Angiotensin II Induces Catecholamine Release by Direct Ganglionic Excitation

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Abstract—Angiotensin II (ANG) is known to facilitate catecholamine release from peripheral sympathetic neurons by enhancing depolarization-dependent exocytosis. In addition, a direct excitation by ANG of peripheral sympathetic nerve activity has recently been described. This study determined the significance of the latter mechanism for angiotensin-induced catecholamine release in the pithed rat. Rats were anesthetized and instrumented for measuring either hemodynamics and renal sympathetic nerve activity or plasma catecholamine concentrations in response to successively increasing doses of angiotensin infusions. Even during ganglionic blockade by hexamethonium (20 mg/kg), angiotensin dose-dependently elevated sympathetic nerve activity, whereas blood pressure–equivalent doses of phenylephrine were ineffective. Independently of central nervous sympathetic activity and ganglionic transmission, angiotensin (0.1 to 1 μg/kg) also induced an up-to 27-fold increase in plasma norepinephrine levels, reaching 2.65 ng/mL. Preganglionic electrical stimulation (0.5 Hz) raised basal norepinephrine levels 11-fold and further enhanced the angiotensin-induced increase in norepinephrine (4.04 ng/mL at 1 μg/kg ANG). Stimulation of sympathetic nerve activity and norepinephrine release were suppressed by candesartan (1 mg/kg) or tetrodotoxin (100 μg/kg), respectively. Angiotensin enhanced plasma norepinephrine, heart rate, and sympathetic nerve activity at similar threshold doses (0.3 to 1 μg/kg), but raised blood pressure at a significantly lower dose (0.01 μg/kg). It is concluded that direct stimulation of ganglionic angiotensin type 1 (AT₁) receptors arouses electrical activity in sympathetic neurons, leading to exocytotic junctional catecholamine release. In both the absence and presence of preganglionic sympathetic activity, this mechanism contributes significantly to ANG-induced enhancement of catecholamine release. (Hypertension. 2002;40:348-354.)

Key Words: angiotensin II ■ angiotensin antagonist ■ catecholamines ■ sympathetic nervous system ■ electric stimulation ■ rats

Angiotensin II (ANG) potently enhances catecholamine release from the peripheral sympathetic system, an action that implies important pathophysiological consequences. Catecholamines released by this mechanism contribute to the vasoconstricting and sodium-retaining properties of ANG. In particular, the chronic effects of ANG at moderate to the vasoconstricting and sodium-retaining properties of ANG. In particular, the chronic effects of ANG at moderate to high doses have been implicated in the development of hypertension and in the concomitant myocardial damage that has been attributed to a stimulation of cardiac β-adrenoceptors.

ANG activates the sympathetic system via several mechanisms. Central nervous sympathetic tone is increased by circulating or locally produced ANG in nuclei responsible for autonomic control. In the peripheral sympathetic system, the termi of adrenergic neurons are equipped with prejunctional angiotensin type 1 (AT₁) receptors whose activation enhances the efficacy of catecholamine discharge induced by each action potential. Such facilitation of norepinephrine (NE) release has frequently been investigated in isolated tissues under electrical stimulation and appears to depend on a presynaptic autinhibitory tone that is relieved in response to ANG. The inability of ANG to induce catecholamine release from isolated tissues in the absence of electrical excitation provoked the common view that facilitation of depolarization-induced catecholamine release represents the essential interaction of ANG with the peripheral sympathetic system in intact animals as well.

However, an alternative pathway also exists through which ANG can stimulate peripheral sympathetic neurons. Activation at the level of the sympathetic ganglia has been described as the mechanism responsible for the cardiac effects of ANG, which comprise increases in heart rate and inotropy. Recently, such ganglionic effects of ANG were further characterized by Ma et al, who demonstrated a direct induction of electrical activity in renal sympathetic nerves of mice even during blockade of cholinergic ganglionic transmission and an increase in intracellular Ca²⁺ in isolated ganglionic cells. These observations indicate that ANG may directly depolarize sympathetic nerves via activation of an-
angiotensin receptors located at the postganglionic cell body. However, it still must be confirmed that ANG will excite intact sympathetic ganglia when preganglionic innervation is abolished more efficiently than just by cholinergic blockade. Furthermore, it is unknown whether ANG-induced ganglionic excitation may be able to provoke peripheral catecholamine release and whether such an effect may involve nonexocytotic modes of catecholamine secretion. Most importantly, the 2 proposed interactions of ANG with the peripheral sympathetic system, ganglionic excitation and prejunctional facilitation, need to be compared with respect to their significance for ANG-induced catecholamine release.

To this end, we investigated ANG-induced catecholamine release in pithed rats after surgical and pharmacological disruption of preganglionic sympathetic activity and in the absence and presence of electrical preganglionic stimulation.

Methods

Pithed Rat Preparation
Male Wistar rats (280 to 350 g, Charles River, Sulzfeld, Germany) were pithed as described earlier. Briefly, the animals were anesthetized with ether, and artificial respiration was initiated with an endotracheal tube. Pentobarbital (50 mg/kg IP) was used as an anesthetic in rats that were to be instrumented for renal sympathetic nerve activity (RSNA) determinations. The thoracolumbar medulla was destroyed using a stainless steel pithing rod, which also served as an electrode for preganglionic electrical stimulation. Catheters were placed into a carotid artery and a femoral vein, and both vagal nerves were severed. Blood pressure was measured via the carotid catheter and was sampled digitally. Only in experiments aiming at the determination of plasma catecholamine concentrations, neuronal catecholamine uptake1 and presynaptic autophosphorylation were suppressed by initial pretreatment with desipramine (0.5 mg/kg, IV) and phenoxybenzamine (10 mg/kg, IV), respectively. Under these conditions, plasma catecholamine concentrations can be considered as a close parameter of total sympathetic outflow. Sympathetic nerve excitation was evaluated at a branch of the left renal nerve, whose electrical activity was picked up by extracellular steel electrodes as recently described. The investigation had been approved by the authorities of the State of Schleswig-Holstein, and the experiments conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Experimental Protocol

Rats of separate groups were stimulated either by IV bolus injections of ANG (up to 3 μg/kg) or phenylephrine (up to 30 μg/kg), or by application of electrical current to preganglionic nerves (0.1 to 3 Hz, 1 ms, 20 V) or after ganglionic electrical stimulation (0.5 Hz, 1 ms, 20 V). Stimulations were applied with increasing intensities at application of electrical current to preganglionic nerves (0.1 to 3 Hz, 1 ms, 20 V). Stimulations were applied with increasing intensities at

Plasma Catecholamine Determinations

Blood samples were taken from the carotid artery and stabilized with reduced glutathione (4 mmol/L) and EDTA (6 mmol/L) to prevent catecholamine oxidation. Catecholamine concentrations were determined by reversed-phase high-performance liquid chromatography (HPLC) and electrochemical detection, as described elsewhere.19

Substances

The AT1 receptor antagonist candesartan (1 mg/kg), the ganglionic blockade with 20 mg/kg hexamethonium. In some experiments, the AT1 receptor antagonist candesartan, and the α-adrenoceptor antagonist phenoxybenzamine (10 mg/kg) were given IV 5 minutes before commencement of the ANG stimulations.

Results

Basal and Control Parameters

After surgical preparation, pithed animals stabilized out to an average mean arterial pressure (MAP) of 58±2 mm Hg and a heart rate of 351±11 beats per minute (bpm). No hemodynamic alterations were provoked by application of hexamethonium (57±2 mm Hg, 339±11 bpm) or addition of atenolol and phenoxybenzamine (62±3 mm Hg, 338±6 bpm), whereas candesartan significantly reduced blood pressure (29±2 mm Hg, 372±18 bpm). The basal levels of renal sympathetic activity in pithed and hexamethonium-treated animals (9.0±0.9 μV) could be ascribed to electrical noise of the experimental setup, because identical signals were also registered post mortem (9.7±1.1 μV).

ANG-Induced Sympathetic Excitation

After suppression of central nervous sympathetic tone by destruction of the spinal medulla and additional ganglionic blockade, ANG, at doses ≥0.3 μg/kg, induced a robust electrical excitation of the renal nerve (Figures 1 and 2). The RSNA signal increased simultaneously with blood pressure. Typically, RSNA peaked and declined earlier than MAP so that it was reset during the slow phase of blood pressure decay (Figure 1). The arousal of RSNA was a specific action of ANG and was not related to blood pressure or heart rate alterations, because it was not provoked by phenylephrine injected at doses up to 30 μg/kg, which developed equivalent vasopressor efficacies (up to 107 mm Hg MAP, data not depicted). Blockade of sympathetic excitation by candesartan also indicated the essential involvement of neuronal AT1 receptors (Figure 2).
ANG-Induced Norepinephrine Release
Postganglionic induction of sympathetic activity by ANG resulted in a dose-dependent, up to 27-fold increase of plasma NE, as assessed in rats treated with desipramine and phenoxybenzamine during ganglionic blockade (Figure 3). Imitation of sympathetic tone by preganglionic electrical stimulation at 0.5 Hz elevated plasma NE concentrations 11-fold to 1.14±0.22 ng/mL (control stimulation in Figure 3) and increased MAP by more than 40 mm Hg (Figure 4). In the presence of this preganglionic stimulation, the efficacy of ANG for elevating plasma NE levels was further increased compared with the condition of ganglionic blockade (Figure 3, \(P<0.05\) for ANG at 0.3 and 1\(\mu\)g/kg). NE release resulting from neuronal excitation by ANG during ganglionic blockade was mediated by \(\text{AT}_1\) receptors and involved depolarization-dependent propagation and exocytosis, because it was abolished by pretreatment with candesartan as well as tetrodotoxin (Figure 3). Direct stimulation by ANG was more effective in postganglionic sympathetic neurons rather than in the adrenal medulla, because plasma levels of epinephrine showed minor responses. Basal plasma levels of epinephrine in ganglion-blocked animals (0.16±0.019 ng/mL) increased 3.6-fold after application of ANG at 1\(\mu\)g/kg.

**Figure 1.** Exemplary registration of mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) in response to a 10-second bolus injection of ANG (3\(\mu\)g/kg). The primary nerve signal (RSNA direct) is depicted along with the signal derived by integration as described in Methods (RSNA integrated).

**Figure 2.** Dose-dependent arousal of RSNA by angiotensin II (ANG) in pithed rats. Intravenous bolus injection of ANG at doses \(\geq 0.3\) \(\mu\)g/kg enhanced the electrical activity of the renal nerve under control conditions (△), but not after treatment with candesartan (1 mg/kg, ○). Despite equivalent hemodynamic efficacy, phenylephrine at doses up to 30\(\mu\)g/kg did not enhance RSNA (not depicted). Data represent mean±SEM of 5 experiments. *\(P<0.05\) versus control stimulation.

**Figure 3.** Dose-dependent enhancement of norepinephrine (NE) overflow by ANG in pithed rats. ANG was administered by IV infusion for 1-minute periods, after which plasma was sampled for NE determination. ANG dose-dependently increased plasma NE concentration during preganglionic stimulation (0.5 Hz, △), as well as after ganglionic blockade by hexamethonium (○), whereas blockade of \(\text{Na}^+\) channels by tetrodotoxin (□) or of \(\text{AT}_1\) receptors by candesartan (●) abolished this response. The ANG-induced increase of NE concentrations relative to the pre-stimulation levels was higher during electrical stimulation than after ganglionic blockade (\(P<0.05\) for 0.3 and 1\(\mu\)g/kg ANG), so that the contribution of ganglionic excitation to ANG-enhanced NE release (at 1\(\mu\)g/kg) during preganglionic stimulation (0.5 Hz) can be estimated to 52%. Data represent mean±SEM of 5 experiments. *\(P<0.05\) versus control stimulation.

**Figure 4.** Functional blockade of ganglionic transmission by hexamethonium. Preganglionic electrical stimulation provoked frequency-dependent increases of mean arterial pressure (MAP) under control conditions (△). Pretreatment with hexamethonium (○) abolished the vasopressor effects of electrical stimulation. MAP values correspond to basal levels of 60±5 mm Hg and 57±7 mm Hg in the control and ganglion-blocked groups, respectively. Data represent mean±SEM of 5 experiments. *\(P<0.05\) versus control stimulation.

**Hemodynamic Responses**
To verify the efficacy of preganglionic electrical stimulation and ganglionic blockade, frequency-dependent responses of MAP were monitored in supplementary experiments. Stimulation intensities of 0.1 to 3 Hz elevated MAP by up to 105 mm Hg, thus covering the full physi-
These findings extend earlier investigations addressing the interactions of the renin angiotensin system and the sympathetic system. Clear evidence for such interactions exists with regard to the enhancement by ANG of central nervous sympathetic tone and to the facilitation of catecholamine release brought about by stimulation of prejunctional ANG receptors on terminals of postganglionic neurons. These mechanisms and the direct ganglionic excitation can be summarized as a neuronal adrenergic component of ANG actions, which may be of great pathophysiological significance for conditions with an activated renin-angiotensin system, such as renal hypertension and heart failure.

The contributions of these 3 potential mechanisms to sympato-excitation by ANG are not easy to dissect. Although prejunctional mechanisms can be excluded by investigating renal or muscle sympathetic nerve activity rather than catecholamine release, the influence of ANG on central nervous sympathetic activity comprises both, a direct attenuation of the baroreceptor reflex as well as its indirect activation provoked by the vasopressor effects of ANG. Experimental correction for this hemodynamic influence revealed that enhancement of sympathetic tone by ANG may be related to baroreceptor reflex sensitivity but can also occur in the absence of blood pressure alterations. In volunteers, even the inhibition of endogenous ANG by the AT1 receptor antagonist losartan attenuated the sympato-excitatory reflex elicited by adenosine without involving any changes in blood pressure.

Such sympathetic activation independent of the baroreceptor reflex may include influences of ANG on peripheral ganglionic transmission. The potential of such actions has been demonstrated with regard to the positive inotropic and chronotropic cardiac responses to ANG that were provoked by local applications to the stellate or caudal cervical ganglia. Although ANG may enhance sympathetic activity by direct spinal actions and by facilitation of acetylcholine release from preganglionic neurons, the postganglionic neurons have been considered to be the relevant target of ANG actions. This was later confirmed by stimulation studies on isolated ganglionic cells. Recently, direct excitation of murine ganglion cells by ANG has been shown to induce Ca2+ influx, which may be linked as a trigger or consequence to neuronal depolarization and thus may initiate propagated electrical activity in intact postganglionic neurons.

In accordance with these interpretations, the mechanism of ANG-mediated peripheral sympathetic excitation by direct ganglionic actions is now extended to a rat model that provides a well-controlled surgical and pharmacological interruption of central nervous sympathetic activity and ganglionic transmission. The ganglion-located cell body of the postganglionic neuron was identified as the target of direct neuronal ANG actions, because ANG is unable to elicit catecholamine release from peripheral sympathetic reticulum in the absence of electrical depolarization and AT1 receptors are located at high densities in sympathetic ganglia. The interpretation of the measured nerve signal as an effenter activity of sympathetic fibers is supported by the previously confirmed absence of afferent signals in ANG-stimulated RSNA and by the ANG-induced enhancement of catecholamine overflow described in this study. The causal link between enhancement by ANG of electrical nerve activity and NE release is also confirmed by the ability of tetrodotoxin to abolish the
latter reaction completely. This effectiveness of the Na\(^{+}\)-channel inhibitor demonstrates that NE release in this condition proceeds by classical action potential-triggered exocytosis.

This study presents the first quantification of peripheral sympathetic activity and catecholamine release caused by ANG-mediated ganglionic excitation. This mechanism effectively triggers NE release, resulting in plasma NE levels that exceed those provoked by preganglionic electrical stimulation at 0.5 Hz (Figure 3). Thus, the efficacy of ganglionic stimulation by ANG (at 1 \(\mu g/kg\)) is comparable to a markedly elevated efferent sympathetic tone that is able to raise MAP by more than 40 mm Hg (Figure 4). This interpretation was also confirmed by the robust increase in RSNA in response to ANG (4.1 \(\mu g\) at 1 \(\mu g/kg\) ANG). Similar extracellular nerve signals (3.6 \(\mu V\)) have been described as indicative of profound sympathetic activation induced by the baroreceptor reflex.\(^2\) The mechanism of ganglionic excitation by ANG does not appear to be restricted to the renal nerve. As revealed by the increase in heart rate, the same doses of ANG (0.3 to 1 \(\mu g/kg\)) stimulate cardiac adrenergic innervation, as well as RSNA and systemic NE release.

The inclusion of experiments under preganglionic stimulation also permits an estimation of the efficacy of catecholamine release by direct ganglionic stimulation in comparison with that by depolarization-dependent prejunctional facilitation. Because the enhancement of plasma NE by ANG was more effective in the presence than in the absence of preganglionic stimulation, it can be concluded that both ganglionic and prejunctional mechanisms of release had become activated by ANG in the dose range investigated (Figure 3). The relative contribution of each mechanism can be estimated when the ANG-induced increase in plasma NE during ganglionic blockade (2.65 ng/mL at 1 \(\mu g/kg\) ANG) is interpreted as a combined effect of ANG-induced postganglionic activity and prejunctional facilitation. A simultaneous preganglionic electrical stimulation at 0.5 Hz adds to the postganglionic activity, while leaving the efficacy of release constant. This combined stimulation increases plasma NE levels to 5.08 ng/mL, so that the additional increase (2.43 ng/mL) can be attributed to the gain in postganglionic activity. It can thus be concluded that direct ganglionic excitation contributes 52\% to NE release even in the presence of a substantial preganglionic activity. In turn, plasma NE concentrations evoked by purely preganglionic stimulation (1.04 ng/mL) would be increased by ANG to calculated NE levels of 2.17 ng/mL if a proportional increase in postganglionic activity is presumed in the absence of prejunctional facilitation. Consequently, the actual excess in the increase of plasma NE to 5.08 ng/mL under these conditions can be attributed to presynaptic facilitation, which therefore seems to enhance NE release at a given postganglionic activity by 134\%. The magnitude of this effect is consistent with the efficacy of ANG to enhance NE release from isolated and electrically stimulated tissue preparations.\(^6\)\(^8\) Because of the requirement of postganglionic activity for NE release, the significance of ganglionic excitation by ANG increases with lower intensities of preganglionic stimulation. During ganglionic blockade in our model, the direct excitation by ANG is able to enhance the basal levels of plasma NE by as much as 27-fold.

Despite the effective induction of NE release by ganglionic excitation, the physiological significance of this mechanism remains elusive. In accordance with previous findings,\(^2\) our data demonstrate that adrenergic pathways are relevant for the increase in heart rate, but not for the acute vasopressor actions of ANG in the lower dose range (Figure 5), and thus cast doubt on the significance of ganglionic effects in ANG-dependent blood pressure regulation. Even though the renin-angiotensin system in the pithed rat is activated because of the low blood pressure,\(^3\) we found no evidence for a pertinent stimulation of ganglia, which could have emerged in terms of a basal level of RSNA, or by an ability of candesartan to decrease the basal plasma levels of NE. The high doses of ANG necessary to provoke ganglionic excitation may correspond to plasma levels that will not be reached in vivo. Although plasma concentrations of ANG have not yet been determined after bolus injections, it may be estimated that a dose of 1 \(\mu g/kg\) may be roughly equivalent to a continuous infusion of 270 ng/kg per minute. This dose has been shown to raise plasma levels of ANG by 2400 pg/mL,\(^2\) thus increasing the elevated levels in the pithed rat (about 400 pg/mL) by a factor of 6. Interestingly, this high dose of ANG was also required to enhance adrenergic pressor responses to preganglionic stimulation in the pithed rat, an action that should predominantly be related to prejunctional facilitation.\(^3\) It must be stated that the overall unphysiological conditions of our model do not permit conclusions as to the significance of ganglionic excitation under long-term physiological or pathophysiological conditions in vivo.

Few observations can be put forward to advocate such a significance. The clear evidence that enhancement of sympathetic tone by ANG is of significance for chronic hypertension\(^1\) has been attributed to a resetting of the baroreceptor reflex\(^12\) or to an impairment in reflex gain,\(^3\) so that ANG actions on autonomic brain nuclei, such as the area postrema, appeared to be involved\(^3\) rather than peripheral ganglia. Ganglionic excitation would be expected to increase the minimum levels of sympathetic nerve activity that can be determined after full activation of the baroreceptor reflex. A study by Xu et al\(^15\) has found this parameter in sodium-deprived rats to be suppressed by losartan in a manner that is independent of intact area postrema function. This effect may be interpreted as a baroreceptor-independent stimulation by circulating ANG of basal adrenergic tone, possibly provoked by ganglionic excitation. Another issue is that of a possible local generation and action of ANG in peripheral nerve tissues. Sympathetic ganglia are equipped with ANG receptors\(^2\) and may belong to the tissues that express ANG generating systems, as has been demonstrated for various cell types, including neurons.\(^3\) The release of immunoreactive ANG on preganglionic stimulation was recently demonstrated in the canine stellate ganglion.\(^3\) The functional consequences of this release were described as an arousal of adrenergic
cardiac reactions that were insensitive to ganglionic blockade but could be antagonized by an AT₁ receptor antagonist.³⁷ This observation seems to indicate that excitation of postganglionic neurons and the concomitant release of catecholamines, as demonstrated in the present study, may be due to an effect of ANG produced locally within sympathetic ganglia.

Perspectives
The present study was able to demonstrate that ganglionic excitation contributes significantly to ANG-induced catecholamine release in a whole animal model. In the absence of preganglionic sympathetic tone, this ganglionic action is a prerequisite for the induction of catecholamine release by ANG and is still responsible for about half of the ANG-induced catecholamine release occurring under physiological preganglionic activity. Although this study was not designed to yield any indication of a physiological occurrence of ganglionic excitation itself, it pointed out that a potentially important mechanism has been neglected in our previous considerations of the interactions between the renin-angiotensin and the sympathetic systems. This omission is most evident in the multitude of pharmacological investigations performed in isolated cells or tissues, which inevitably excluded ganglionic effects. As such, the antiadrenergic actions of AT₁ receptor antagonists may receive guidance for the treatment of chronic autonomic dysregulation, such as hypertension. If ANG takes place within the ganglia, this would result in a tonic postsynaptic activity that may be barely detectable, but may be involved over the long term in diseases of chronic autonomic dysregulation, such as hypertension. The proposal of such intraganglionic renin-angiotensin systems may bear the most promise of discovering new physiopathological and pathophysiological functions of ganglionic angiotensin II.

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References


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