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Enhancement by exogenous and locally generated angiotensin II of purinergic neurotransmission via angiotensin type 1 receptor in the guinea-pig isolated mesenteric artery

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1 Angiotensin II is known to enhance sympathetic neurotransmission in the vasculature by increasing the release of noradrenaline, but little is known about the effect on the co-released transmitter, adenosine 5′-triphosphate (ATP). In the present study we have examined the effect of angiotensin II on the excitatory junction potential (e.j.p.) elicited by repetitive field stimulation in the guinea-pig isolated mesenteric artery, to establish the angiotensin II receptor subtype involved in modulating the release of ATP and the role of the endothelium in converting angiotensin I to angiotensin II.

2 Suramin (300 μM), a P2 purinoceptor antagonist, abolished both the e.j.ps and depolarizing response to α,β-methylene-ATP, a stable analogue of ATP, without affecting the resting membrane potential and noradrenaline-induced depolarization.

3 Angiotensin II (0.1 μM) affected neither the resting membrane potential nor the amplitude of the first e.j.p., but increased the amplitudes of the subsequent e.j.ps. This enhancing effect of angiotensin II was abolished by CV-11974 (0.1 μM), an angiotensin II type 1 (AT_1) receptor antagonist, but unaffected by PD 123319 (1 μM), an angiotensin II type 2 (AT_2) receptor antagonist, or CGP 42112A (1 μM), AT_2 receptor ligand.

4 Angiotensin I (0.1 μM) exerted a similar effect on e.j.ps to that of angiotensin II. CV-11974 (0.1 μM) or temocaprilat (10 μM), an angiotensin converting enzyme (ACE) inhibitor, abolished the effect of angiotensin I. Removal of the endothelium did not alter the action of angiotensin I.

5 The results of the present study indicate that the release of ATP from sympathetic nerves innervating the guinea-pig isolated mesenteric artery, as determined from the magnitude of the e.j.p., can be enhanced by angiotensin II via activation of prejunctional AT_1 receptors. Qualitatively similar effects were observed with angiotensin I, which appears to be converted into angiotensin II by a subendothelial process.

Keywords: Purinergic neurotransmission; angiotensin receptors; excitatory junction potential; renin-angiotensin system; guinea-pig mesenteric artery; endothelium

Introduction

Angiotensin II (AII) plays an important role in the regulation of vascular tone by a number of mechanisms (Dzau, 1988). Besides its direct effect on vascular smooth muscle, AII has been shown to enhance noradrenergic neurotransmission via presynaptic AII receptors (Malik & Nasjletti, 1976; Zimmerman, 1978). The existence of the local renin-angiotensin system is now well recognized (Dzau, 1988; Arnal *et al*., 1994). Local generated AII may also play a role in the modulation of neurotransmission (Malik & Nasjletti, 1976). AII receptors have been classified into two major subtypes, i.e., type 1 (AT_1) and type 2 (AT_2) (de Gasparo *et al*., 1995). Selective antagonists for AII receptors have been developed (de Gasparo *et al*., 1995), and this development prompted many investigators to determine the specific roles of AII receptor subtypes.

There is now considerable evidence indicating that adenosine 5′-triphosphate (ATP) is a co-transmitter with noradrenaline (NA) in sympathetic neuromuscular junctions (von Kügelgen & Starke, 1991a). In the human saphenous vein, electrical stimulation produced an ATP overflow along with NA (Rump & von Kügelgen, 1994). In certain smooth muscle cells, such as those from the guinea-pig vas deferens (Sneddon & Westfall, 1984) and the rat tail artery (Sneddon, 1992), ATP (or a related purine nucleotide), released in response to nerve stimulation, is thought to act on purinoceptors (P2X receptors) on smooth muscle cells to evoke transient depolarization, which is known as the excitatory junction potential (e.j.p.) (Stjärne, 1986).

Earlier studies concerning the effects of AII on sympathetic neurotransmission were mainly based on measurements of vasoconstricting responses or NA efflux upon electrical field stimulation (Ziogas *et al*., 1984; Saye *et al*., 1986; Trachte, 1988; Cox *et al*., 1995). The receptor subtype that mediates the enhancing effect of AII on noradrenergic transmission has been suggeted to be AT_1 (Brasch *et al*., 1993). Although the presynaptic modulation of noradrenergic and purinergic transmission appears to have much in common, several studies have suggested notable differences in the regulation of these two systems (Trachte, 1988; Ellis & Burnstock, 1989). Thus, further studies are needed to clarify the presynaptic modulation of purinergic transmission by the renin-angiotensin system. In this context, if the e.j.p. is mediated by a purine nucleotide, the recording of e.j.ps may serve as a useful probe for the investigation of purinergic neurotransmission.

The present study aims to address three questions. First, to determine whether the e.j.p. in the guinea-pig isolated mesenteric artery is mediated by ATP or a related purine nucleotide. Second, to clarify whether the effect of AII on purinergic neurotransmission, as assessed by magnitude of the e.j.ps, is mediated by AT_1 or AT_2 receptors. Third, to determine whether angiotensin I (AI) can mediate a similar effect on purinergic neurotransmission through its local conversion by angiotensin converting enzyme (ACE) to AII.

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Methods

Preparation of arteries

This study was approved by the Animal Care Committee of Kyushu University. Female Hartley guinea-pigs (body weight 250 to 350 g) were killed by exsanguination followed by decapitation. The second or third branch of the mesenteric artery was excised and bathed in cold Krebs solution of the following composition (in mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. In some preparations, the endothelium was removed by careful rubbing of the intimal surface of the lumen with a fine insect pin. The absence of the endothelium was confirmed by the lack of hyperpolarizing responses to carbachol (10 μM) and additionally by light microscopic examination.

Recording of e.j.p.

The isolated mesenteric artery branches were pinned out on a rubber base attached to the bottom of the experimental chamber (capacity 2 ml). The chamber was superfused with 36°C Krebs solution bubbled with 95% O_2-5% CO_2 (pH 7.2–7.3) at the rate of 3 ml min^{-1}. After an equilibration of at least 60 min membrane potentials of vascular smooth muscle cells were recorded by use of conventional glass capillary microelectrodes filled with 3 M KCl, with tip resistances of 50–80 MΩ as previously described (Fujii, 1988). Criteria for successful impalement included: an abrupt drop in voltage upon impalement of the microelectrode into the vascular smooth muscle cell, a stable membrane potential for at least 2 min, and a sharp return to zero potential upon withdrawal of the electrode. To record e.j.ps, the periarterial nerves were stimulated by drawing the proximal part of the artery into a suction electrode (Ag-AgCl). An electrical stimulator (SEN-3201, Nihon Koden, Tokyo, Japan) was used to supply a train of pulses (1 Hz, 11 pulses, 30–70 μs, 30–60 V) every 2.5 min. At this interval, the e.j.p. amplitudes remained constant. Signals were amplified through an amplifier (MEZ-7200, Nihon Koden), monitored on an oscilloscope (VC-11, Nihon Koden), and recorded with a pen recorder (RJG-4002, Nihon Koden). In addition, in order to compare the enhancing effects of AII on e.j.ps under different treatments, we calculated the AII-induced % increases in the ratio of the fully facilitated e.j.p. amplitude (E_{max}) to the first e.j.p. amplitude (E_1) in each cell. All experiments were conducted in the presence of indomethacin (3 μM) to inhibit the formation of prostaglandins.

Drugs

The following drugs were used: angiotensin I, angiotensin II, indomethacin, noradrenaline hydrochloride, α,β-methylene-ATP lithium salt, carbamylcholine chloride, tetrodotoxin (Sigma Chemical, St. Louis, MO, U.S.A.), suramin sodium (Wako Pure Chemical Industries, Ltd., Osaka, Japan), temocaprilat, an active metabolite of the ACE inhibitor temocapril (Oizumi *et al*., 1988) (a gift from Sankyo Co. Ltd., Tokyo, Japan), CV-11974 (2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4yl]methyl]-1*H*-benzimadazole-7-carboxylic acid), an active metabolite of TCV-116 (Shibouta *et al*., 1993) (a gift from Takeda Chemical Industry, Ltd., Osaka, Japan), CGP 42112A (N-alpha-nicotinoyl-Tyr-(N-alpha-CBZ-Arg)Lys-His-Pro-Ile-OH) (Neosystem, Strasbourg, France), and PD 123319 (1-[[4-(dimethylamino)-3-methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazol[4,5-c]-pyridine-6-carboxylic acid, ditrifluoroacetate, monohydrate) (a gift from Parke-Davis Co. Ltd., Ann Arbor, MI, U.S.A.).

Stock solutions of AI and AII were prepared in concentrations of 0.1 mM in distilled water and stored at −20°C. Indomethacin was freshly prepared in 0.01 M Na_2CO_3. Tetrodotoxin was dissolved in 0.1 M acetic acid. Temocaprilat was prepared in methanol. CV-11974 was dissolved in 0.9% saline containing 0.025 M Na_2CO_3. CGP 42112A was prepared in 9.95% dimethyl sulphoxide. PD 123319, α,β-methylene-ATP and carbachol were dissolved in distilled water. Noradrenaline was dissolved in distilled water containing an equimolar concentration of ascorbic acid. All drugs were further diluted 1000 times or more in the Krebs solution to give the final chamber concentrations. The solvents used to dissolve drugs did not affect electrical responses at their final bath concentrations. The peak effects of AII and AI on e.j.ps were obtained within 5 min after application of the agents. In the experiments in which the effects of drug treatment on responses to angiotensins were examined, the drugs were added to the superfusate at least 15 min before the application of the angiotensins. The values were accepted only when the continuous recordings of e.j.ps were successful from a single cell throughout the application of AII or AI. The values at the peak effect for the angiotensins were used for statistical analysis.

Statistics

Data are expressed as mean ± s.e.mean; n refers to the number of animals examined. A comparison of the facilitatory curves of e.j.ps was performed by two-way ANOVA for repeated measures. AII-induced % increases in the ratio of E_{max} to E_1 (E_{max}/E_1) were analysed by one-way ANOVA followed by Scheffé's test. Other variables were analysed by the paired or unpaired Student's t test. Probability values of less than 0.05 were considered statistically significant.

Results

The mean resting membrane potential of the guinea-pig isolated mesenteric artery was -69.3 ± 0.6 mV ($n=23$). The membrane potentials in the presence of 0.1 μM AII and 0.1 μM AI were -69.1 ± 1.0 mV ($n=9$) and -69.9 ± 0.8 mV ($n=8$), respectively, which did not differ significantly from the control resting membrane potential. All other drugs used in the present study did not significantly alter the resting membrane potential at the concentrations used.

Periarterial nerve stimulation evoked e.j.ps in the guinea-pig isolated mesenteric artery, which were sensitive to 0.3 μM tetrodotoxin (data not shown). Suramin (300 μM), a P2 purinoceptor antagonist, abolished the e.j.ps after 70–90 min of application (Figure 1a). At the same time, suramin did not affect the resting membrane potential (-70.8 ± 0.5 mV, $n=6$). In addition, suramin abolished the depolarizing response to α,β-methylene-ATP (1–10 μM), a stable analogue of ATP, without inhibiting NA (30 μM)-induced depolarization (Figure 1b, representative of other 3 experiments).

Figure 2 shows typical recordings of the effect of AII on e.j.ps in the absence or presence of CV-11974, an AT_1 receptor antagonist. Under control conditions, the e.j.p. amplitude showed facilitation process, i.e., increase with repetitive stimulation, reaching the maximum and steady level after about 4–6 stimuli (Figures 1a and 2). AII 1 nM did not significantly affect the amplitude of e.j.ps (Figure 3a). AII 10 nM and 0.1 μM, did not alter the amplitude of the first e.j.p. (control 5.5 ± 0.6 mV, 10 nM AII 5.4 ± 0.3 mV, $n=4$; control 4.8 ± 0.5 mV, 0.1 μM AII 4.6 ± 0.4 mV, $n=6$), but enhanced the amplitude of subsequent e.j.ps (Figures 2a and 3b,c). CV-11974 (0.1 μM), an AT_1 receptor antagonist, abolished the enhancing effect of AII (0.1 μM) on e.j.ps (Figures 2b and 4b). In the presence of PD 123319 (1 μM), a non-peptide AT_2 receptor antagonist, and CGP 42112A (1 μM), a peptide AT_2 receptor ligand, AII still significantly enhanced e.j.ps (Figure 4c,d).

CV-11974 virtually abolished the AII (0.1 μM)-induced increase in the ratio of the fully facilitated e.j.p. amplitude to the first e.j.p. amplitude (E_{max}/E_1) (Table 1). On the other hand, neither PD 123319 nor CGP 42112A affected the AII-induced increase in E_{max}/E_1 (Table 1).

AI (0.1 μM) did not alter the amplitude of the first e.j.p. (control 5.2±0.8 mV, $n=6$; AI 5.4±0.7 mV, $n=6$), but significantly enhanced the amplitudes of the subsequent e.j.ps ($P<0.05$) (Figure 5a); an effect similar to that of AII. CV-11974 (0.1 μM) abolished the enhancing effect of AI (0.1 μM) (Figure 5b). The enhancing effect of AI on e.j.ps was also abolished by temocaprilat (10 μM) (Figure 5c). AI still enhanced the e.j.ps in preparations without the endothelium (Figure 5d) and this effect was also abolished by temocaprilat (data not shown).

CV-11974 and temocaprilat abolished the increase in E_{max}/E_1 produced by AI (0.1 μM) (Table 2). The endothelium removal did not affect the increase in the E_{max}/E_1 by AI (Table 2).

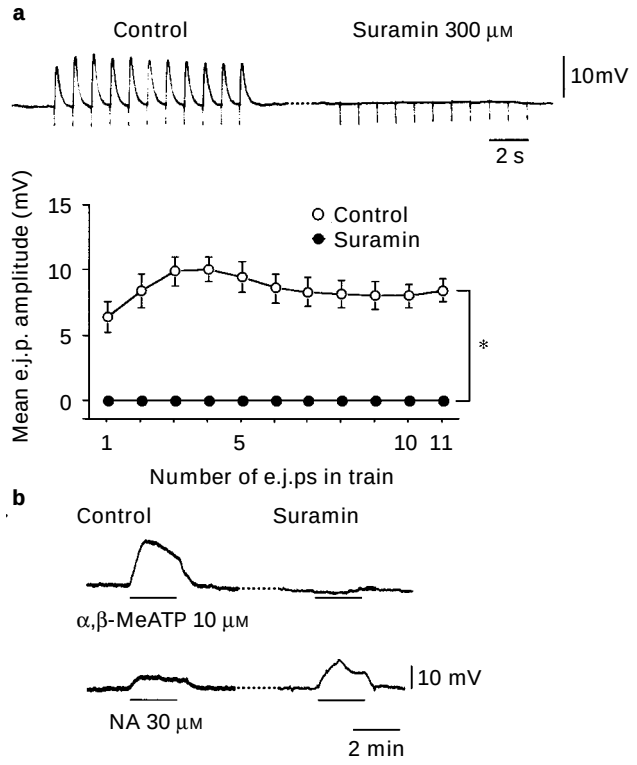

Figure 1 (a) Top: effects of suramin (300 μM) on excitatory junction potentials (e.j.ps) in guinea-pig isolated mesenteric artery. Suramin was applied to the bath for 70 to 90 min. Recordings were obtained from the same preparation. Bottom: line graphs summarizing the effect of suramin (300 μM) on the e.j.ps. Responses are shown as mean with vertical lines indicating s.e.mean of 6 observations. *Denotes a significant difference. (b) Effects of suramin (300 μM) on α,β-methylene-ATP (MeATP)-induced depolarization (top) and noradrenaline (NA)-induced depolarization (bottom) in guinea-pig isolated mesenteric artery. Suramin was applied to the bath for 70 to 90 min. Recordings were obtained from the same preparation.

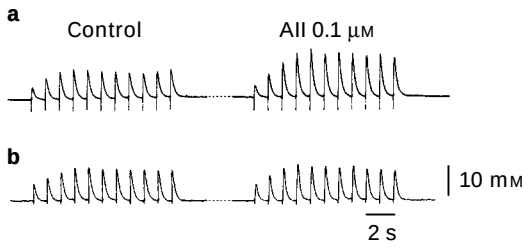

Figure 2 Excitatory junction potentials elicited by repetitive nerve stimulation in guinea-pig isolated mesenteric artery: (a) before (left) and after (right) application of 0.1 μM angiotensin II (AII); (b) before (left) and after (right) application of AII in the presence of CV-11974 (0.1 μM).

Discussion

The present findings support the proposal that the e.j.p. is mediated by ATP, or a related purine nucleotide, in the guinea-pig isolated mesenteric artery; thus the e.j.p. may be used as a marker of purinergic neurotransmission in this artery. AII-induced enhancement of e.j.ps was abolished by the AT_1 receptor antagonist, CV-11974 (Noda *et al*., 1993; Shibouta *et al*., 1993), but was not affected by the AT_2 receptor antagonist, PD 123319 (Martens *et al*., 1996), or the AT_2 receptor ligand, CGP 42112A (Martens *et al*., 1996), suggesting that AII enhances purinergic transmission via the AT_1 receptor. AI exerted a similar effect on e.j.ps most likely through a local conversion by ACE to AII, as the effect of AI was abolished by an ACE inhibitor temocaprilat. The removal of the endothelium did not alter the enhancing effect of AI on e.j.ps.

E.j.p. as a measure of purinergic neurotransmission

Perivascular nerve stimulation evokes e.j.ps in many sympathetically innervated smooth muscle cells (Ishikawa, 1985;

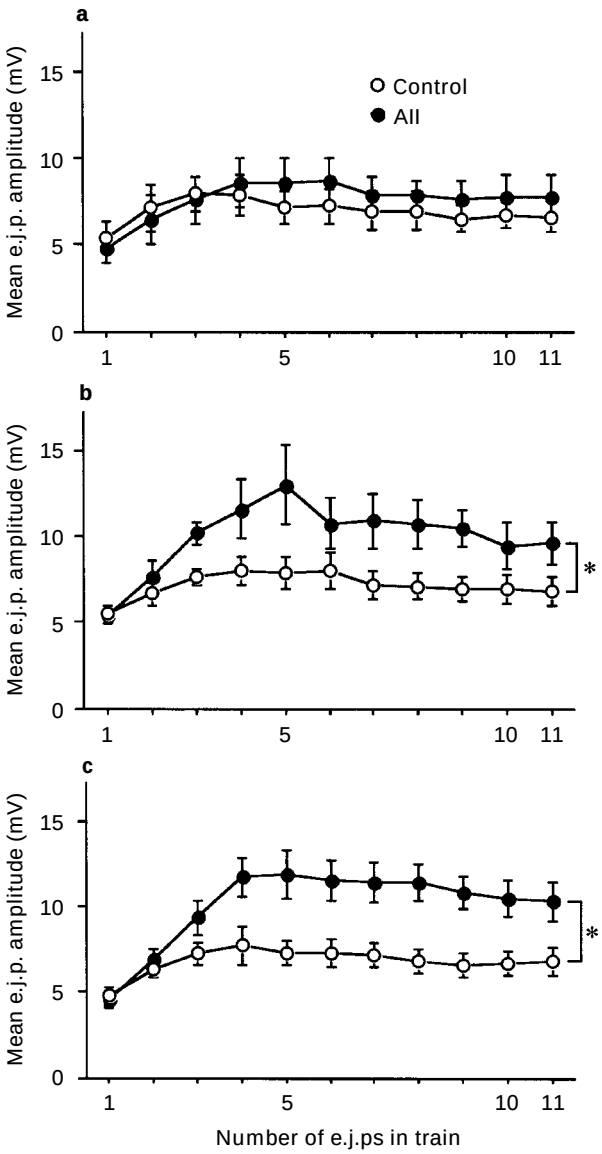

Figure 3 The effect of (a) 1 nM angiotensin II (AII), (b) 10 nM AII and (c) 0.1 μM AII on the excitatory junction potentials (e.j.ps) of the guinea-pig isolated mesenteric artery. Responses are shown as mean and vertical lines indicate s.e.mean of 4–6 observations. *Denotes a significant difference.

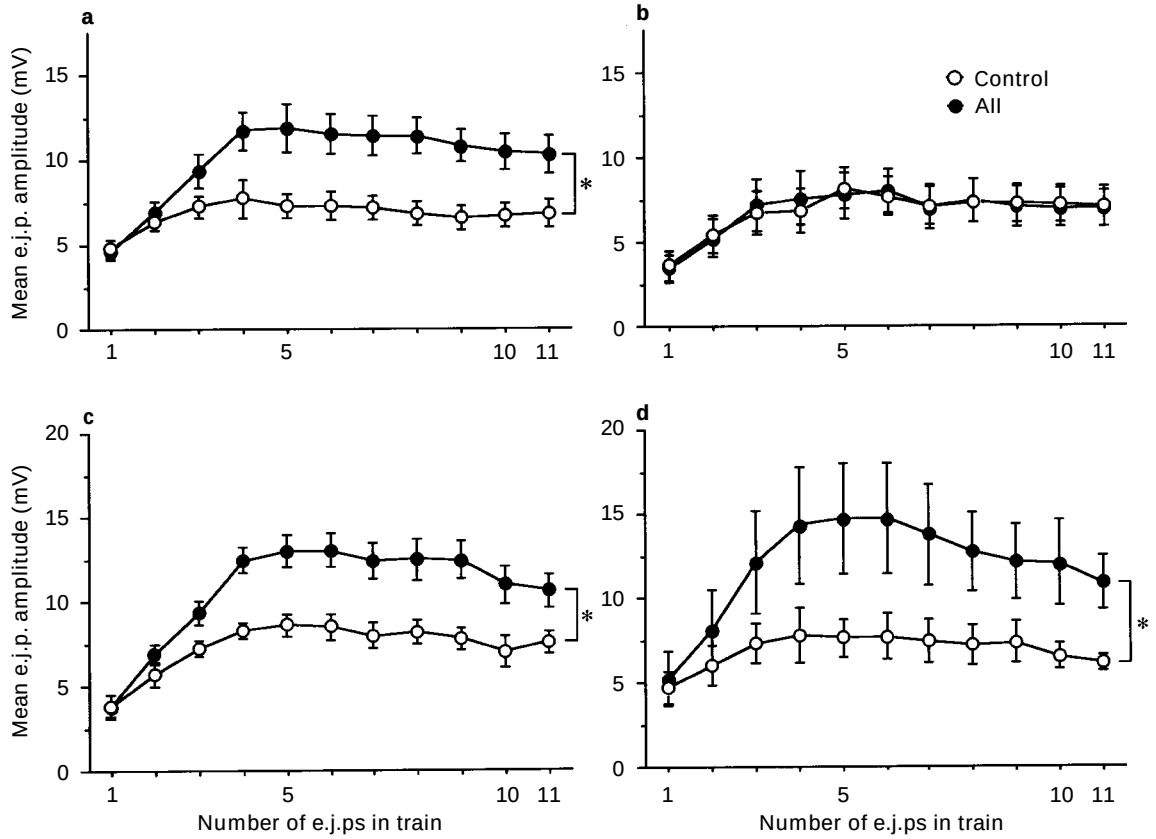

Figure 4 Effects of angiotensin II (AII, 0.1 μM) on the excitatory junction potentials (e.j.ps) in the absence (a) and presence of CV-11974 0.1 μM (b), PD 123319 1 μM (c) and CGP 42112A 1 μM (d). Responses are shown as mean and vertical lines indicate s.e.mean of (a) 6, (b,c) 5 or (c) 4 observations. *Denotes a significant difference.

Table 1 The effects of CV-11974, PD 123319 and CGP 42112A on angiotensin II (AII)-induced enhancement in E_{max}/E_1

No treatment	*CV-11974* (0.1 μM)	*PD 123319* (1 μM)	*CGP 42112A* (1 μM)
54.2±5.6	5.4±3.7*	48.2±4.4	76.2±13.1

E_{max}/E_1 represents the ratio of the fully facilitated e.j.p. amplitude (E_{max}) to the first e.j.p. amplitude (E_1). Values shown are mean increase above control response (%)±s.e.mean; $n=4-6$. *$P<0.05$ vs no treatment.

Ziogas & Cunnane, 1991; Sneddon, 1992; McLaren *et al*., 1995). Previous investigators demonstrated that e.j.ps are resistant to α-adrenoceptor antagonists (Starke *et al*., 1989; McLaren *et al*., 1995), but are inhibited by suramin (Sneddon, 1992; McLaren *et al*., 1995), a selective P2-purinoceptor antagonist (Dunn & Blakeley, 1988), or by α,β-methylene-ATP, a stable analogue of ATP, which desensitizes P2X-purinoceptors (Ishikawa, 1985; Fujii, 1988). Based on these findings, it has been proposed that ATP, but not NA, mediates e.j.ps by acting on P2-purinoceptors on smooth muscle cells (Sneddon & Burnstock, 1984; Ishikawa, 1985; Suzuki, 1985; Fujii, 1988; Sneddon, 1992; McLaren *et al*., 1995). However, in the previous study on the guinea-pig mesenteric artery (Ishikawa, 1985), the author was unable to conclude that e.j.ps are mediated by ATP, because α,β-methylene-ATP produced a persistent depolarization, which might itself partly explain its inhibitory action on e.j.ps. In the present study, 300 μM suramin abolished e.j.ps without affecting the resting membrane potential. In addition, suramin abolished the depolarizing response to α,β-methylene-ATP, but did not inhibit the noradrenaline-induced depolarization. These findings strongly support the contention that the e.j.p. in the guinea-pig isolated mesenteric artery is mediated by ATP, or a related purine nucleotide, acting on P2-purinoceptors. Hence, the recording of e.j.ps may serve as a probe for the investigation of purinergic neurotransmission in this artery. The degree of contribution of purinergic mechanisms to the contractions to sympathetic nerve stimulation may vary depending on the vascular bed studied, species or stimulation conditions (Sjöblom-Widfeldt, 1990); in general, purinergic mechanisms appear to contribute to the fast, twitch-like contractions, whereas noradrenergic mechanisms may account for the second phase of contractions (Sneddon & Burnstock, 1984; Burnstock & Warland, 1987).

Effects of AII on e.j.ps

AII enhanced the facilitation process of e.j.ps without altering the amplitude of the first e.j.p. and the resting membrane potential in the guinea-pig isolated mesenteric artery. Our findings are consistent with those obtained in the guinea-pig vas deferens and uterine artery (Bell, 1972; Ziogas & Cunnane, 1991). In both preparations, AII enhanced e.j.ps without altering the amplitude of the first e.j.p. and the spontaneously occurring e.j.ps, indicating that AII primarily acts presynaptically to enchance e.j.ps, but not by altering the electrical properties of smooth muscle cells. Ziogas & Cunnane (1991) suggested that the AII-induced increase in transmitter release is due to an increase in the probability of transmitter release from individual varicosities in the guinea-pig vas deferens. On the other hand, with respect to noradrenergic transmission, AII might exert additional effects postsynaptically, as it has been shown that AII uncovered neurogenic contractions mediated by postjunctional α_2-adrenoceptors in the presence of blockade of α_1-adrenoceptors in the rabbit saphenous artery (Dunn *et al*., 1991).

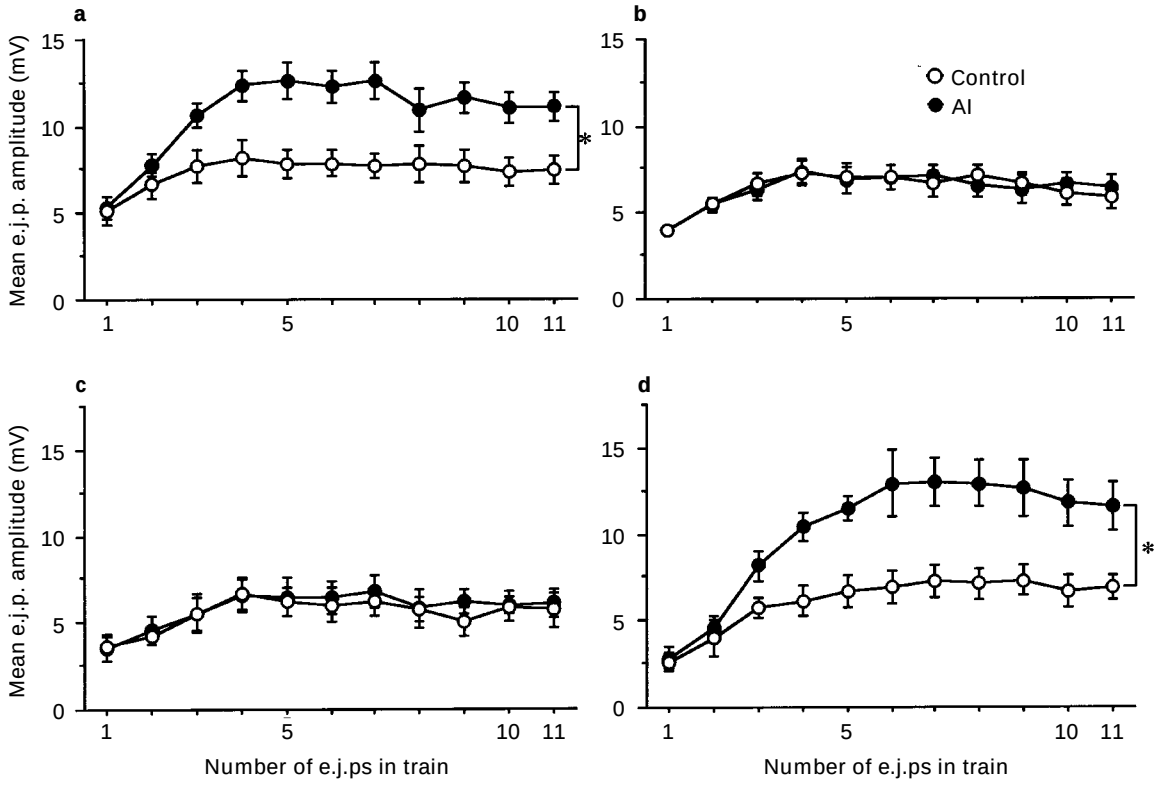

Figure 5 Effects of angiotensin I (AI, 0.1 μM) on excitatory junction potentials (e.j.ps) in the absence (a) or presence of CV-11974 0.1 μM (b), temocaprilat 10 μM (c) and in preparations without the endothelium (d). Responses are shown as mean and vertical lines indicate s.e.mean of (a,b) 6, (d) 5 or (c) 4 observations. *Denotes a significant difference.

Table 2 The effect of CV-11974, temocaprilat and removal of endothelium on angiotensin I (AI)-induced enhancement in E_{max}/E_1

No treatment	*CV-11974*	*Temocaprilat*	*Removal of endothelium*
46.7±10.8	−3.7±2.8*	6.3±4.9*	73.5±8.9

E_{max}/E_1 represents the ratio of the fully facilitated e.j.p. amplitude (E_{max}) to the first e.j.p. amplitude (E_1). Values shown are mean increase above control response (%)±s.e.mean; $n=4-6$. *$P<0.05$ vs responses in endothelium-intact preparations without treatments.

Although the potentiating effect of AII on noradrenergic neurotransmission has been consistently shown (Ziogas *et al*., 1984; Brasch *et al*., 1993; Cox *et al*., 1995), previous studies concerning the effects of AII on purinergic neurotransmission, using either twitch contractions or ATP overflow to nerve stimulation, have given variable results. In the guinea-pig vas deferens, AII enhanced purinergic transmission elicited by low frequencies of stimulation, but not that by higher frequencies of stimulation (Ellis & Burnstock, 1989). In the rabbit vas deferens, the purinergic component of neurogenic contraction was inhibited by application of AII (Trachte, 1988), while in the other study it was enhanced under the administration of indomethacin (Saye *et al*., 1986).

It should be noted that the present experiments were conducted in the presence of indomethacin. In preliminary experiments, we found that during prolonged application of AII, the amplitude of e.j.ps decreased after an initial increase in amplitude. This inhibitory action of AII was prevented by indomethacin (data not shown). Similar observations were also noted in the guinea-pig vas deferens (Cunnane *et al*., 1990; Ziogas & Cunnane, 1991). AII is known to stimulate prostaglandin release (Saye *et al*., 1986). Kuriyama & Makita (1982) showed that indomethacin enhanced e.j.ps in the rabbit and guinea-pig mesenteric artery. Furthermore, Brock & Cunnane (1996) recently showed that prostaglandin E_1 (PGE_1) and PGE_2 reduced the amplitude of e.j.ps by inhibiting quantal ATP release in the guinea-pig vas deferens. These findings suggest that endogenous and exogenous prostaglandins may act presynaptically to inhibit e.j.ps. Another factor that should be considered is that a part of the ATP released might derive from non-neural tissues, presumably smooth muscle cells, especially in the case of high-frequency, long-trains of nerve stimulation (von Kügelgen & Starke, 1991b). It is thus conceivable that the above mentioned discrepancies regarding the effects of AII on purinergic transmission may be, in part, attributable to differences in experimental conditions, e.g., frequency and number of stimuli and presence or absence of cyclo-oxygenase inhibitors. Our experimental conditions, short trains of stimulation with a low frequency in the presence of indomethacin, allowed us to investigate the presynaptic action of AII on purinergic transmission with a lesser influence of other confounding factors.

AII receptor subtype involved in AII effect on purinergic transmission

In a previous study, the AII-induced enhancement of e.j.ps in the guinea-pig vas deferens was prevented by the nonselective AII receptor antagonist saralasin (Ziogas & Cunnane, 1991). However, the AII receptor subtype involved was not elucidated in that study. The present study showed that the enhancing effect of AII on e.j.ps was abolished by CV-11974, a selective non-peptide antagonist of the AT_1 receptor (Noda *et al*., 1993; Shibouta *et al*., 1993), but was not affected by either PD 123319, a selective non-peptide antagonist of the AT_2 receptor, or by CGP 42112A, a peptide AT_2 receptor ligand. In the rabbit aorta, CV-11974 inhibited the specific binding of [^{126}I]-[Sar^1-Ile^8] AII to the AT_1 receptor with an inhibition constant (K_1) of 0.56 nM, while in the bovine cerebellum, CV-11974 (up to 10 μM), did not bind to the AT_2 receptor (Noda *et al*., 1993). Thus, at the concentration used in this study, CV-

11974 may act selectively on the AT_1 receptor. On the other hand, in the rat cultured hypothalamus and brain stem neurones, PD123319 (1 μM) blocked the AT_2 receptor-mediated effect of AII on whole cell K^+ current, while CGP 42112A (100 nM) mimicked the effect of AII (Martens *et al*., 1996); hence the lack of effects of PD123319 (1 μM) and CGP 42112A (1 μM) on the AII action on e.j.ps in this study excludes the involvement of AT_2 receptors. Thus, the present findings, together with previous results indicate that AII enhances purinergic neurotransmission via activation of presynaptic AT_1 receptors in the guinea-pig isolated mesenteric artery.

Several studies have investigated the presynaptic AII receptor subtype involved in the potentiating effect of AII on noradrenergic neurotransmission (Tofovic *et al*., 1991; Brasch *et al*., 1993). In the isolated left atrium of the guinea-pig, the facilitatory effect of AII on the stimulation-induced NA efflux was antagonized by losartan, a selective non-peptide antagonist of the AT_1 receptor, but was unaffected by PD 123319 (Brasch *et al*., 1993). In Wistar Kyoto rats, AII potentiated increases in mesenteric perfusion pressure elicited by perivascular nerve stimulation *in vivo* (Tofovic *et al*., 1991) and this effect of AII was abolished by Dup 753 (losartan), but not by PD 123177, a selective non-peptide antagonist of the AT_2 receptor (de Gasparo *et al*., 1995). Thus, these studies suggested that the effect of AII on noradrenergic transmission is mediated by presynaptic AT_1 receptors.

Studies specifically designed to determine the presynaptic AII receptor subtype that modulates purinergic transmission are limited and the results are inconsistent. Trachte (1988) and Trachte *et al*. (1990) demonstrated that AII depressed the non-adrenergic components of neurogenic contraction in the rabbit vas deferens and this effect was inhibited by Dup 753. Concomitantly, the AII-induced increases in PGE release also inhibited by Dup 753 (Trachte *et al*., 1990). The discrepancy between our results and those obtained previously may arise from differences in experimental conditions, as mentioned previously. Recently, two types of the AT_1 receptor have been cloned, termed AT_{1A} and AT_{1B} (de Gasparo *et al*., 1995). In a recent study, Cox *et al*. (1995) suggested that the presynaptic action of AII on purinergic and noradrenergic transmission is mediated by the AT_{1B} receptor in the rat vas deferens and tail artery, since AII-induced enhancement was inhibited by a high concentration of PD 123319, a dose they supposed to have an inhibitory action on AT_{1B}. In the present study, PD 123319, even at its highest concentration used, did not affect the action of AII on e.j.ps. The reason for these discrepant findings is unknown, but species and/or regional differences might partly account for these differences.

Local renin-angiotensin system and purinergic transmission

It is now well recognized that some of the components of the renin-angiotensins system are present in tissues, such as the vasculature and cardiac cells (Dzau, 1988; Arnal *et al*., 1994). In the rat isolated mesenteric artery, it has been shown that both AII and AI potentiated vasoconstrictor responses to nerve stimulation. The effect of AI was abolished by ACE inhibitors and [Sar^1-Ile^8] AII (Malik & Nasjletti, 1976). Similarly, AI as well as AII enhanced the stimulation-induced NA efflux in guinea-pig isolated atria, and this effect of AI was abolished by captopril and saralasin (Ziogas *et al*., 1984). These results indicate that AII locally generated from AI could enhance sympathetic transmission. In the present study, AI also enhanced the amplitude of the e.j.ps. This effect of AI was abolished by both ACE inhibitors and the AT_1 antagonist, CV-11974. The present study is the first to demonstrate that AI can enhance purinergic transmission, as assessed by e.j.ps, through its local conversion to AII.

AI is primarily converted to AII by ACE. However, recent studies suggest that there is a chymostatin-sensitive AII generation, presumably by chymase, in some mammalian tissues (Urata & Ganten, 1993). In the present study, the enhancing effect of AI on e.j.ps was abolished by ACE inhibitors. It is, therefore, unlikely that enzymes other than ACE are involved in AII generation from AI in the guinea-pig isolated mesenteric artery.

The enhancing effect of AI on e.j.ps was still observed in preparations without the endothelium. This is consistent with the observations of Story & Ziogas (1986); they showed that the removal of the endothelium, by gas perfusion, did not alter the facilitatory effect of AI on noradrenergic transmission in the rat tail artery. ACE activities have been detected not only in the endothelium, but also in the media and adventitia (Arnal *et al*., 1994). Our observations thus suggest that the intact endothelium may not be essential for the local generation of AII that gains access to purinergic nerve terminals. However, as we did not construct complete dose-response curves for the effects of AI, and as the AI was added to the superfusate, i.e., extraluminal route, our findings do not exclude the involvement of the endothelium in this conversion.

In conclusion, AII enhances purinergic neurotransmission, as assessed by e.j.ps, via presynaptic AT_1 receptors in the guinea-pig isolated mesenteric artery. Locally generated AII from AI by ACE also exerts similar effects on purinergic neurotransmission. In addition, an intact endothelium may not be essential for the local generation of AII.

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