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Intracisternally applied angiotensin II does not excite reticulospinal vasomotor neurons in anesthetized rats

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Abstract

We examined whether vasomotor neurons in the rostroventrolateral reticular nucleus of the medulla oblongata might be responsible for an acute increase in arterial pressure, elicited by application of angiotensin II in the central nervous system, as suggested by others. In urethane-pentobarbital-anesthetized and ventilated rats, intracisternal administration of angiotensin II (1–30 nmol, infused over a period of 30 s) produced a dose-dependent pressor response, which was abolished by intracisternal application of [Sar^1,Thr^8]angiotensin II (100 nmol), an angiotensin II receptor antagonist. The pressor response, however, was neither preceded by nor associated with increased discharges of vasomotor neurons with slow- and fast-conduction axons in the rostroventrolateral reticular nucleus and of lumbar sympathetic chain and renal sympathetic nerves. Intravenous injections of [β-mercapto-β,β-cyclopentamethylenepropinyl^1,-*O*-Et-Tyr^2,Val^4,Arg^8]vasopressin, a vasopressin receptor antagonist, largely abolished the central angiotensin II-induced pressor response, while a blockade of ganglionic transmission with hexamethonium and disruption of descending sympathoexcitatory output were ineffective. We conclude that central administration of angiotensin II, under the experimental conditions and at the doses, evokes an acute pressor response largely through the release of vasopressin, not by exciting vasomotor and sympathetic neurons.

Keywords: Angiotensin II; Pressor response; Medullary vasomotor neuron; Rostral ventrolateral medulla; Vasopressin

1. Introduction

Much interest has been raised to roles of angiotensins in the generation and maintenance of hypertension. Angiotensin II is a powerful vasoconstrictor and regulates salt and water balance. It releases vasopressin and increases arterial pressure, drinking, and proximal tubular sodium reabsorption (Severs et al., 1966; Unger et al., 1985; Reid and Rubin, 1987; Phillips, 1987; Van Giersbergen et al., 1992; Timmermans et al., 1993). Blocking the angiotensin receptors and inhibiting the angiotensin-converting enzyme, the enzyme that converts angiotensin I to angiotensin II thus reduce arterial pressure (Adams et al., 1990; Harrap et al., 1990; Oddie et al., 1992; Wu and Berecek, 1993; Unger and Gohlke, 1994; O'Sullivan and Harrap, 1995).

Cardiovascular responses elicited by administration of angiotensin II directly into the central nervous system have been proposed to depend on responses of neurons in either (a) the area postrema or subfornical organ (Allen et al., 1988; Gutman et al., 1989; Gorbea-Oppliger and Fink, 1995); (b) the rostroventrolateral reticular nucleus of the medulla oblongata to increase arterial pressure (Allen et al., 1988; Sasaki and Dampney, 1990; Li et al., 1992), or (c) the caudal ventrolateral medulla or the nucleus tractus solitarius to decrease arterial pressure (Fow et al., 1994; Sesoko et al., 1995). The nucleus tractus solitarius and the caudal ventrolateral medulla in mammals represent major brain stem areas involved in sympathetic baroreflex and stimulation of neurons in these specific areas inhibits the spinal cord-projecting vasomotor neurons in the rostroventrolateral reticular nucleus and thereby sympathetic nerve activity (Sun, 1995). The area postrema and subfornical organ, on the other hand, are circumventricular organs and are believed by some to play an essential role in linking sensing angiotensin II levels in the circulation and cerebrospinal fluid and controlling cardiovascular functions. Activation of neurons in the area postrema may result in an excitation or inhibition of the vasomotor neurons in the rostroventrolateral reticular nucleus (Sun and Spyer, 1991).

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While chronic administration of angiotensin II results in increased sympathetic nerve activity and arterial pressure (Bishop et al., 1995), studies addressing whether enhanced sympathetic tone contributes to the immediate increases in arterial pressure evoked by centrally administrated angiotensin II remain inconclusive (Kline et al., 1990; Reid, 1992). Relative contribution of vasopressin and the sympathetic nervous system to the pressor response may also vary with experimental conditions, species, and route of injections (Severs et al., 1966; Van Giersbergen et al., 1992; Lee et al., 1995). Thus, the pressor response to intracisternally applied angiotensin II in conscious rabbits was three orders of magnitudes more sensitive after sinoaortic denervation (Elghozi and Head, 1990).

A potential mechanism for angiotensin II-induced pressor response is suggested by several investigators (see below) to involve vasomotor neurons in the rostroventrolateral reticular nucleus of the medulla oblongata. These neurons are believed to play a critical role in generating resting sympathetic nerve activity and integrate a variety of centrally and reflexively initiated sympathetic responses (Sun, 1995). They project to the intermediolateral cell column of the spinal cord, probably monosynaptically innervating the sympathetic preganglionic neurons and their excitation is known to elicit immediate, powerful increases in arterial pressure. Thus, acute microinjections of angiotensin II into the nucleus were reported to increase arterial pressure and sympathetic nerve activity (Allen et al., 1988; Sasaki and Dampney, 1990; Li et al., 1992; Chan et al., 1994) and of an angiotensin II receptor antagonist, to reduce arterial pressure (Sasaki and Dampney, 1990). The latter observation suggests a tonic activity of angiotensin II release in the rostroventrolateral reticular nucleus. However, it has not been directly examined whether an excitation of vasomotor neurons underlies the centrally administered angiotensin II-induced increases in arterial pressure at effective doses. Microinjections of high concentrations of peptides may produce non-specific effects. In addition, angiotensin II is a potent vasoconstrictor and its microinjections into the rostroventrolateral reticular nucleus may constrict vessels and induce ischemia-hypoxia. Ischemia-hypoxia is known to evoke a powerful sympathoexcitatory response, by exciting reticulospinal vasomotor neurons in the rostroventrolateral reticular nucleus (Sun and Reis, 1994b). The response may be pathophysiologically significant, such as at hyper-angiotensinemia, and is, however, sensitive to the systemic administration of ganglionic blockers and adrenoceptor antagonists.

In the present study, we, therefore, investigated whether vasomotor neurons in the rostroventrolateral reticular nucleus might mediate centrally administered angiotensin II-induced pressor response by directly monitoring the neuronal activity at an effective dose. We demonstrated that central angiotensin II-induced increases in arterial pressure were abolished by a systemic blockade of vasopressin receptors but neither preceded by nor associated with increased activities of the vasomotor and sympathetic neurons. A brief abstract of part of this study has been presented (Sun and Reis, 1995b).

2. Materials and methods

2.1. General procedure

Adult male Sprague-Dawley rats (325–370 g) were anesthetized with urethane-sodium pentobarbital (20% and 0.24%, respectively), administered slowly via a tail vein. The initial dose (usually 1.3–1.5 ml) was adjusted to eliminate the hind paw-pinch retraction reflex. Cannulas were inserted into the left brachial artery for recording arterial pressure, the left femoral vein for injections of supplemental anesthetics and drugs, and the trachea for artificial ventilation. They were paralyzed with tubocurarine (2.5–3.0 mg/kg, i.v.) and ventilated with a rodent respirator with 100% O_2. End-tidal CO_2 was maintained between 3.5 and 4.5% by adjusting tidal volume and/or respiratory rate. An inflatable snare was placed around the descending aorta below the diaphragm to briefly constrict the aorta, elevate arterial pressure, and thereby stimulate the arterial baroreceptors (Sun and Reis, 1994a). Rectal temperature was monitored and maintained at 37.5 ± 0.5°C with a heating pad. The adequacy of anesthesia under paralysis was assured by the observation that arterial pressure was stable and unresponsive to pinching the hind paw. Whenever small but obvious changes in arterial pressure ($\geq$ 5 mm Hg) were observed in response to the pinching, an additional dose of anesthetic was given (5–15 mg/kg sodium pentobarbital, i.v., adjusted to eliminate the response).

In rats in which single-unit recordings of reticulospinal vasomotor neurons in the rostroventrolateral reticular nucleus were performed, rats were placed on a stereotaxic frame and a hole of 5–6 mm in diameter was drilled into the interparietal bone with its center 2 mm lateral to the midline and 3.5 mm posterior to the lambdoid suture. A bipolar concentric stimulating electrode (NE 100, David Kopf Institute, tip separation 0.5 mm) was placed in the fascia surrounding the mandibular branch of the facial nerve to locate the motor nucleus of the facial nerve by recording the antidromic field potentials. The location of the field potentials was used as an internal landmark to search for reticulospinal vasomotor neurons in the rostroventrolateral reticular nucleus. A second identical bipolar stimulating electrode was inserted on the right side (the ipsilateral side of the single-unit recordings) of the spinal cord at T_2-T_3 with its tip 1.0 mm below the dorsolateral sulcus to test for antidromic activation of single units recorded in the rostroventrolateral reticular nucleus.

Intracisternal (i.c.; 20 μl/about 30 s) infusions of agents were made via PE-10 tubing inserted through the atlanto-occipital membrane as described previously (Sun et

al., 1988). Such a volume and a period of injection seem to produce reliable, effective concentrations of the injected agents in the rostroventrolateral reticular nucleus (Sun et al., 1988) and a lasting pressor response so that effects of baroreceptor unloading can be tested. We have demonstrated that reticulospinal vasomotor neurons in the rostroventrolateral reticular nucleus are within the reach of i.c. administered agents since such an application of kynurenate, a weak receptor antagonist of the excitatory amino acid receptors with EC_{50} values of the range of 200–500 μM (Stone and Burton, 1988), effectively abolished responses of the vasomotor neurons to a variety of reflexes and iontophoretically applied *N*-methyl-D-aspartate (Sun et al., 1988; Sun and Reis, 1993).

Angiotensin II (human, synthetic; Sigma) and [Sar^1,Thr^8]angiotensin II (Sigma) were dissolved in a solution containing (in mM) 124 NaCl, 4.9 KCl, 25.6 $NaHCO_3$, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 3.1 $CaCl_2$, and 10 glucose, with pH of the solution adjusted to 7.3.

2.2. Single-unit recordings and iontophoresis

Single-unit recordings were performed by using a glass electrode filled with 3 M NaCl (4–8 MΩ) and protruded from 5-barreled electrodes by 25–35 μm (Sun and Reis, 1994a, 1995a). Signals were filtered (200–4000 Hz band pass) and monitored on an oscilloscope. The unit discharges, digitized by a window discriminator, were counted during intervals of 1 s and recorded as an integrated activity histogram. 5-barreled pipettes were used for iontophoresis. Current balancing was performed via a channel filled with 3 M NaCl. The remaining four channels were filled with one of the following solutions: 0.8 M γ-aminobutyric acid (GABA; Sigma), pH 4.5; 0.2 M L-glutamate (Sigma), pH 8.5; in 160 mM NaCl. Ejecting current intensities were limited to 100 nA at maximum to avoid the influence of non-specific current effects (Sun and Reis, 1994a). A retaining current of 10 nA was applied between periods of drug ejections.

Designation of neurons in the rostroventrolateral reticular nucleus as the reticulospinal vasomotor neurons was established by well recognized criteria (Sun and Reis, 1994a, Sun and Reis, 1995a): (a) they were localized within the cardiovascular region of the nucleus; (b) they were antidromically excited from stimulation of the intermediolateral columns of the thoracic spinal card; (c) they fired spontaneously with discharge probability related to the cardiac rhythm; (d) their activity showed an identical pattern and sensitivity as that of the sympathetic nerves to reflex inhibition evoked by activation of the arterial baroreceptors; and (e) they were immediately and markedly inhibited by microiontophoresis of GABA and excited by iontophoresis of L-glutamate, indicating that the signals were not recorded from axons of passage in the area. The rostro-ventrolateral reticular nucleus in rats contains two subpopulations of spinal cord-projecting barosensitive neurons, among neurons with respiratory rhythms and a large majority of non-cardiovascular non-respiratory neurons. The majority of the reticulospinal barosensitive neurons are intrinsic pacemakers with relatively fast-conducting axons (2–8 m/s) to the spinal cord, while the remaining reticulospinal barosensitive neurons have slow conducting axons (about 0.5 m/s). It has not been clearly defined whether the latter are intrinsic pacemakers. Efforts were made to examine responses of both subpopulations of the barosensitive neurons to an effective dose of angiotensin II, by intensively searching for the slow-conducting barosensitive neurons.

2.3. Nerve recordings

Sympathetic nerve activity was recorded either from the lumbar sympathetic chain, isolated adjacent to the bifurcation of the aorta and iliolumbar arteries (between L_3 and L_5) or from the renal sympathetic nerve. The nerves were placed on bipolar silver electrodes flooded with warm mineral oil. Electrical activity was recorded using a wide band AC pre-amplifier and integrator (Model 7P3C, Grass Institute). Neural activity was full-wave rectified, integrated, and displayed as arbitrary voltages relative to the values of a 500 Hz internal square wave calibration pulse (Sun and Reis, 1994a).

2.4. Production of hypoxia

Systemic hypoxia was induced by rapidly switching the input gas to the respirator from 100% O_2 to 100% N_2 for 20 s, followed by switching back to 100% O_2. This procedure was found to reduce arterial blood O_2 partial pressure, on average, to 27 mm Hg, measured at the end of N_2 inhalation (Sun and Reis, 1994a).

2.5. Statistical analysis

Statistical analysis was performed using the Student's *t* test for paired data whenever appropriate. The values are expressed as means ± S.E. of the mean. Differences were judged significant at $P < 0.05$.

3. Results

3.1. Intracisternal administration of angiotensin II dose- and receptor-dependently increases arterial pressure

Intracisternal administration of angiotensin II produced a pressor response, which lasted 1–4 min, depending on the doses (Fig. 1). The response was dose-dependent. At 1 nmol, i.c. administered angiotensin II over a period of about 30 s did not evoke any obvious changes in arterial pressure, while higher doses of the agent significantly increased arterial pressure. For instance, at 30 nmol, i.c.

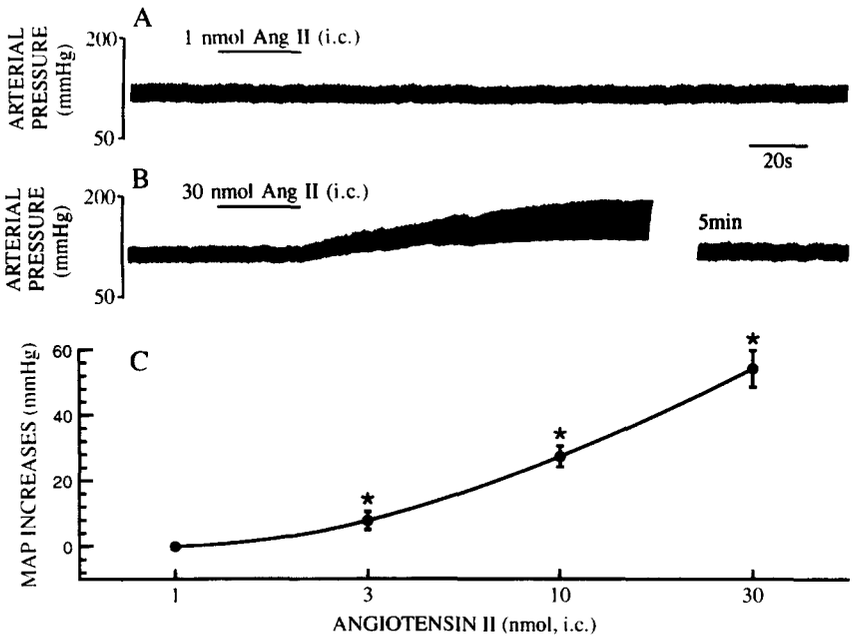

Fig. 1. Intracisternal infusion of angiotensin II produces dose-dependent acute increases in arterial pressure. A: a small dose of angiotensin II (1 nmol/20 μl; Ang II at bar, administered over a period of about 30 s) did not produce any obvious changes in arterial pressure. B: angiotensin II at 30 nmol (20 μl; Ang II at bar; administered over a period of about 30 s) produced a lasting marked increase in arterial pressure. A full recovery was observed 5 min after the end of the administration. C: dose-dependent increases in mean arterial pressure (MAP). Each value represents maximal increases in mean arterial pressure (mean ± S.E.M.; $n = 6$) evoked by the doses of intracisternal angiotensin II, slowly administered over a period of 30 s.

administered angiotensin II over a period of 30 s evoked a maximal, lasting increase in mean arterial pressure by 54.2 ± 5.6 mm Hg on average ($n = 6$, $P < 0.05$) from mean arterial pressure of 105.3 ± 2.7 mm Hg. The pressor response was reproducible since a second injection of the same dose at an interval of 30 min after the previous dose elicited the same magnitude of response (not shown). Whenever two or more doses of angiotensin II were given in the same rat, a 30 min interval was always allowed before a subsequent dose was given.

Table 1
Effects of intracisternal infusion of an angiotensin II receptor blocker and intravenous administration of a ganglionic blocker and a vasopressin receptor antagonist on intracisternal angiotensin II (30 nmol)-induced acute increases in arterial pressure

Treatments	n	Resting MAP (mm Hg)	Δ in MAP (mm Hg)	P [d]
Control	6	104.3 ± 3.0	+53.9 ± 5.4 [a]	
[Sar[1],Thr[8]]Ang II (i.c.)	6	103.8 ± 2.9 [b]	+2.3 ± 2.5 [b]	< 0.05
Control	7	105.0 ± 3.2	+52.4 ± 5.3 [a]	
Hexamethonium + PE	7	104.2 ± 3.1 [b]	+51.8 ± 5.2 [a]	> 0.05
Control	5	103.7 ± 3.3	+52.1 ± 5.7 [a]	
Cervical transection + PE	5	104.5 ± 3.6 [b]	+51.6 ± 5.5 [a]	> 0.05
Control	6	106.2 ± 2.6	+54.2 ± 5.2 [a]	
Vasopressin R. antagonist [c]	6	105.0 ± 2.5 [b]	+10.6 ± 2.7 [a]	< 0.05

MAP: mean arterial pressure; Ang: angiotensin; PE: L-phenylephrine (i.v.); R.: receptor. [a] Significant changes ($P < 0.05$). [b] Changes not significant ($P > 0.05$). [c] [β-Mercapto-β,β-cyclopentamethylenepropinyl[1],-O-Et-Tyr[2],Val[4],Arg[8]]vasopressin. [d] P levels of significance for i.c. angiotensin II-induced responses when compared with those in the absence of the treatments.

The receptor specificity of the i.c. angiotensin II-induced pressor response was examined by i.c. administration of [Sar[1],Thr[8]]angiotensin II (100 nmol/20 μl per 25–30 s, i.c.), an angiotensin II receptor antagonist. 30 min after the angiotensin II administration (i.c., 30 nmol/20 μl per 30 s) and 5 min after the injection of the antagonist, a second dose (30 nmol/20 μl per 30 s) of angiotensin II was administered. No significant increase in arterial pressure was evoked by i.c. angiotensin II after the antagonist administration, while injections of the angiotensin II receptor antagonist alone did not produce any obvious changes in arterial pressure (Table 1).

3.2. Intracisternal administration of angiotensin II decreases activities of vasomotor neurons in the rostroventrolateral reticular nucleus and of lumbar and renal sympathetic nerves

Effects of i.c. angiotensin II (30 nmol/20 μl per 30 s) on activities of the vasomotor neurons in the rostroventrolateral reticular nucleus were examined on 12 vasomotor neurons recorded. Eight of the neurons had conduction velocity of axons ranging from 2.0 to 6.2 m/s, therefore, the fast-conduction vasomotor neurons, calculated by dividing the distance between the recording electrode in the medulla and stimulating electrode in the spinal cord. The remaining four were slow-conduction barosensitive neurons with the calculated conduction velocities between

0.48–0.64 m/s. They all exhibited an identical pattern of response during the i.c. angiotensin II-induced pressor response, as illustrated in Fig. 2, so that their values were pooled together. Briefly, they discharged at relatively constant rate (12.5 ± 2.7 spikes/s) under the resting conditions. Their basal firing rate (14.8 ± 3.4 spikes/s) was defined when sodium nitroprusside was injected (i.v.) to unload the arterial baroreceptors. During i.c. angiotensin II-induced pressor response, no increases in their firing rate were observed. Instead, their firing rate was gradually reduced as the pressor response increased in magnitudes until finally the neurons all became silent (Fig. 2B; therefore -12.5 ± 2.7 spikes/s, $n = 12$, $P < 0.05$). Sodium nitroprusside was then injected (i.v.) to unload the arterial baroreceptors to determine whether the inhibition was largely the result of baroreflex inhibition in nature. The pressor responses were, thus, cut short (before the response peak was actually reached) so that their values were not analyzed. The baroreceptor unloading reversed the responses of the vasomotor neurons (Fig. 2B) in all the 12 neurons tested. Their firing rate during the baroreceptor unloading was 13.9 ± 3.2 spikes/s on average, lower than but not significantly different ($n = 12$, $P > 0.05$) from their basal firing rate before the angiotensin II administration. The same pattern of neuronal responses, decreased activity corresponding to the magnitudes of the evoked increases in arterial pressure, during the i.c. administration of angiotensin II was observed when the peptide was given at smaller doses (3 or 10 nmol; not shown).

Effects of i.c. angiotensin II (30 nmol/20 μl per 30 s) on sympathetic nerve activity were also examined. In six rats, resting sympathetic nerve activity recorded from the lumbar sympathetic chain exhibited an integrated activity of 19.6 ± 2.4 μV on average. The nerves were inhibited by activation of the arterial baroreceptors. Unloading the arterial baroreceptors with i.v. injections of sodium nitroprusside increased their discharges to a basal level of 22.9 ± 2.6 μV on average. Intracisternal administration of 30 nmol (20 μl/30 s) increased arterial pressure and gradually decreased the sympathetic nerve activity as the pressor response gained in amplitudes. The nerves finally became silent (therefore, -19.6 ± 2.4 μV, $n = 6$, $P < 0.05$; Fig. 3B). Much as the responses of the vasomotor neurons in the rostroventrolateral reticular nucleus, unloading the arterial baroreceptors with i.v. injections of sodium nitroprusside reversed the inhibition (Fig. 3B). The integrated sympathetic nerve activity during the baroreceptor unloading was 21.5 ± 3.0 μV on average, lower but not significantly different from the basal activity before the i.c. administration of angiotensin II. The same pattern of nerve response, decreased activity corresponding to the magnitudes of the evoked increases in arterial pressure, during the i.c. administration of angiotensin II was also observed when the peptide was given at smaller doses (3 or 10 nmol; not

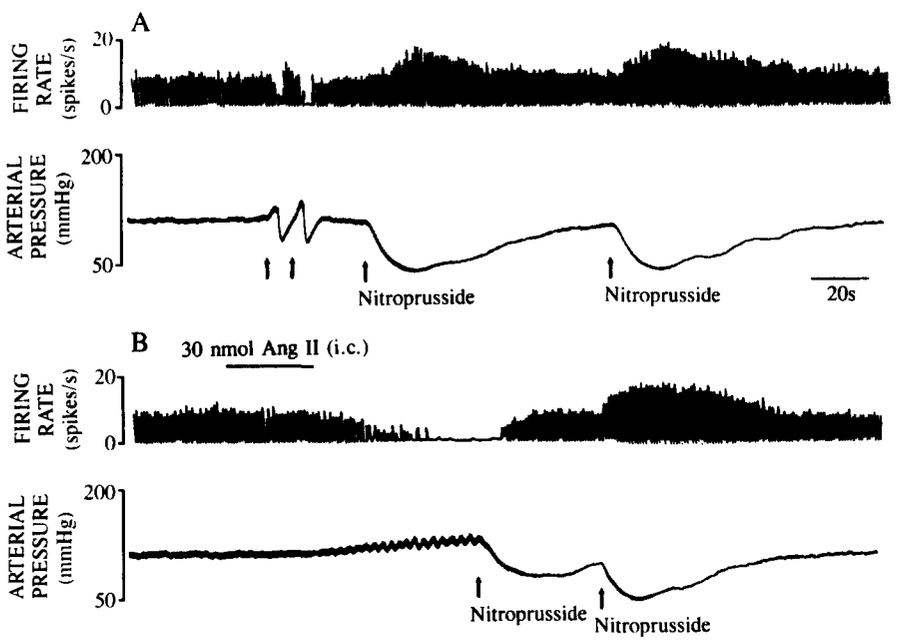

Fig. 2. Intracisternal infusion of angiotensin II induces acute increases in arterial pressure but not in the activity of reticulospinal vasomotor neurons in the rostroventrolateral reticular nucleus. A: a reticulospinal vasomotor neuron was inhibited by a brief constriction (started at the first two arrows and released at each peak of the increases) of the descending aorta to increase arterial pressure but increased its firing rate upon baroreceptor unloading during decreases in arterial pressure evoked by i.v. injections of sodium nitroprusside (10 μg). B: intracisternal administration of angiotensin II (30 nmol; Ang II at bar) evoked an increase in arterial pressure. The neuron, however, was inhibited during the angiotensin II-induced acute increases in arterial pressure and no increase in its firing rate was observed. When the pressor response became obvious, unloading the arterial baroreceptors with i.v. injections of sodium nitroprusside returned activity of the neuron to its basal firing rate.

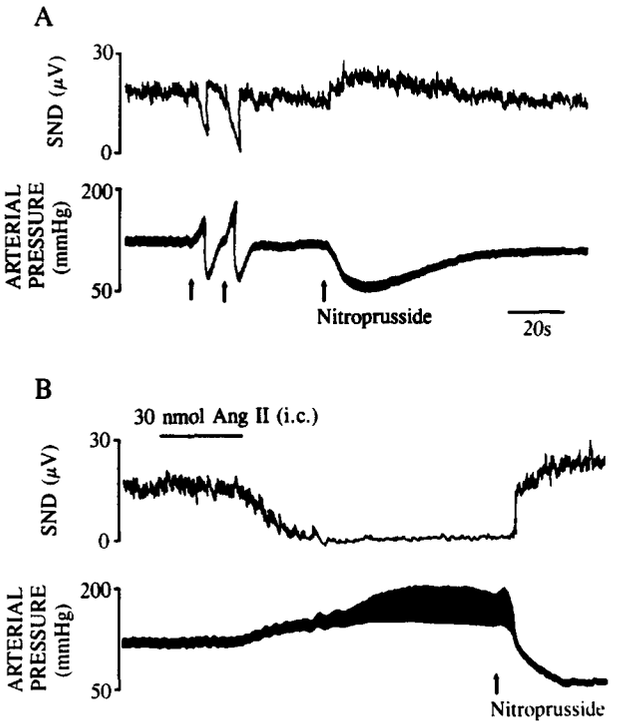

Fig. 3. Intracisternal infusion of angiotensin II induces acute increases in arterial pressure but not in the activity of the sympathetic nerves. A: the sympathetic nerve was inhibited by a brief constriction (started at the first two arrows and released at each peak of the increases) of the descending aorta. The nerve increased its activity upon baroreceptor unloading during decreases in arterial pressure evoked by i.v. injections of sodium nitroprusside (10 μg). B: intracisternal administration of angiotensin II (30 nmol; Ang II at bar) evoked an increase in arterial pressure. The nerve, however, was inhibited during the angiotensin II-induced acute increases in arterial pressure and no increase in its activity was observed. The decrease in the nerve activity during the pressor response could be attributed to baroreflex inhibition of sympathetic neurons by the evoked increase in arterial pressure. When the pressor response became obvious, unload the arterial baroreceptors with i.v. injections of sodium nitroprusside returned the nerve activity to its control level. SND: sympathetic nerve discharge.

shown). In five rats, recording of the renal sympathetic nerves showed an identical response pattern and magnitudes to i.c. angiotensin II (30 nmol/20 μl per 30 s) as the lumbar sympathetic chain. No increases in the renal nerve activity were observed during the evoked pressor response. The nerves decreased their activity as the pressor response gained in amplitudes and became silent. These data were therefore not presented in detail.

The results, an inhibition during the evoked pressor response and an almost full reversal of the inhibition of the vasomotor neurons and sympathetic nerves by baroreceptor unloading to their control basal levels, indicate that the decreases in activities of the vasomotor neurons and sympathetic nerves during i.c. angiotensin II-induced acute increases in arterial pressure can be attributed entirely to the baroreflex inhibition of the vasomotor and sympathetic neurons, evoked by the hemodynamic changes.

3.3. Blocking ganglionic transmission and cervical spinal transection do not abolish i.c. angiotensin II-induced pressor responses

The i.c. administered angiotensin II-induced acute increases in arterial pressure were further examined when ganglionic transmission was blocked with hexamethonium and after the spinal cord was transected at the cervical level. The effectiveness of the procedures was indicated by an immediate decrease in mean arterial pressure to 50–60 mm Hg and evaluated by examining the hypoxic pressor response, which has been shown to depend entirely on a rapid excitation of the vasomotor neurons in the rostroventrolateral reticular nucleus (Sun and Reis, 1993, 1994a, 1995a; Sun, 1995).

In seven rats, N_2 inhalation for 20 s evoked an oscillated maximal increase in arterial pressure (not shown) by 36.8 ± 4.9 mm Hg ($P < 0.05$) on average, from their resting arterial pressure (Table 1), as described previously (Sun and Reis, 1993, 1994a). After an i.v. injection of hexamethonium (200 mg/kg) to block the ganglionic transmission, arterial pressure (reduced initially to 57.7 ± 3.6 mm Hg on average) was restored to the control levels and maintained by an i.v. infusion of L-phenylephrine (0.5–2.0 mg/kg per h). Under such conditions, intracisternal administration of angiotensin II produced a pressor response, which did not differ significantly from the control response (Table 1). N_2 inhalation for 20 s, however, evoked a brief decrease in arterial pressure by 46.5 ± 4.7 mm Hg ($n = 7$, $P < 0.05$) at maximum, a vasodilatory response to hypoxia (Sun and Reis, 1993, 1994a). No obvious increases in arterial pressure were observed.

A complete spinal cord transection at the cervical C_1 level was performed in five rats, after observing the responses to i.c. angiotensin II (30 nmol/20 μl per 30 s) and to acute hypoxia ($+35.4 \pm 5.6$ mm Hg, $P < 0.05$). Arterial pressure (reduced to 56.9 ± 3.2 mm Hg on average after the transection) of these rats was restored to their control level and maintained by an i.v. infusion of L-phenylephrine (0.5–2.0 mg/kg per h). Under such conditions, i.c. administration of the same dose of angiotensin II elicited a pressor response, not significantly different from the response before the transection (Table 1). N_2 inhalation, however, did not produce a pressor response. The acute hypoxic response in arterial pressure after the spinal transection was a maximal decrease in mean arterial pressure by 48.2 ± 4.6 mm Hg ($n = 5$, $P < 0.05$) on average.

3.4. Systemic administration of a vasopressin receptor antagonist reduces i.c. angiotensin II-induced pressor responses

The possibility that the i.c. administered angiotensin II might produce pressor responses through release of vasopressin into the blood was evaluated by examining the pressor response after i.v. injections of an vasopressin

receptor antagonist, [β-mercapto-β,β-cyclopentamethyleneprop inyl[1],-O-Et-Tyr[2],Val[4],Arg[8]]vasopressin (3 mg/kg). The injections of the vasopressin receptor antagonist alone had no significant effects on the resting arterial pressure but largely reduced (by 80% on average; 5 min after the end of the injection) the i.c. angiotensin II-induced pressor response (Table 1).

4. Discussion

In the present study, we have demonstrated that responses of the vasomotor neurons in the rostroventrolateral reticular nucleus do not underlie central angiotensin II-induced acute pressor response in urethane-anesthetized normotensive rats. First, the i.c. angiotensin II-induced pressor response, evoked in evaluating the neuronal and nerve involvements, was sufficiently large (an over 50 mm Hg increase in mean arterial pressure). The relatively higher doses of angiotensin II may reflect the way of administration (a period of infusion rather a bolus injection, to produce a sustained pressor response for testing effects of baroreceptor unloading), remote sites of actions, restricted diffusion to the sites, and possible less potent of the synthesized human form in the species. Second, the pressor response depends on the activation of the angiotensin II receptors since the response was abolished by the application of the receptor antagonist. The application of the angiotensin II receptor antagonist alone did not produce any detectable changes in arterial pressure, consistent with the general contention that the angiotensin system plays a minimal role in the control of arterial pressure in normotensive animals (Timmermans et al., 1993). Third, the i.c. angiotensin II-induced pressor response was neither preceded by nor associated with increased discharges of the vasomotor neurons, including the fast- and slow-conduction neurons, and of the sympathetic nerves. This can not be attributed to the evoked big pressor response that led to an inhibition of sympathetic outflow and overshadowed a sympathoexcitatory response, for (a) smaller doses of intracisternal angiotensin II evoked smaller pressor responses and corresponding inhibition of the activities of the vasomotor neurons and sympathetic nerves; (b) the pressor response evoked by the big dose of angiotensin II developed rather slowly without an early increased activity of the vasomotor neurons and sympathetic nerves, which otherwise should have been evident; and (c) no increased activities of the vasomotor neurons and sympathetic nerves were observed when the hemodynamic responses were abolished during baroreceptor unloading. The results are consistent with the finding that micromolar concentrations of angiotensin II did not markedly excite the vasomotor pacemaker neurons in the rostroventrolateral reticular nucleus in vitro (Sun and Guyenet, 1989). In a recent study, 61% of the spontaneously active neurons with irregularly non-bursting firing in the rostroventrolateral reticular nucleus in vitro were excited by angiotensin II (Li and Guyenet, 1995). However, spinal cord-projecting spontaneously active neurons in the rostroventrolateral reticular nucleus with an irregular non-bursting pattern of discharges are not cardiovascular for their activity does not show a cardiac rhythm in vivo (Granata, 1994). Fourth, blocking the ganglionic transmission or disrupting the descending sympathoexcitatory output from the medulla to the spinal cord did not reduce i.c. angiotensin II-induced acute pressor response, while a systemic blockade of vasopressin receptors largely reduced the pressor response, indicating that release of vasopressin is the major mediator in the pressor response.

I.c. administered angiotensin II may act at three major sites to evoke vasopressin release: hypothalamic supraoptic and paraventricular nuclei and median preoptic nucleus, the subfornical organ, and A_1 and A_6 noradrenergic cells. Angiotensin II directly inhibits the transient outward I_A and I_K currents of rat supraoptic neurosecretory neurons (Nagatomo et al., 1995) and excites them (Okuya et al., 1987). There is in fact an angiotensinergic excitatory projection from the subfornical organ to the hypothalamus supraoptic and paraventricular nuclei and the median preoptic nucleus (Swanson and Sawchenko, 1983; Jhamandas et al., 1989; Lippoldt et al., 1993) and the pathway can be excited by applying angiotensin II in the subfornical organ (Tanaka and Nomura, 1993; Wright et al., 1993). The paraventricular nucleus, on the other hand, receives noradrenergic inputs from the A_1 and A_6 cell groups (Jones and Moore, 1977; Swanson and Sawchenko, 1983) and release of noradrenaline in the paraventricular nucleus activates vasopressinergic projections from the paraventricular nucleus to the hypophysis, resulting in release of vasopressin into the systemic circulation and increases in arterial pressure and drinking (Veltmar et al., 1992). In addition, intracerebroventricular administration of angiotensin II in pressor doses has been found to induce norepinephrine release in the paraventricular nucleus (Stadler et al., 1992; Steckelings et al., 1992).

Our study, however, does not rule out the possibility that chronic administration of angiotensin II may raise activities of the vasomotor neurons and sympathetic nerves. Several days were reported to be required for angiotensin II to reset sympathetic baroreflexes (Bishop et al., 1995), though Kline et al. reported that in rats chronic angiotensin II-induced increases in arterial pressure (10 days of infusion) were not associated with enhanced norepinephrine turnover (Kline et al., 1990). Neither do the present results rule out the possibility that diffusion of the agent into the systemic circulation may contribute to part of the pressor response. Angiotensin II, like vasopressin (Reid and Rubin, 1987), is known to act on adrenal chromaffin cells (Marley and Bunn, 1988), sympathetic ganglia (Wong et al., 1992) to facilitate catecholamine release, and on vessels to enhance responses of the vascular smooth muscle to norepinephrine (Reid and Rubin, 1987; Purdy and Weber,

1988). These actions can be blocked by systemic administration of ganglionic blockers and adrenoceptor antagonists. It also remains to be studied whether the reticulospinal vasomotor neurons in some hypertensive animals might be more sensitive to acute increases in angiotensin II concentrations (Muratani et al., 1993).

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