Angiotensin II acting on brain AT₁ receptors induces adrenaline secretion and pressor responses in the rat

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Angiotensin II (AngII) plays an important role in the regulation of cardiovascular function, both peripheral and central. Both peripheral and central actions of AngII are involved in this regulation, but the mechanisms of the latter actions as a neurotransmitter/neuromodulator within the brain are still unclear. Here we show that (1) intracerebroventricularly (i.c.v.) administered AngII in urethane-anesthetized male rats elevates plasma adrenaline derived from the adrenal medulla but not noradrenaline with valsartan-sensitive brain mechanisms, (2) peripheral AT₁ receptors are not involved in the AngII-induced elevation of plasma adrenaline, although AngII induces both noradrenaline and adrenaline secretion from bovine adrenal medulla cells, and (3) i.c.v. administered AngII elevates blood pressure but not heart rate with the valsartan-sensitive mechanisms. From these results, i.c.v. administered AngII acts on brain AT₁ receptors, thereby inducing the secretion of adrenaline and pressor responses. We propose that the central angiotensinergic system can activate central adrenomedullary outflow and modulate blood pressure.

Angiotensin II (AngII) plays an important role in the regulation of cardiovascular function. Both peripheral and central actions of AngII are involved in this regulation, but mechanisms of the latter actions as a neurotransmitter/neuromodulator within the brain are still unclear. Here we show that (1) intracerebroventricularly (i.c.v.) administered AngII in urethane-anesthetized male rats elevates plasma adrenaline derived from the adrenal medulla but not noradrenaline with valsartan-sensitive brain mechanisms, (2) peripheral AT₁ receptors are not involved in the AngII-induced elevation of plasma adrenaline, although AngII induces both noradrenaline and adrenaline secretion from bovine adrenal medulla cells, and (3) i.c.v. administered AngII elevates blood pressure but not heart rate with the valsartan-sensitive mechanisms. From these results, i.c.v. administered AngII acts on brain AT₁ receptors, thereby inducing the secretion of adrenaline and pressor responses. We propose that the central angiotensinergic system can activate central adrenomedullary outflow and modulate blood pressure.
in rats\textsuperscript{21,22}. These findings suggest a possibility that sympathetic and adrenomedullary systems can be separately regulated by the central nervous system. In the present study, we examined the effects of AngII directly administered into the rat brain on both systems by measuring plasma NA and Ad, blood pressure and heart rate.

**Results**

**Centrally administered AngII elevates plasma Ad but not NA.** Treatment with vehicle [10 µl saline per animal, intracerebroventricularly (i.c.v.)] had no effect on the plasma levels of NA and Ad (Figure 1a and 1b). AngII (1 and 3 nmol per animal, i.c.v.) dose-dependently elevated plasma Ad, but AngII at a dose of 10 nmol per animal (i.c.v.) showed similar effects on plasma Ad compared with the effects of AngII at 3 nmol (Figure 1a and 1b). The Ad responses peaked at 10 min after the administration of AngII and then declined towards their basal levels (Figure 1a). On the other hand, AngII (1, 3 and 10 nmol per animal, i.c.v.) had no significant effects on plasma NA (Figure 1a and 1b). The actual values for NA and Ad at 0 min were 413 ± 21 and 253 ± 14 pg ml\(^{-1}\) (n = 23).

**Ad elevated by AngII is derived from adrenal medulla.** In previous preliminary studies, we measured the plasma concentration of corticosterone and cortisol\textsuperscript{23}. Three hours after sham-operation or adrenalectomy plus hydrocortisone, corticosterone and cortisol were 358.0 ± 21.6 and 23.3 ± 4.1 ng ml\(^{-1}\) in sham-operated rats (n = 3) and 28.1 ± 3.1 and 484.0 ± 151.5 ng ml\(^{-1}\) in adrenalectomized rats with hydrocortisone (5 mg kg\(^{-1}\), i.m.) (n = 3), respectively. In the present study, the basal plasma level of Ad was effectively reduced by acute bilateral adrenalectomy: the actual values for Ad at 0 min were 222 ± 64 pg ml\(^{-1}\) in the sham-operated group (n = 4), and 71 ± 7 pg ml\(^{-1}\) in the adrenalectomized group (n = 4). AngII (3 nmol per animal, i.c.v.) induced elevation of plasma Ad in the sham-operated group was abolished by the bilateral adrenalectomy (Figure 2a and 2b).

**AngII induces Ad elevation through brain AT\(_1\) receptors.** Preliminary, we checked that central treatment with valsartan, an AT\(_1\) receptor blocker, or PD123319, an AT\(_2\) receptor blocker, only had no obvious effect on plasma levels of Ad (data not shown). Pretreatment with valsartan (100 nmol per animal, i.c.v.) almost abolished AngII- (3 nmol per animal, i.c.v.) induced elevation of plasma Ad (Figure 3a and 3b). On the other hand, pretreatment with PD123319 (100 nmol per animal, i.c.v.) had no significant effect on the AngII-induced response (Figure 3c and 3d). The actual values for Ad at 0 min were 303 ± 22 pg ml\(^{-1}\) in the vehicle-1- [3 µl of 100% N,N-dimethylformamide (DMF) per animal, i.c.v.] pretreated group (n = 5), 118 ± 21 pg ml\(^{-1}\) in the valsartan-pretreated group (n = 5), 184 ± 52 pg ml\(^{-1}\) in the vehicle-2- (5 µl saline per animal, i.c.v.) pretreated group (n = 4) and 148 ± 28 pg ml\(^{-1}\) in the PD123319-pretreated group (n = 6), respectively.

**AngII evokes NA and Ad secretion from adrenal medulla cells.** In comparison with the control group, the AngII- (3.3 or 10 µM) treated group showed significant increments of spontaneous secretion of NA and Ad from the cultured bovine adrenal chromaffin cells at 60, 90 and 120 min after administration (Figure 4a and 4b). The degree of increments in Ad was larger than those in NA (Figure 4a and 4b).

![Figure 1 | Effects of centrally administered angiotensin II (AngII) on plasma noradrenaline and adrenaline levels.](image-url)
Centrally acting AngII induces Ad elevation. Preliminary, we checked that peripheral treatment with valsartan only had no obvious effect on plasma levels of Ad (data not shown). In rats pretreated with vehicle (300 μl of 1% DMF per saline per animal, i.v.), centrally administered AngII- (3 nmol per animal, i.c.v.) induced elevation of plasma Ad (Figure 5a and 5b) but not NA.

Figure 2 | Effect of acute bilateral adrenalectomy on the centrally administered AngII-induced elevation of plasma adrenaline levels. Acute bidirectional adrenalectomy [plus hydrocortisone (5 mg kg\(^{-1}\) per animal, i.m.)] or sham-operation (plus 200 μl saline per animal, i.m.) was done 3 h before the application of AngII (3 nmol per animal, i.c.v.). (a) Increments of plasma adrenaline above the basal level. Arrow indicates the administration of AngII. (b) AUC of the elevation of plasma adrenaline above the basal level for each group. *P < 0.05, when compared to the sham-operated group with an unpaired Student’s t-test. Other conditions are the same as those of Figure 1.

Figure 3 | Effects of valsartan and PD123319 on the centrally administered AngII-induced elevation of plasma adrenaline levels. Valsartan (Val) (AT\(_1\) receptor blocker) (100 nmol per animal), vehicle-1 (3 μl DMF per animal), PD123319 (PD) (AT\(_2\) receptor blocker) (100 nmol per animal), or vehicle-2 (5 μl saline per animal) was i.c.v. administered 30 min before the administration of AngII (3 nmol per animal, i.c.v.). (a and c) Increment of plasma adrenaline above the basal level. Arrows indicate the administration of Val (a)/PD (c)/vehicle-1/vehicle-2 and AngII. (b and d) AUC of the elevation of plasma adrenaline above the basal level for each group of (a) and (c), respectively. *P < 0.05, when compared to the vehicle-1- and AngII-treated group with an unpaired Student’s t-test. The other conditions are the same as those of Figures 1 and 2.
The actual values for Ad at 0 min were 174 animal, i.c.v.) induced elevation of plasma Ad (Figure 5a and 5b). Animal, i.v.) had no significant effect on the AngII- (3 nmol per vehicle-pretreated group (n = 5), and 271 ± 39 pg ml⁻¹ in the valsartan-pretreated group (n = 4), respectively.

Figure 4 | Effect of AngII on secretion of noradrenaline and adrenaline from cultured bovine adrenal chromaffin cells. Cells were incubated with (3.3 or 10 μM) or without (None) indicated concentrations of AngII for up to 120 min at 37°C. Subsequently, both noradrenaline (a) and adrenaline (b) secreted spontaneously in the incubation medium were measured by HPLC. Data represent the mean ± s.e.m. *P < 0.05, when compared to the “None” group with an unpaired Student’s t-test.

Centrally acting AngII induces pressor responses. In the vehicle- (300 μl saline containing 1% DMF per animal, i.v.) pretreated group, systolic, mean and diastolic blood pressure were significantly elevated 5 min after administration of AngII (3 nmol per animal, i.c.v.) compared with the valsartan- (100 nmol per animal, i.v.) pretreated group, but no significant blood pressure changes between the two groups were observed 0 min after the administration of AngII (Table 1). On the other hand, heart rate in the vehicle-pretreated group was significantly lower than that in the valsartan-pretreated group 0 and 5 min after the administration of AngII (Table 1).

Discussion
In this study, we demonstrated that i.c.v. administered AngII elevated plasma Ad, but not NA, secreted from the rat adrenal medulla. The AngII-induced Ad secretion was inhibited by central pretreatment with valsartan, an AT₁ receptor blocker, but not with PD123319, an AT₂ receptor blocker. AngII stimulated the spontaneous secretion of both Ad and NA from bovine adrenal chromaffin cells, but the centrally administered AngII-induced secretion of Ad was not influenced by peripheral pretreatment with valsartan. I.c.v. administered AngII elevated systolic, mean and diastolic blood pressure but not heart rate and the elevations were abolished by valsartan. Our data suggest that centrally administered AngII acting on brain AT₂ receptors induced Ad secretion from the rat adrenal medulla and pressor responses.

Studies in which AngII was administered directly into the cerebroventricles showed increases in blood pressure in many species24,25. On the other hand, acute i.v. administered AngII shows a different pattern of peripheral sympathetic outflow. AngII transiently inhibits renal sympathetic nerve activity, but increases splanchnic and cardiac sympathetic nerve activities26-27. These findings suggest that centrally acting AngII modulates sympathetic nerve activity in a regionally selective way. In the present study, i.c.v. administered AngII significantly elevated plasma Ad but not NA, indicating a possibility that selective activation and inhibition of sympathetic nerve activities induced by AngII counteracts significant changes in plasma NA levels. Previous reports of this laboratory showed that i.c.v. administered neuromedin U, a stress-related neuropeptide28, also elevated plasma Ad, but not NA29, and that i.c.v. administered CRF activated celiac andstellate ganglia but not superior cervical ganglia in the rat30. Taken together, central regulation of sympathetic outflow can occur in a region/organ-selective manner and sympathetic and adrenomedullary systems can be regulated separately by the central nervous system.

Plasma Ad originates exclusively from the Ad-containing cells in the adrenal medulla, however, the contribution of the extramedullary chromaffin tissues cannot be excluded. Therefore, we examined the effect of acute bilateral adrenalectomy supplemented with hydrocortisone on the i.c.v. administered AngII-induced elevation of plasma Ad. Cortisol (hydrocortisone) supplementation in adrenalectomized rats resulted in similar concentrations compared to corticosterone in sham-operated rats. Since cortisol and corticosterone have similar efficacy to corticosteroid, this supplementation can counteract the deficiency of corticosterone in adrenalectomized rats. In the present study, adrenalectomy abolished the AngII-induced elevation of plasma Ad, suggesting that centrally administered AngII activates the secretion of Ad from the rat adrenal medulla.

Typical receptors for AngII are divided into AT₁ and AT₂ subtypes, which are also distributed within the brain32-34. In rodents, two AT₁ isoforms, AT₁a and AT₁b, have been identified and an overlapping localization of the isoforms is observed in the brain32; however, the ligand specificities and signal-effector coupling are virtually identical33,34. Therefore, it seems to be difficult to distinguish the two isoforms using pharmacological approaches. There is a growing consensus that the balance between AT₁; and AT₂ receptor signalling can determine the biological response induced by AngII. Brain AT₁ receptors have been implicated in the regulation of blood pressure35, while brain AT₂ receptors seem to modulate the inhibition of the sympathetic nervous system36. Actually, increasing central AT₂ receptor expression attenuated the development of renovascular hypertension in the rat37. In the present study, we characterized which subtype (AT₁ or AT₂) in the brain is involved in the i.c.v. administered AngII-induced elevation of plasma Ad using valsartan or PD123319. Valsartan is a highly selective blocker for AT₁ recep-
AT2 receptors, exhibits Ki values of 210 nM at AT2 in the rat brain and 1,800-fold higher selectivity than AT1. PD123319 is a potent and selective blocker for AT2 receptors, exhibits Ki values of 210 nM at AT2 in the rat brain and 1,800-fold higher selectivity than AT1. In the present study, central pretreatment with valsartan strongly attenuated the AngII-induced elevation of plasma Ad. On the other hand, PD123319 had no effect on the AngII-induced response. These results suggest that brain AT1 receptors are involved in the centrally administered AngII-induced secretion of Ad from the rat adrenal medulla.

Subsequently, we examined a possibility that AngII administered into the cerebroventricles can leak into the systemic circulation, thereby acting on the adrenal medulla directly. In the present experiment, i.c.v. administered AngII (1 and 3 nmol per animal) dose-dependently elevated plasma Ad in the rat. Considering the volume of the cerebrospinal fluid in the rat (about 300 μl41), the amount of AngII given i.c.v. in our study would result in a cerebrospinal fluid concentration of 3.5 and 10 μM, respectively. In an in vitro assay, treatment with AngII at these doses significantly induced spontaneous secretion of both NA and Ad from the bovine adrenal chromaffin cells, and the degree of Ad increments was larger than that of NA ones. These results suggest that AngII has the ability to induce secretion of both catecholamines, especially Ad, from the adrenal medulla directly. On the other hand, peripheral pretreatment with valsartan, which hardly crosses the blood brain barrier, had no effect on the i.c.v. administered AngII-induced elevation of plasma Ad in the rat. Taken together, centrally administered AngII induces Ad secretion from the rat adrenal medulla through the brain, but not peripheral, AT1 receptors, whereas i.c.v. administered AngII rapidly elevated blood pressure, in accordance with previous reports24,25. Moreover, the pressor responses were abolished by peripheral pretreatment with valsartan, although the pretreatment had no effect on AngII-induced Ad secretion. This discrepancy is probably explained by a vasorelaxant effect of peripherally administered valsartan, which can counteract the vasoconstrictive effect of Ad secreted by the centrally administered AngII. Actually, compared with the vehicle pretreated group, tachycardia was observed in the valsartan-pretreated group, indicating a possibility that the tachycardia may be a compensatory action after peripherally administered valsartan-induced vasorelaxation.

In the brain, AT1 receptors are distributed in circumventricular organs and regions influencing the central regulation of cardiovascular function such as the nucleus tractus solitarius, the rostral and caudal ventrolateral medulla and the hypothalamus31,42. In the hypothalamus, the paraventricular nucleus (PVN) has been considered as a regulatory centre of the central sympatho-adrenomedullary outflow43,44. Actually, microinjected AngII into the PVN increased mean blood pressure45 and specific knockdown of AT1a receptors in the PVN by infusion of interfering RNA against the receptors prevents hypertension induced by AngII treated peripherally46,47. These findings suggest a possibility that the angiotensinergic system in the PVN is critical for AngII-induced elevation of blood pressure and also for the central sympatho-adrenomedullary outflow. However, the PVN is a heterogeneous structure containing different types of output neurons including projecting neurons to brain stem autonomic centres and to sympathetic preganglionic neurons located in the spinal cord48,49. Oldfjeld et al. reported that the AT1 receptors are not

![Figure 5](image.png)

**Figure 5** | Effect of peripherally administered valsartan on the centrally administered AngII-induced elevation of plasma adrenaline levels. Valsartan (Val) (AT1 receptor blocker) (100 nmol/animal) or vehicle (300 μl of 1% DMF in saline per animal) was i.v. administered 30 min before the administration of AngII (3 nmol per animal, i.c.v.). (a) Increments of plasma adrenaline above the basal level. Arrows indicate the administration of Val/vehicle and AngII. (b) AUC of the elevation of plasma adrenaline above the basal level for each group. Other conditions are the same as those of Figures 1–3.

| Table 1 | Blood pressure and heart rate at 0 and 5 min after central administration of angiotensin II (AngII) |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Intravenous pretreatment         | SBP (mmHg)           | MBP (mmHg)           | DBP (mmHg)           | HR (beats/min)           |
| 0 min after AngII treatment     |                               |                               |                               |                               |
| Vehicle (n = 5)                 | 124.8 ± 4.8                   | 99.6 ± 5.0                   | 87.0 ± 5.2                   | 336 ± 9*                     |
| Valsartan (n = 4)               | 123.0 ± 6.3                   | 96.7 ± 3.8                   | 83.5 ± 2.6                   | 380 ± 7                      |
| 5 min after AngII treatment     |                               |                               |                               |                               |
| Vehicle (n = 5)                 | 140.3 ± 4.4*                  | 114.6 ± 2.0*                 | 101.8 ± 0.9*                 | 324 ± 6*                     |
| Valsartan (n = 4)               | 120.0 ± 6.9                   | 96.3 ± 5.2                   | 83.3 ± 3.4                   | 371 ± 12                     |

*Values are means ± s.e.m. Valsartan was administered at 100 nmol per animal and AngII (3 nmol per animal, i.c.v.) was administered 30 min after the pretreatment.

*P < 0.05, when compared to the Valsartan group with an unpaired Student's t-test. SBP: systolic blood pressure, MBP: mean blood pressure, DBP: diastolic blood pressure, HR: heart rate.
expressed in the PVN neurons directly projecting to sympathetic rat adrenal medulla and pressor responses. These findings suggest a possibility that adrenomedullary outflow can be separately regulated by the central nervous system including brain angiotensinergic system, which can also modulate blood pressure.

Methods

Animals. All animal care and experiments were conducted in compliance with the guidelines for the care and use of laboratory animals approved by Kochi University (No. G-5 and H-39) which are in accordance with the “Guidelines for proper conduct of animal experiments” of the ARRIVE guidelines for reporting experiments involving animals. All efforts were made to minimize the suffering of the animals and the number of animals needed to obtain reliable results. A total of 60 animals were used in the experiments described here. Twelve-week-old male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 300–350 g were housed at two per cage and were maintained in an air-conditioned room 22–24°C under a constant day-night rhythm (14/10 h light-dark cycle, lights on at 05:00) for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum.

Experimental procedures for drug administration. In the morning (09:00–10:00), under urethane anaesthesia (1,0 g kg–1, i.p.), the femoral vein was cannulated for saline injection and intravenous administration of drugs, and the femoral artery was cannulated in order to collect blood samples. In some experiments, acute bilateral adrenalectomy (plus hydrocortisone (5 mg kg–1, i.p.), i.m.) or sham-operation (plus 200 μl saline per animal, i.m.) was done just before cannulation by an abdominal midline incision56–58. Subsequently, every rat was placed in a stereotaxic apparatus (SR-6R; Narishige, Tokyo, Japan) until the end of each experiment, as described in a published work of this laboratory63. The skull was drilled for intracerebroventricular administration of drugs using a stainless-steel cannula (outer diameter of 0.3 mm). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP: –0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas52. Three hours were allowed to elapse before the application of drugs.

Drug administration in vivo. AngII dissolved in sterile saline was slowly administered into the right lateral ventricle at 10 μl per animal using a cannula connected to a 50 μl Hamilton syringe at a rate of 0.5 ml min–1. The cannula was retained until the end of the experiment. Valsartan, an AT1 receptor blocker, or PD123319, an AT2 receptor blocker, dissolved in 3 μl of DMF or 5 μl of sterile saline, respectively, was i.c.v. administered using the cannula connected to a 10-μl Hamilton syringe at a rate of 10 μl min–1, which was retained in the ventricle for 15 min to avoid the leakage of these blockers and then removed from the ventricle. Subsequently, AngII was slowly administered as described above 30 min after the application of the blockers. When valsartan was i.v. injected, the drug dissolved in 1% saline was slowly injected via a cannula inserted into the femoral artery, after cannulation and placement in a stereotaxic apparatus, and the femoral artery was cannulated in order to estimate blood pressure and heart rate.

Treatment of data and statistics. All values are expressed as means ± s.e.m. Statistical differences were determined using repeated-measure (treatment × time) or one-way analysis of variance, followed by post hoc analysis with the Bonferroni method. When only two means were compared, an unpaired Student’s t-test was used. P values less than 0.05 indicate statistical significance.

Drugs and chemicals. The following materials were used: synthetic AngII (Peptide Institute, Osaka, Japan); valsartan ([S]-3-3-methyl-2-[N-([4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl]methyl)pentanamido]butanoic acid) (Cayman Chemical, Ann Arbor, MI, USA); PD123319 (PD123319 ditrifluoroacetate) ([S]-[[4-(dimethylinamino)-3-methylphenyl]methyl]-(5-diphenylacetyl)-4,6,7-tetrahydro-1H-imidazo(4,5-c)pyridine-6-carboxylic acid ditrifluoracetate) (R&D Systems, Inc., Minneapolis, MN, USA). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

1. Nishimura, H. Angiotensin receptors – evolutionary overview and perspectives. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 128, 11–30 (2001).
2. Fournier, D., Luft, F. C., Bader, M., Ganten, D. & Andrade-Navarro, M. A. Emergence and evolution of the renin-angiotensin-aldosterone system. J. Mol. Med. 90, 493–508 (2012).
3. Hendel, M. D. & Collister, J. P. Contribution of the subfornical organ to angiotensin II-induced hypertension. Am. J. Physiol. Heart Circ. Physiol. 288, H680–H685 (2005).
4. Stocker, S. D. & Toney, G. M. Median preoptic neurons projecting to the hypothalamic paraventricular nucleus mediate osmotic, circulating Ang II and baroreceptor input in the rat. J. Physiol. 569, 599–610 (2005).
5. Ganten, D., Herrman, K., Beynen, A., van der, T. & Lang, R. E. Angiotensin synthesis in the brain and increased turnover in hypertensive rats. Science 221, 869–871 (1983).
6. Grobe, J. L., Xu, D. & Sigmund, C. D. An intracellular renin-angiotensin system in the hypothalamus. Hypertension 23, 187–193 (1994).
7. Song, K., Allen, A. M., Paxinos, G. & Mendelsohn, F. A. Mapping of angiotensin II receptor subtype heterogeneity in rat brain. J. Comp. Neurol. 316, 467–484 (1992).
8. Allen, A. M., O’Callaghan, E., Mendelsohn, F. A. & Chai, S. Y. [Intercellular Communication/Neuropeptides and neurotrophic factors/Neural...
39. Blankley, C. J. et al. Synthesis and structure–activity relationships of a novel series of non-peptide angiotensin II receptor binding inhibitors specific for the AT2 subtype. J. Med. Chem. 34, 3248–3260 (1991).
40. Bosnyak, S. et al. Relative affinity of angiotensin peptides and novel ligands at AT1 and AT2 receptors. Clin. Sci. 121, 297–303 (2011).
41. Shapiro, J. S. et al. Cisterna magna cannulated repeated CSF sampling rat model—effects of a gamma-secretase inhibitor on AJ levels. J. Neurosci. Methods. 205, 36–44 (2012).

42. Lenkei, Z., Palkovits, M., Corvol, P. & Lorens-Cortés, C. Expression of angiotensin type-1 (AT1) and type-2 (AT2) receptor mRNAs in the adult rat brain: a functional neuroanatomical review. Front. Neuroendocrinol. 18, 383–439 (1997).
43. Swanson, L. W. & Sawchenko, P. E. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology 31, 410–417 (1980).
44. Jansen, A. S., Nguyen, X. V., Karptiskij, V., Mettenleiter, T. C. & Loewy, A. D. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 270, 644–646 (1995).
45. Bains, J. S., Potyok, A. & Ferguson, A. V. Angiotensin II actions in paraventricular nucleus: functional evidence for neurotransmitter role in efferents originating in subfornical organ. Brain Res. 599, 223–229 (1992).
46. Northcott, C. A. et al. Adrenalin inhibition of AT1 receptors in the paraventricular nucleus induces acute increases in mean arterial blood pressure in the rat. J. Physiol. Regul. Integr. Comp. Physiol. 299, R1202–R1211 (2010).
47. Chen, A., Huang, B. S., Wang, H. W., Ahmad, M. & Leenin, F. H. Knockdown of mineralocorticoid or angiotensin II type 1 receptor gene expression in the paraventricular nucleus prevents angiotensin II hypertension in rats. J. Physiol. 592, 3532–3536 (2014).
48. Runge, R. N., Motawee, K., Pyner, S. & Coote, J. H. The paraventricular nucleus of the hypothalamus sends efferents to the spinal cord of the rat that closely oppose sympathetically preganglionic neurons projecting to the stellate ganglion. Exp. Brain Res. 120, 164–172 (1998).
49. Hardy, S. G. Hypothalamic projections to cardiovascular centers of the medulla. Brain Res. 894, 233–240 (2003).
50. Oldfield, B. I. et al. Efferent neural projections of angiotensin receptor (AT1) expressing neurons in the hypothalamic paraventricular nucleus of the rat. J. Neuroendocrinol. 13, 139–146 (2001).
51. Shimizu, T., Okada, S., Yamaguchi-Shima, N. & Yokotani, K. Brain phospholipase A2 generated eicosanoids elevate sympathetic nerve activity in conscious rats. Auton. Neurosci. 159, 152–156 (2009).
52. Andersson, B. & Eriksen, L. Conjoint action of sodium and angiotensin on brain mechanisms controlling water and salt balance. Acta. Physiol. Scand. 81, 18–29 (1971).

53. May, C. N. & McAllan, R. M. Baroreceptor-independent renal nerve inhibition by intracerebroventricular angiotensin II in conscious sheep. Am. J. Physiol. 273, R560–R567 (1997).
54. Ungar, T. et al. Differential effects of central angiotensin II and substance P on sympathetic nerve activity in conscious rats. Implications for cardiovascular adaptation to behavioral responses. Circ. Res. 56, 563–575 (1985).
55. Watson, A. M., Mogulko, R., McAllan, R. M. & May, C. N. Stimulation of cardiac sympathetic nerve activity by central angiotensinergic mechanisms in conscious sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, R1051–R1056 (2004).
56. Mittendorfer, R., D. M., Mattei, J. J. & Davenport, A. P. Emerging pharmacology and physiology of neuromedin U and the structurally related peptide neuromedin S. Br. J. Pharmacol. 158, 87–103 (2009).
57. Sasaki, T., Shimizu, T., Wakahuchi, H. & Yokotani, K. Centrally administered neuromedin U elevates plasma adrenaline by brain prostanoid TP receptor-mediated mechanisms in rats. Eur. J. Pharmacol. 592, 81–86 (2008).
58. Usui, D. et al. Selective activation of the sympathetic ganglia by centrally administered corticotropin-releasing factor in rats. Auton. Neurosci. 146, 111–114 (2009).
59. Wright, J. H. & Harding, J. W. Brain renin-angiotensin—a new look at an old system. Prog. Neurobiol. 95, 49–67 (2011).
60. Davison, R. L. Physiological genomic analysis of the brain renin-angiotensin system. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285, R498–R511 (2003).
61. Sasamura, H. et al. Cloning, characterization, and expression of two angiotensin receptor (AT1) isoforms from the mouse genome. Biochem. Biophys. Res. Commun. 215, 253–259 (1995).
62. Burson, J. M., Aguilar, G., Gross, K. W. & Sigmund, C. D. Differential expression of angiotensin receptor 1A and 1B in mouse. Am. J. Physiol. 267, E260–E267 (1994).
63. Mayrov, D. N. Brain angiotensin AT1 receptors as specific regulators of cardiovascular function to acute psychomotor stress. Clin. Exp. Pharmacol. Physiol. 38, 126–135 (2011).
64. Gao, L. & Zucker, L. H. AT1 receptor signaling and sympathetic regulation. Curr. Opin. Pharmacol. 11, 124–130 (2011).
65. Blanch, G. T. et al. Increased Expression of Angiotensin II Type2 Receptors in the Solitary-Vagal Complex Blunts Renovascular Hypertension. Hypertension. 64, 777–778 (2014).
66. Criscione, L. et al. Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT1 receptor subtype. Br. J. Pharmacol. 110, 761–771 (1993).
