An electrophysiological study of the actions of angiotensin II at the sympathetic neuroeffector junction in the guinea-pig vas deferens

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1 The effects of angiotensin II on sympathetic neuroeffector transmission in the guinea-pig vas deferens have been investigated by the use of intracellular and focal extracellular recording techniques to measure indirectly, the release of adenosine 5'-triphosphate (ATP).

2 Angiotensin II (10–100 nM) did not alter the amplitude of the first excitatory junction potential (e.j.p.) in a train but increased the amplitude of subsequent e.j.ps. There was a corresponding increase in the probability of occurrence of extracellularly recorded evoked excitatory junction currents (e.j.cs). Spontaneous quantal transmitter release was unaffected by angiotensin II.

3 The enhancement of transmitter release produced by angiotensin II was prevented by the angiotensin receptor antagonist, saralasin.

4 The increase in transmitter release produced by angiotensin II was due to an increase in the probability of transmitter release from individual varicosities and not due to any detectable change in the configuration of the nerve terminal impulse or to the induction of repetitive firing.

5 There was no overall enhancement of e.j.ps or e.j.cs by angiotensin II in reserpinized tissues. Surprisingly, the predominant effect of angiotensin II in reserpinized vasa deferentia was to inhibit evoked transmitter release, an effect reversed by indomethacin (3 µM).

6 The results show that angiotensin II increases the release of sympathetic transmitter by activating prejunctional angiotensin II receptors. However, when the co-transmitter noradrenaline was depleted, angiotensin II now inhibited transmitter release indirectly, presumably by stimulating prostaglandin formation in the smooth muscle cells which then inhibited release transjunctionally.

Keywords: Angiotensin II; Electrophysiology; ATP; Sympathetic nerves; Transmitter release; Vas deferens

Introduction

Many substances can modulate transmitter release from sympathetic nerve terminals by activating specific prejunctional receptors (see Starke, 1977; Westfall, 1977). However, surprisingly little is known about the precise mechanism of action of many of these agents at the level of the individual varicosity. In part this is because methods have not been available to study transmitter release mechanisms in sympathetic nerve terminals on an impulse-to-impulse basis. Novel electrophysiological approaches have shown that electrically evoked transmitter release from individual varicosities in the vas deferens occurs intermittently and is monoquantal (Blakeley & Cunnane, 1979; Cunnane & Stjärne, 1982; Cunnane, 1984). Focal extracellular recording techniques have further shown that intermittence is not caused by a failure of the nerve impulse to invade the varicosity each time the parent axon is stimulated but is due to a low probability of release in the invaded varicosity (Brock & Cunnane, 1987; 1988). Hirst & Nield (1980) also concluded from studies of junction potentials in guinea-pig mesenteric arterioles that evoked transmitter release occurs intermittently. With electrophysiological techniques it is now possible to define more precisely the site of drug action at the sympathetic neuroeffector junction. In particular, the effects of drugs applied to just a few varicosities in the terminal arborization can be studied. In the present investigation the effects of angiotensin II on sympathetic transmission in the guinea-pig vas deferens have been investigated.

Angiotensin II, an octapeptide which plays a role in the regulation of blood pressure (Martin, 1985), is known to enhance sympathetic neuroeffector transmission (Ziogas *et al.*,

1984) by activating prejunctional angiotensin II receptors (reviewed by Starke, 1977; Westfall, 1977; Ziogas, 1991) but the mechanisms involved are unclear. For example, angiotensin II may alter the configuration of the action potential in the nerve terminals or induce repetitive firing. Alternatively, angiotensin II may modify one of the steps involved in depolarization-secretion coupling. Focal extracellular recording techniques can distinguish unequivocally between these possibilities. Furthermore, it is possible to determine whether the enhancement of sympathetic neurotransmission by angiotensin II involves an alteration in the size of the transmitter quantum or in the probability of a varicosity releasing a quantum of transmitter, effects which have not previously been considered.

Earlier studies of the effects of angiotensin II on transmitter release largely involved measurements of noradrenaline overflow from electrically stimulated tissues (see Starke, 1977; Westfall, 1977). However, it is now widely believed that the electrical changes recorded in smooth muscle cells following sympathetic nerve stimulation are due to the action of adenosine 5'-triphosphate (ATP) or a related purine nucleotide, released as a co-transmitter with noradrenaline (Stjärne, 1986; White, 1988). Thus, it is important to remember that in the present study the effects of angiotensin II on ATP rather than noradrenaline release have been investigated. The effects of angiotensin II on purinergic transmission when the noradrenaline stores of sympathetic nerves were depleted by reserpine were also investigated.

Methods

General

Male Duncan-Hartley guinea-pigs (250–350 g) were killed by a blow to the head and bled. Vasa deferentia and the associated

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hypogastric nerve trunks were removed and placed in Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, NaH_2PO_4 0.4, NaHCO_3 25.0, MgCl_2 1.2, CaCl_2 1.8 and glucose 11.1. The Krebs solution was continuously bubbled with 95% O_2 /5% CO_2 to pH 7.4.

Reserpine pretreatment

Guinea-pigs were given a single intraperitoneal injection of reserpine (5 mg kg^{-1}) 18 h before removal of the vasa deferentia. The extent of the noradrenaline depletion was assessed by Falck-Hillarp fluorescence histochemistry or by high performance liquid chromatography (h.p.l.c.) analysis of the noradrenaline content of the vas deferens (Allcorn *et al.*, 1986). This reserpine pretreatment protocol routinely produced depletion of catecholamines below detectable levels.

Extracellular recording

Vasa deferentia were pinned out on the Sylgard (Dow-Corning) covered base of a 4 ml organ bath and the outer smooth muscle layer exposed by careful removal of the surface connective tissue in a small region. The organ bath was mounted on the stage of a Zeiss ACM microscope and the tissues perfused at a rate of $2\text{--}3 \text{ ml min}^{-1}$ with Krebs solution maintained at $36\text{--}37^\circ\text{C}$. The postganglionic sympathetic nerves innervating the vas deferens were stimulated through a suction electrode placed close to the prostatic end (Ferry, 1967; Brock & Cunnane, 1988). A digital stimulator (Applegarth Electronics) was used to deliver rectangular pulses of $0.05\text{--}0.1 \text{ ms}$ duration and $5\text{--}20 \text{ V}$ through an optically isolated stimulation unit (Square One Instruments).

A bevelled glass electrode (tip diameter $\leq 50 \mu\text{m}$) filled with Krebs solution was positioned on the surface of the vas deferens and slight suction applied (seal resistance $< 1 \text{ M}\Omega$). A sintered Ag/AgCl pellet in the bath served as the indifferent electrode. The electrode holder was modified to enable a polyethylene cannula to be passed down the barrel of the recording electrode. In this way it was possible to perfuse ($75\text{--}100 \mu\text{L min}^{-1}$) the tip of the electrode with ordinary Krebs solution or Krebs solution containing the appropriate drugs. Approximately 5 min was required for drug solutions to reach the tip as determined by local perfusion with tetrodotoxin to block nerve impulse conduction or by the use of fluorescent dyes. Signals were amplified through an a.c. amplifier (Neurolog NL 104, low frequency cut off 0.1 Hz) and stored on a Panasonic video recorder (AG6200) after digitization (20 kHz) through a Sony digital audio processor unit (PCM-701ES).

Intracellular recording

Vasa deferentia were pinned out on the Sylgard (Dow-Corning) covered base of a 2 ml organ bath and a small window cut in the surface connective tissue in order to expose the outer smooth muscle cells. The bath was perfused at a rate of $2\text{--}3 \text{ ml min}^{-1}$ with Krebs solution maintained at $36\text{--}37^\circ\text{C}$. In this case the sympathetic nerves were stimulated by gently pulling the hypogastric nerve trunk through a Ag/AgCl ring electrode positioned close to the prostatic end of the vas deferens. A stimulator was used to deliver rectangular pulses as described above.

Changes in membrane potential of smooth muscle cells, on or near the surface of the vas deferens, were recorded by conventional intracellular recording techniques (see Cunnane & Manchanda, 1988a). Capillary-glass micro-electrodes filled with 5 M potassium acetate (resistances $40\text{--}80 \text{ M}\Omega$) were positioned over the vas deferens with a Leitz micromanipulator and observed through a Wild dissecting microscope. A sintered Ag/AgCl pellet in the bath served as the indifferent electrode. Signals were amplified through a d.c. amplifier (Neurolog NL 102), digitized and stored as described for extracellular recording.

Analysis

A system based on a Zenith.386 microcomputer and an analogue-to-digital converter card (Data Translation – DT2801A) was used to analyse data previously stored on tape. Records were filtered (d.c. – 3 kHz , Neurolog NL 125) and amplified (Neurolog NL 105) as required for analysis by the computer programme SCAN and DVIEW kindly supplied by John Dempster (Strathclyde University).

A paired *t* test was used to compare data within individual experiments because of the variability in the amplitude and number of events which occurred from attachment to attachment. This variability arises from such factors as the seal resistance of the electrode, the number of functional varicosities in each attachment and their relationship to individual smooth muscle cells in a three dimensional functional syncytium.

Drugs

Angiotensin II (Sigma) and saralasin (Sigma) were prepared as 10^{-3} M stock solutions in distilled water, frozen as $100 \mu\text{L}$ aliquots, and serially diluted in Krebs solution as required. Reserpine (Sigma) was dissolved in 200 mg ml^{-1} ascorbic acid immediately prior to use. Indomethacin (Sigma) was dissolved in 0.1 M Na_2CO_3 and used immediately.

Results

Effects of angiotensin II on electrical activity recorded with a focal extracellular electrode

The pattern of electrical activity recorded when a focal extracellular electrode is placed on the surface of the vas deferens is shown in the left panel of Figure 1. Following electrical stimulation of the hypogastric nerve trunk with trains of 25 stimuli at 1 Hz , stimulus locked excitatory junction currents (e.j.cs) are evoked which are preceded by a non-intermittent nerve impulse. The e.j.c. is the response of the smooth muscle to evoked quantal release of transmitter and the preceding bi- or tri-phasic spike is the extracellular equivalent of the action potential in the nerve terminals. A detailed account of the intermittent nature of the transmitter release mechanism has been given elsewhere (Brock & Cunnane, 1987; 1988).

Control trains of 25 pulses at 1 Hz were delivered every 2 min until a relatively stable pattern of release was obtained, 10–15 trains usually being sufficient. When angiotensin II ($10\text{--}100 \text{ nM}$) was perfused into the tip of the recording electrode for 25 min (12 trains) a clear enhancement of evoked electrical activity was recorded. Representative trains of 25 pulses at 1 Hz from an experiment in which 30 nM angiotensin II was applied are shown in Figure 1. To improve the signal to noise ratio, the traces were averaged (Figure 1b) and the records show that angiotensin II does not produce any detectable change in the configuration of the nerve terminal impulse, nor induce repetitive firing. The effect on transmitter release was reversed by wash.

In each experiment, negative deflections of sufficient magnitude (twice baseline noise) and duration ($> 50 \text{ ms}$) were counted as e.j.cs and the number of e.j.cs occurring during 4 successive trains of 25 pulses at 1 Hz were summed to minimize variability between individual trains. The data from one such experiment is shown in Figure 2 which clearly indicates that angiotensin II increased the number of e.j.cs occurring per 100 stimuli. The pooled data from 4 experiments are summarized in Table 1.

It was important to establish that there was no change in the postjunctional sensitivity of the smooth muscle membrane to angiotensin II which might have accounted for the 'apparent' increase in e.j.c. probability. In the absence of stimulation spontaneous excitatory junction currents (s.e.j.cs) are recorded, which are a measure of the spontaneous quantal release of

Figure 1 Effects of locally applied angiotensin II on the nerve terminal impulse and transmitter release in the guinea-pig vas deferens. (a) Nerve terminal impulse (NTI) and excitatory junction currents (e.j.c.s) evoked by 25 pulses at 1 Hz, before, during 20 min perfusion with angiotensin II (30 nM) and 30 min after wash. Single attachment (b). Averages of the records shown in (a).

transmitter. The amplitude distributions of e.j.c.s and s.e.j.c.s obtained in the absence and presence of angiotensin II in the same attachment are compared in Figure 3. The e.j.c.s evoked by the last 8 control trains of 25 pulses at 1 Hz (200 pulses) before the addition of angiotensin II were analyzed together with the last eight trains of stimuli in the presence of angiotensin II. S.e.j.c.s were counted for periods of 5 min before and after the application of angiotensin II. In the absence of angio-

tensin II, both the e.j.c. and s.e.j.c. amplitude distributions were skewed, a characteristic which makes interpretation of frequency and amplitude changes less certain. Nevertheless, it is clear that angiotensin II increased the number of evoked e.j.c.s per train of stimuli and shifted the amplitude distribution to the right, and occasional e.j.c.s larger than those seen in the absence of angiotensin II were observed. In contrast, the amplitude distribution of s.e.j.c.s was largely unaffected by angiotensin II. These changes indicate that angiotensin II increases the probability of evoked transmitter release.

Figure 2 Effects of local application of angiotensin II on electrically evoked transmitter release in the guinea-pig vas deferens. The histogram shows the number of e.j.c.s recorded during 4 successive trains of 25 pulses at 1 Hz before, during local perfusion with angiotensin II (30 nM) and after wash in the same attachment.

Effects of saralasin

In three experiments, the angiotensin II receptor antagonist saralasin (100 nM) was added to both the Krebs solution perfusing the recording electrode and the organ bath. Saralasin alone did not significantly alter the configuration of the nerve terminal impulse or the pattern of transmitter release. It should be noted that on one occasion, an increase in transmitter release was observed. This finding was not rigorously pursued in the present study and may be due to the partial agonist properties of saralasin (Ziogas *et al.*, 1984). The local application of 30 nM angiotensin II in the presence of saralasin did not now enhance transmitter release (Table 1).

Effect of angiotensin II on extracellular activity recorded in reserpinized vasa deferentia

Since ATP and not noradrenaline probably mediates the e.j.c. (Brock & Cunnane, 1988) it was of interest to study the effects of angiotensin II in catecholamine-depleted vasa deferentia. In an earlier study, the noradrenaline content of untreated vasa deferentia was $10.1 \pm 1.1 \mu\text{g g}^{-1}$ wet weight ($n = 6$) (Cunnane

Figure 3 Effect of angiotensin II on the amplitude distribution of s.e.j.cs and e.j.cs recorded in the same attachment. (a) Histograms showing the amplitude distribution of e.j.cs recorded in 8 consecutive trains of 25 pulses at 1 Hz before (left panel) and during the addition (10–24 min) of angiotensin II (30 nM) (right panel). Events less than 15 μV were not discernible from the noise level and were classified as failures of transmitter release. (b) Histograms showing the amplitude distribution of s.e.j.cs recorded in a 5 min period before and during local perfusion with angiotensin II (30 nM).

Figure 4 Effects of angiotensin II on e.j.p. amplitude in the guinea-pig vas deferens. (a) E.j.p.s evoked by trains of 10 pulses at 1 Hz before and during the addition of angiotensin II (30 nM) in a single cell. (b) The effects of angiotensin II on a population of cells ($n \geq 5$ for each group) recorded in single vas deferens before (○), during the application of angiotensin II (10 nM) (●) and after wash (□).

et al., 1989). In the present study, pretreatment of guinea-pigs with reserpine reduced the noradrenaline content of vasa deferentia to undetectable levels by either h.p.l.c. analysis or by Falck-Hillarp fluorescence histochemistry. Spontaneous and evoked electrical activity was recorded in vasa deferentia taken from animals pretreated with reserpine. Surprisingly, in reserpinized tissues, when angiotensin II was applied locally there was no overall increase in the probability of occurrence of e.j.cs (Table 1). Table 1 shows the pooled data from three experiments in which an inhibitor of prostaglandin synthesis, indomethacin (3 μM) was present. Indomethacin alone did not significantly affect transmitter release. However, in the presence of indomethacin, angiotensin II enhanced transmitter release in a manner analogous to that in non-reserpinized vasa. That is, angiotensin II increased the number of e.j.cs evoked per train of stimuli without affecting the configuration of the nerve terminal impulse. In untreated vasa deferentia the average increase in e.j.c. number was $51 \pm 8.6\%$ ($n = 4$) and in indomethacin-treated reserpinized vas deferentia the increase was $44.7 \pm 10.5\%$ ($n = 3$).

Effect of angiotensin II on electrical activity recorded with intracellular electrodes

The resting membrane potential of individual smooth muscle cells lay in the range -65 to -75 mV. Figure 4 shows the

Table 1 The effect of angiotensin II on the number of e.j.cs evoked when trains of 25 pulses at 1 Hz were given at 2 min intervals in untreated and reserpinized vasa deferentia

		n	Number of e.j.cs/100 pulses		
			Pre-AII	AII (30 nM)	Mean difference
Untreated vasa deferentia	Control	4	50 ± 9	74 ± 10	24 ± 4*
	Saralasin (100 nM)	3	58 ± 12	55 ± 12	-3 ± 6
Reserpine-treated vasa deferentia	Control	4	36 ± 8	39 ± 7	3 ± 3
	Indomethacin (3 μM)	3	37 ± 8	54 ± 9	16 ± 4*

Four successive trains were summed to minimize variability between trains (Number of e.j.cs/100 pulses). *Significantly different from pre-angiotensin II (AII) values ($P < 0.05$ paired t test).

characteristic pattern of facilitation of e.j.ps evoked by a train of 10 stimuli at 1 Hz. E.j.ps increased in amplitude during the train until a constant amplitude was reached after about 5–10 stimuli. As previously reported (Bell, 1972; Cunnane *et al.*, 1989), there was no clear effect of angiotensin II on the first e.j.p. in a train but subsequent e.j.ps were enhanced (Figure 4). The resting membrane potential and spontaneous excitatory junction potentials (s.e.j.ps) were unaffected by angiotensin II (not shown).

Effect of angiotensin II on intracellularly recorded activity in reserpinized vasa deferentia

In reserpinized vasa deferentia the resting membrane potential was the same as in untreated tissues (65–75 mV) and e.j.ps of normal amplitude were recorded during trains of stimuli at 1 Hz. However, the pattern of facilitation was prolonged compared with untreated vasa deferentia (Figure 4 cf. Figure 5), presumably because of removal of α_2 -adrenoceptor autoinhibition (Brock *et al.*, 1990). Angiotensin II (30 nM) produced

variable effects on e.j.p. amplitude in reserpinized vasa deferentia. The predominant effect of angiotensin II was to depress e.j.p. amplitude ($n = 4$). Figure 5a shows the effects of angiotensin II (30 nM) on e.j.ps recorded in a single cell. The effects of angiotensin II on the mean e.j.p. amplitude measured in a population of cells is shown in the left panel of Figure 5b. In 4 experiments when indomethacin (3 µM) was present in the bathing solution, angiotensin II always enhanced e.j.p. amplitude. The results of an individual experiment in which the effects of angiotensin II were assessed in a population of cells, first in the absence and then in the presence of indomethacin are shown in Figure 5b. The initial inhibition of e.j.ps produced by angiotensin II in reserpinized tissues was clearly reversed to an enhancement in the presence of indomethacin, suggesting that prostaglandins are involved in transjunctional modulation of purinergic transmission. It should be noted that the enhancement of transmitter release produced by angiotensin II in untreated vasa deferentia was observed once and no effect of angiotensin II was observed on another occasion, in experiments with reserpinized vasa deferentia.

Figure 5 Inhibition of e.j.p. amplitude by angiotensin II in vasa deferentia taken from reserpine pretreated guinea-pigs. (a) Single cell showing the rapid inhibition of e.j.ps by angiotensin II (30 nM). (b) Effect of angiotensin II (30 nM) on a population of cells in the same preparation. The effects of angiotensin II are shown in the absence (left panel) and in the presence (right panel) of indomethacin (3 µM) ($n \geq 4$ cells for each group). Control (○); angiotensin II (●); after washout (□). It is clear that angiotensin II reduces e.j.p. amplitude in reserpinized vasa deferentia and that indomethacin treatment reverses this inhibition to an increase.

Discussion

The finding that angiotensin II increased the amplitude of e.j.ps evoked by trains of stimuli in the guinea-pig vas deferens agrees with the earlier report of Bell (1972). Angiotensin II enhanced e.j.ps without affecting the amplitude or frequency of occurrence of s.e.j.ps which indicates a prejunctional site of action. Surprisingly, angiotensin II did not increase the amplitude of the first e.j.p. in a train of stimuli at 1 Hz, a result not found with other agonists which alter transmitter release. For example, clonidine which inhibits (Blakeley *et al.*, 1981), and tetraethylammonium which enhances (Stjärne *et al.*, 1990) e.j.p. amplitude each have marked effects on the first e.j.p. in a train. It would seem that the varicosity has first to be invaded by a nerve impulse before angiotensin II can enhance release to subsequent stimuli. Similar conclusions can be drawn from the studies with focal extracellular electrodes in which the relationship between the arrival of the nerve terminal impulse and transmitter release was examined. The number of e.j.cs recorded per train of stimuli was increased in the presence of angiotensin II with no marked effect on the configuration of the nerve terminal impulse or without the induction of repetitive firing. Once again, there was no obvious effect on the e.j.cs evoked by the first pulse in a train, even when many trains of stimuli were averaged to minimise variability because of intermittence. The enhancing effect on transmitter release of angiotensin II was blocked by the angiotensin II receptor antagonist saralasin which supports the idea that activation of prejunctional angiotensin II receptors modifies the mechanisms involved in depolarization-secretion coupling.

Starke (1977) concluded that the prejunctional action of angiotensin II involves an increase in the amount of transmitter released per nerve impulse. Based on the assumption that every varicosity released transmitter on stimulation, the enhancement by angiotensin II would require an increase in the amount of transmitter released from each varicosity. It is now evident that transmitter release from individual varicosities in response to trains of low frequency stimuli is intermittent and monoquantal (see Introduction). Therefore, other mechanisms have to be considered to explain the facilitatory effects of angiotensin II on transmitter release mechanisms. Transmitter release may be enhanced by increasing the probability of release from active varicosities or by recruiting previously silent varicosities. Angiotensin II increased the number of e.j.cs recorded per train of stimuli and produced a shift to the right of the amplitude distribution of e.j.cs. If angiotensin II was solely affecting the quantal content of the same number of active varicosities, a shift to the right of the amplitude distribution without a change in the number of e.j.cs would have been expected. Caution needs to be exercised when interpreting changes in shape of skewed distributions, but it is worth noting that the effects of angiotensin II are similar to the effects of increasing the frequency of stimulation (Brock & Cunnane, 1988) or removing α_2 -autoinhibition (Brock *et al.*, 1990), procedures which also increase transmitter release.

E.j.ps and e.j.cs were also recorded from reserpinized vasa deferentia suggesting that the transmitter responsible for the generation of the e.j.p. and the e.j.c. is not noradrenaline. Furthermore e.j.ps and e.j.cs are abolished by α,β -methylene ATP which desensitizes P_2 -purinoceptors and can be mimicked by the local application of ATP (Brock & Cunnane, 1988; Cunnane & Manchanda, 1988a). ATP is likely to be the transmitter generating the e.j.p. in the vas deferens and is thought to be co-released with noradrenaline from sympathetic nerve terminals (Burnstock, 1986; White, 1988).

A surprising finding in the present study was that following reserpine pretreatment angiotensin II did not significantly

enhance e.j.ps or e.j.cs in the guinea-pig vas deferens. Rather, an inhibitory effect on evoked transmitter release was the predominant effect. This inhibitory effect of angiotensin II in reserpinized tissues was reversed to an enhancement by indomethacin treatment suggesting an increased role for stimulation of prostaglandin production by angiotensin II in noradrenaline-depleted preparations. Previous studies indicate that angiotensin II may stimulate prostaglandin synthesis. In tissues such as the, rat mesenteric bed (Jackson & Campbell, 1981), rat heart (Lanier & Malik, 1982) and dog heart (Lanier & Malik, 1983), stimulation of prostaglandin production by angiotensin II has an opposing inhibitory effect on sympathetic noradrenergic transmission, similar to that reported in the present study for sympathetic purinergic transmission. An inhibitory effect of angiotensin II on purinergic transmission has previously been reported. In the rabbit vas deferens, angiotensin II and angiotensin III inhibit the initial purinergic contraction evoked by electrical stimulation, an effect which is also reversed by inhibition of prostaglandin synthesis (Saye *et al.*, 1986; Trachte, 1988).

Interestingly, in the rabbit vas deferens angiotensin II and angiotensin III inhibited the purinergic but enhanced the noradrenergic component of contraction evoked by electrical stimulation. These effects were presumed to be prejunctional since the responses to exogenous noradrenaline and ATP were unaffected by the angiotensins. It has been suggested that this could represent indirect evidence that ATP and noradrenaline are stored and released from different locations in sympathetic nerve terminals (Trachte *et al.*, 1989). This suggestion was also made by Ellis & Burnstock (1989) on the basis of the findings that angiotensin II and angiotensin III could differentially modulate [3 H]-noradrenaline release and ATP release as determined by the luciferin-luciferase technique. In the present inquiry it is clear that, when neuronal noradrenaline stores are depleted by reserpine pretreatment, the predominant effect of angiotensin II is inhibition of purinergic transmission. It is not possible to draw any definitive conclusions from the present data about the question of co-storage and co-release of noradrenaline and ATP.

In conclusion, our electrophysiological studies indicate that at low frequencies of stimulation, angiotensin II enhances purinergic transmission. Angiotensin II increased the probability of release from individual varicosities which normally release transmitter intermittently. This prejunctional action of angiotensin II does not involve a change in the configuration of the nerve terminal impulse and is likely to involve changes in the intracellular processes activated by depolarization. In addition, our findings show that agents which modulate transmitter release by activating prejunctional receptors can have additional postjunctional actions which may become important after certain drug treatments or in specific pathophysiological conditions. Thus, following reserpine pretreatment, angiotensin II inhibited rather than enhanced purinergic transmission presumably by triggering prostaglandin synthesis in the smooth muscle which then transjunctionally inhibited transmitter release. Several questions remain unanswered including: (1) whether depletion of noradrenaline from the sympathetic nerve terminals *per se* or an effect of reserpine on smooth muscle cells produces the increased involvement of prostaglandins as transjunctional modulators of transmitter release; and (2) whether the pharmacological profile of the pre- and postjunctional angiotensin II receptor is different.

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