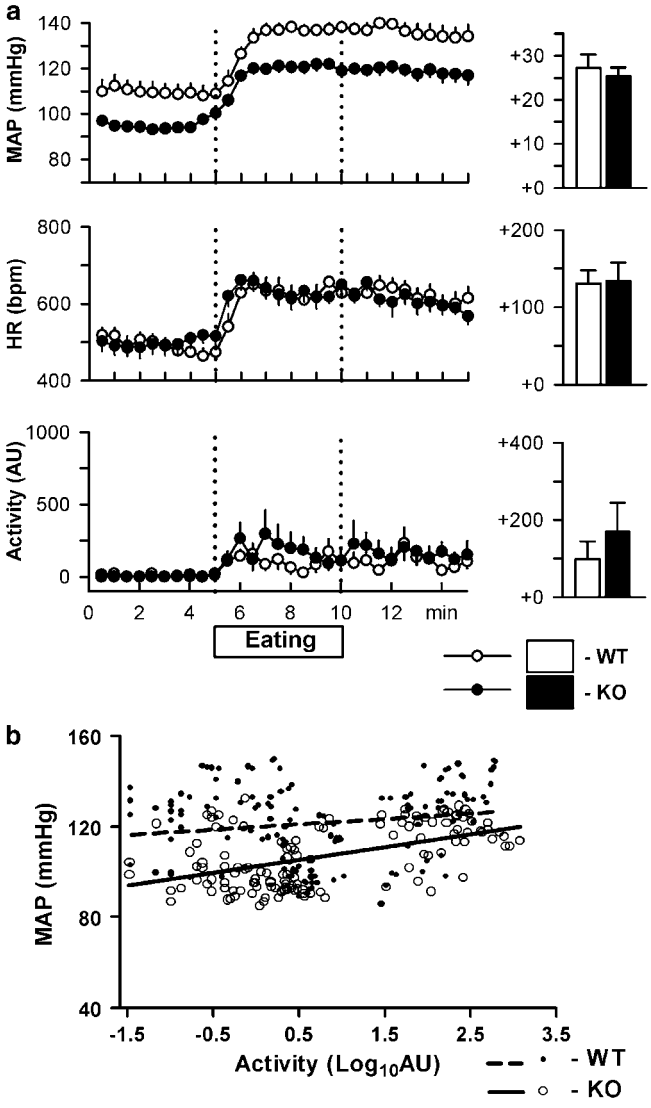

Figure 4 (**a**) Effect of high sodium intake on cardiovascular and locomotor response to feeding in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice. Abbreviations are as in Figure 1. (**b**) Relationship between MAP and locomotion during feeding in $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice.

Effect of high salt intake on response to feeding

The resting pre-feeding values of MAP were increased on high salt intake by 12 ± 4 mm Hg (P=0.02) in $AT_{1A}^{-/-}$ mice, but not in $AT_{1A}^{+/+}$ mice (2 ± 4 mm Hg; Table 1). Thus, on a high-salt diet, resting MAP in $AT_{1A}^{-/-}$ mice remained lower by 14 ± 5 mm Hg than that in $AT_{1A}^{+/+}$ mice (P=0.01). High salt intake did not affect pre-feeding values of HR and locomotor activity, which were thus not different between groups (Table 1).

Under conditions of high salt intake, food presentation and feeding were accompanied by similar increases in MAP in $AT_{1A}^{+/+}$ and $AT_{1A}^{-/-}$ mice (27 ± 3 and 25 ± 2 mm Hg, respectively; Figure 4). These responses were very similar to those on normal salt intake (26 ± 3 and 25 ± 3 mm Hg, respectively; Figure 2). Likewise, the HR responses to feeding in $AT_{1A}^{+/+}$ and $AT_{1A}^{-/-}$ mice (131 ± 17 and 134 ± 23 b.p.m., respectively; Figure 4) were not different from those on normal salt intake (Figure 2).

There was a significant correlation between MAP and locomotion in $AT_{1A}^{-/-}$ mice (r=0.51, $P<0.001$), but not in $AT_{1A}^{+/+}$ mice (r=0.16, NS) during the test (Figure 4). The responsiveness of MAP to locomotor activity in $AT_{1A}^{-/-}$ mice on a high-salt diet (5.6 ± 0.8 mm Hg per $\log_{10}$ AU) was similar to that on normal salt intake (7.0 ± 0.8 mm Hg per $\log_{10}$ AU).

DISCUSSION

This study shows for the first time that targeted deletion of the AT_{1A} receptor gene in mice leads to an attenuation in the pressor response to negative emotional stress. Conversely, AT_{1A} receptor deficiency has little effect on cardiovascular arousal induced by appetitive events, such as presentation and eating palatable food. Another new finding of this study is that AT_{1A} receptor deficiency might promote the sensitivity of blood pressure to synergistic effects of dietary salt and stress. Finally, we report for the first time that there is little interaction

between the AT_{1A} receptor, excess dietary sodium and feeding-induced cardiovascular arousal.

Stress response

Pharmacological evidence indicates that AT_1 receptors are crucially involved in regulating the cardiovascular response to negative emotional stress.[5,6] In particular, it has been shown that central or systemic administration of AT_1 receptor antagonists attenuated the increase in blood pressure and plasma catecholamine levels caused by several psycho- and physico-emotional stressors in rats and rabbits.[12,24–26] Our data extend these findings by showing that targeted deletion of the AT_{1A} receptor gene selectively attenuates the pressor response to stress in mice. Taken together, the above pharmacological and functional genomic studies strongly suggest that AT_1 receptors, and specifically AT_{1A} subtype for murines, are required for full expression of the cardiovascular component of fight-or-flight response.

The precise mechanisms that underlie the attenuation of the pressor response to restraint stress in $AT_{1A}^{-/-}$ mice are yet to be fully clarified. Little effect of AT_{1A} receptor knockout on the pressor response to feeding suggests that the reduction in cardiovascular reactivity could not simply be attributed to changes in basal levels of blood pressure. Given that locomotor activity is an independent determinant of cardiovascular arousal,[20,23] another possibility would be that this attenuation was because of a diminished responsiveness to locomotion during stress. However, $AT_{1A}^{-/-}$ and $AT_{1A}^{+/+}$ mice have a similar pressor response to a given increase in locomotor activity. Thus, also considering a weak correlation between locomotion and blood pressure during the test, it seems unlikely that the decrease in cardiovascular reactivity in $AT_{1A}^{-/-}$ mice was crucially influenced by locomotion.

It is known that circulating angiotensin II, production of which rapidly increases in response to stress,[27] can influence cardiovascular reactivity at the peripheral vascular level by facilitating catecholamine synthesis and release, and/or increasing vascular reactivity to noradrenaline.[28,29] Hence, AT_{1A} receptor deficiency in our study could attenuate the vascular responsiveness to noradrenaline and possibly other circulating stress mediators. Earlier reports, however, suggested that AT_{1A}-receptor gene knockout does not alter the isolated carotid artery responses to several vasoconstrictor agents, including α-adrenoceptor agonist phenylephrine, thromboxane A2 agonist U46619, serotonin and KCl.[30] Perhaps more importantly, cardiovascular arousal caused by feeding was not altered in our $AT_{1A}^{-/-}$ mice, further indicating that vascular responsiveness to central pressor stimuli remained essentially intact in these animals. Therefore, it seems unlikely that the decrease in cardiovascular reactivity in $AT_{1A}^{-/-}$ mice was principally mediated by changes in the vascular contractile response to stress-induced sympathetic stimulation.

It is thus conceivable that a disrupted CNS regulation of autonomic outputs to the periphery might underlie the reduction in cardiovascular reactivity in $AT_{1A}^{-/-}$ mice. Our preliminary data showing that aversive (cage-switch) stress-induced expression of a marker of neuronal activation c-Fos protein is attenuated in the DMH and RVLM of $AT_{1A}^{-/-}$ mice by 58 and 42%, respectively,[31] are in accord with this notion. Earlier pharmacological studies showing that AT_1 receptors in the DMH and RVLM are required for full expression of the pressor response to aversive (air-jet) stress in rabbits[12,26] further support this possibility.

Food presentation and feeding

It is well established that not only aversive, but also appetitive stimuli are capable of inducing a distinct, sympathetically mediated rise in blood pressure in both animals and human participants.[8–10] In particular, food presentation and feeding are normally accompanied in animals by marked increases in blood pressure and heart rate.[8,12,21] This feeding-associated arousal is initiated by the activation of descending inputs from higher CNS rather than viscero-sympathetic reflexes because of food intake.[32,33] In this study, presentation and eating palatable food elicited distinct pressor and tachycardic responses, which, in wild-type animals, were of similar magnitude to those caused by aversive stimuli. Likewise, we have recently shown that mild aversive (air-jet) and appetitive (straw) stimuli induced very similar cardiovascular arousal in normal rabbits.[12] It is intriguing that in contrast to aversive stress, the pressor response to presentation and eating palatable food (almond) was not altered in $AT_{1A}^{-/-}$ mice. This finding indicates that AT_{1A}-receptor gene deficiency does not affect cardiovascular correlates of normal feeding behavior, which is regarded as appetitive or positively motivated.[34] These results are in accord with our recent observation that *Ren-1*^{c} enhancer knockout mice have an attenuated response to restraint and shaker stress, but not to feeding.[21] Thus, in relation to cardiovascular reactivity, $AT_{1A}^{-/-}$ mice and *Ren-1*^{c} enhancer knockout mice show essentially the same phenotype. This observation indicates that the above phenotypes might specifically relate to the dysregulated renin–angiotensin system, and not to the activation of genetic compensatory mechanisms or disruption of neighboring gene expression. In line with this possibility, we earlier reported that pharmacological blockade of AT_1 receptors in the DMH (a region crucial for the regulation of autonomic cardiovascular arousal) decreased the pressor response to air-jet stress, but not food presentation and feeding in rabbits.[12] Collectively, these data suggest that, with respect to cardiovascular arousal, the brain–angiotensin II system might be primarily involved in regulating the fight-or-flight response, making it a potential therapeutic target for selective attenuation of cardiovascular hyperreactivity to aversive stress.

Effect of high salt intake on stress response

Evidence indicates that high salt intake can reinforce the pressor effect of emotional stress in genetically predisposed animals or human subjects.[13–15] Brain AT_1 receptors are likely to play a crucial role in this synergistic effect of salt and stress, because the enhanced pressor and sympathetic responses to air-jet stress could be prevented by central blockade of these receptors in several salt-sensitive animal models, including spontaneously hypertensive and Dahl salt-sensitive rats.[16,17] Importantly, the same treatment regimen had no effect on the stress response in Dahl salt-resistant rats,[17] indicating that the aforementioned synergistic effect could be attributed to the salt-induced hyperactivity of central AT_1 receptors in susceptible animals.

In this study, a high-salt diet facilitated the pressor response to aversive stress in $AT_{1A}^{-/-}$, but not $AT_{1A}^{+/+}$ mice. It is unlikely that this disparity was mediated by the salt-induced change in basal blood pressure and/or vascular reactivity, because the pressor response to feeding was not altered in $AT_{1A}^{-/-}$ mice on high salt intake. It is therefore conceivable that the increase in cardiovascular reactivity in these animals primarily reflected a central synergistic interaction between acute stress and salt. Thus, apart from AT_1 receptor hyperactivity,[16,17] genetic deficiency of this receptor might increase the sensitivity of blood pressure to synergistic effects of salt and stress. Further research will be necessary to identify the precise mechanisms that underlie the interplay between the functional state of AT_1 receptor and synergistic effect of salt and stress on blood pressure.

Effect of high salt intake on response to feeding

An intriguing new finding of this study is that the salt-induced increase in blood pressure reactivity to negative stress in $AT_{1A}^{-/-}$ mice was not accompanied by a rise in cardiovascular arousal associated with appetitive feeding behavior. This finding indicates that central pressor mechanisms that control feeding-associated arousal are not only independent of AT_{1A} receptors, but also resistant, at least under experimental conditions, to dietary salt loading. Further research is necessary to identify the chemical nature of these mechanisms.

In summary, these results indicate that genetic deficiency of AT_{1A} receptor in mice leads to an attenuated pressor response to negative emotional stress, but not cardiovascular arousal caused by appetitive feeding behavior. The reduction in stress reactivity in $AT_{1A}^{-/-}$ mice seems not to relate to altered locomotion or vascular contractile responsiveness to central pressor stimuli. Our results also indicate that AT_{1A} receptor deficiency is an important factor for the salt-induced augmentation of cardiovascular stress responses. Conversely, there is little relation between the AT_{1A} receptor, excess dietary sodium and cardiovascular arousal associated with feeding. These results might have clinical implications as they suggest that AT_1 receptors could be a selective therapeutic target for preventing detrimental synergistic effects of salt and stress on the cardiovascular system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia and the High Blood Pressure Research Foundation of Australia.

1 Esler M. Stress, stressors and cardiovascular disease. *Repatriation Medical Authority Consensus Conference on Stress and Challenge—Health and Disease*. Repatriation Medical Authority: Brisbane, Australia,, pp. 198–206.
2 Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 1999; **99**: 2192–2217.
3 Hirasawa R, Hashimoto K, Ota Z. Role of central angiotensinergic mechanism in shaking stress-induced ACTH and catecholamine secretion. *Brain Res* 1990; **533**: 1–5.
4 Kregel KC, Stauss H, Unger T. Modulation of autonomic nervous system adjustments to heat stress by central ANG II receptor antagonism. *Am J Physiol* 1994; **266**: R1985–R1991.
5 Watanabe T, Fujioka T, Hashimoto M, Nakamura S. Stress and brain angiotensin II receptors. *Crit Rev Neurobiol* 1998; **12**: 305–317.
6 Saavedra JM, Ando H, Armando I, Baiardi G, Bregonzio C, Jezova M, Zhou J. Brain angiotensin II, an important stress hormone: regulatory sites and therapeutic opportunities. *Ann NY Acad Sci* 2004; **1018**: 76–84.
7 Baltatu O, Campos LA, Bader M. Genetic targeting of the brain renin-angiotensin system in transgenic rats: impact on stress-induced renin release. *Acta Physiol Scand* 2004; **181**: 579–584.
8 Del Bo A, Le Doux JE, Reis DJ. Sympathetic nervous system and control of blood pressure during natural behaviour. *J Hypertens* 1985; **3** (Suppl 3): S105–S106.
9 Braesicke K, Parkinson JA, Reekie Y, Man MS, Hopewell L, Pears A, Crofts H, Schnell CR, Roberts AC. Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. *Eur J Neurosci* 2005; **21**: 1733–1740.
10 Brosschot JF, Thayer JF. Heart rate response is longer after negative emotions than after positive emotions. *Int J Psychophysiol* 2003; **50**: 181–187.
11 Pressman SD, Cohen S. Does positive affect influence health? *Psychol Bull* 2005; **131**: 925–971.
12 De Matteo R, Head GA, Mayorov DN. Angiotensin II in dorsomedial hypothalamus modulates cardiovascular arousal caused by stress but not feeding in rabbits. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R257–R264.
13 Folkow B. Critical review of studies on salt and hypertension. *Clin Exp Hypertens A* 1992; **14**: 1–14.
14 Turkkan JS, Story MK. Blood pressure hyperreactivity in non-human primates during dietary sodium combined with behavioral stress. *Integr Physiol Behav Sci* 1991; **26**: 98–107.
15 DiBona GF, Jones SY. Analysis of renal sympathetic nerve responses to stress. *Hypertension* 1995; **25**: 531–538.
16 Huang BS, Leenen FH. Brain 'ouabain' and angiotensin II in salt-sensitive hypertension in spontaneously hypertensive rats. *Hypertension* 1996; **28**: 1005–1012.
17 Huang BS, Leenen FHH. Both brain angiotensin II and 'ouabain' contribute to sympathoexcitation and hypertension in Dahl S rats on high salt intake. *Hypertension* 1998; **32**: 1028–1033.
18 Oliverio MI, Best CF, Kim HS, Arendshorst WJ, Smithies O, Coffman TM. Angiotensin II responses in AT(1A) receptor-deficient mice: a role for AT(1B) receptors in blood pressure regulation. *Am J Physiol Renal Physiol* 1997; **41**: F515–F520.
19 Chen Y, Joaquim LF, Farah VM, Wichi RB, Fazan Jr R, Salgado HC, Morris M. Cardiovascular autonomic control in mice lacking angiotensin AT1a receptors. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R1071–R1077.
20 Kario K, Schwartz JE, Pickering TG. Ambulatory physical activity as a determinant of diurnal blood pressure variation. *Hypertension* 1999; **34**: 685–691.
21 Jackson K, Head GA, Morris BJ, Chin-Dusting J, Jones E, La Greca L, Mayorov DN. Reduced cardiovascular reactivity to stress but not feeding in renin enhancer knockout mice. *Am J Hypertens* 2007; **20**: 893–899.
22 Butz GM, Davisson RL. Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. *Physiol Genomics* 2001; **5**: 89–97.
23 Leary AC, Donnan PT, MacDonald TM, Murphy MB. The influence of physical activity on the variability of ambulatory blood pressure. *Am J Hypertens* 2000; **13**: 1067–1073.
24 Saiki Y, Watanabe T, Tan N, Matsuzaki M, Nakamura S. Role of central ANG II receptors in stress-induced cardiovascular and hyperthermic responses in rats. *Am J Physiol* 1997; **272**: R26–R33.
25 Cierco M, Israel A. Role of angiotensin AT1 receptor in the cardiovascular response to footshock. *Eur J Pharmacol* 1994; **251**: 103–106.
26 Mayorov DN, Head GA. AT1-receptors in the RVLM mediate pressor responses to emotional stress in rabbits. *Hypertension* 2003; **41**: 1168–1173.
27 Yang G, Xi ZX, Wan Y, Wang H, Bi G. Changes in circulating and tissue angiotensin II during acute and chronic stress. *Biol Signals* 1993; **2**: 166–172.
28 Arribas S, Sanchez-Ferrer CF, Peiro C, Ponte A, Salaices M, Marin J. Functional vascular renin-angiotensin system in hypertensive transgenic rats for the mouse renin gene Ren-2. *Gen Pharmacol* 1994; **25**: 1163–1170.
29 Jezova M, Armando I, Bregonzio C, Yu ZX, Qian S, Ferrans VJ, Imboden H, Saavedra JM. Angiotensin II AT(1) and AT(2) receptors contribute to maintain basal adrenomedullary norepinephrine synthesis and tyrosine hydroxylase transcription. *Endocrinology* 2003; **144**: 2092–2101.
30 Ryan MJ, Didion SP, Mathur S, Faraci FM, Sigmund CD. Angiotensin II-induced vascular dysfunction is mediated by the AT1A receptor in mice. *Hypertension* 2004; **43**: 1074–1079.
31 Davern P, Chen D, Head GA, Walther T, Mayorov DN. Neuronal activation in the hypothalamus is reduced in AT1A receptor knockout mice following aversive stress. 2006 HBPRCA Presentations. *Hypertension* 2007; **49**: 1465.
32 Matsukawa K, Ninomiya I. Changes in renal sympathetic nerve activity, heart rate and arterial blood pressure associated with eating in cats. *J Physiol* 1987; **390**: 229–242.
33 Diamond P, LeBlanc J. Hormonal control of postprandial thermogenesis in dogs. *Am J Physiol Endocrinol Metab* 1987; **253**: E521–E529.
34 Reynolds SM, Berridge KC. Positive and negative motivation in nucleus accumbens shell: bivalent rostrocaudal gradients for GABA-elicited eating, taste 'liking'/'disliking' reactions, place preference/avoidance, and fear. *J Neurosci* 2002; **22**: 7308–7320.