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European Journal of Pharmacology 295 (1996) 61–68

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Purinergic and adrenergic transmission and their presynaptic modulation in canine isolated perfused splenic arteries

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Received 3 April 1995; revised 3 October 1995; accepted 6 October 1995

Abstract

Vasoconstrictions induced by periarterial electrical stimulation were analysed pharmacologically in the canine isolated perfused splenic artery. Phentolamine enhanced the vasoconstrictions at 1 Hz but inhibited those at 10 Hz. Suramin and P_{2x} purinoceptor desensitization with α,β-methylene ATP abolished the phentolamine-enhanced and -resistant vasoconstrictions. α,β-Methylene ATP inhibited the vasoconstrictions at 1 Hz and by exogenous ATP but did not change those at 10 Hz and by exogenous noradrenaline. Suramin reduced the vasoconstrictions by the electrical stimulations and α,β-methylene ATP but did not affect those by exogenous ATP. Prazosin did not affect the vasoconstrictions at 1 Hz but inhibited those at 10 Hz. Rauwolscine enhanced the prazosin-resistant vasoconstrictions. These results suggest that the electrical stimulation at 1 Hz releases purinergic transmitters (ATP or a closely related compound) as a dominant candidate for the vasoconstrictions, and a co-released noradrenaline may inhibit the release of purinergic transmitters through presynaptic α_2-adrenoceptors in the canine splenic artery.

Keywords: Purinergic transmission; P_{2X} purinoceptor; α_2-Adrenoceptor; Autoinhibition; Electrical stimulation; Splenic artery; (Dog)

1. Introduction

Adenosine 5′-triphosphate (ATP) has been proposed as a co-transmitter with noradrenaline in sympathetic nerves of the vas deferens and of many blood vessels (for review; von Kügelgen and Starke, 1991). Biochemical evidence suggests that ATP is stored with noradrenaline in the sympathetic nerves and is co-released with noradrenaline (Helle et al., 1971; Kirkpatrick and Burnstock, 1987; Kasakov et al., 1988; Driessen et al., 1993; Von Kügelgen et al., 1994a). Although the contributions of ATP and noradrenaline to the vasoconstrictor responses to sympathetic nerve stimulation are clearly distinguishable in the vas deferens (McGrath, 1978; Westfall et al., 1978; Sneddon and Westfall, 1984; Burnstock, 1990), the purinergic component of vasoconstriction has not been fully clarified in blood vessels.

The release of noradrenaline from sympathetic nerves and its presynaptic modulation have been widely investigated (Langer, 1980; Starke, 1987; Nicholas et al., 1988), but information on the neural release of ATP and its presynaptic modulation is relatively scanty. It has been shown that the adrenergic and purinergic components of the vasoconstriction evoked by electrical stimulation are influenced by α_2-adrenoceptor-mediated autofeedback in the rabbit ileocolic artery (Von Kügelgen and Starke, 1985; Bulloch and Starke, 1990; MacDonald et al., 1992), the rabbit saphenous artery (MacDonald et al., 1992) and the canine mesenteric artery (Muramatsu et al., 1989). Bulloch and Starke (1990) observed the effects of presynaptic α_2-autoinhibition on the vasoconstrictor responses to noradrenaline and ATP released in the rabbit ileocolic artery, and they suggested that presynaptic α_2-autoinhibition might decrease mainly noradrenaline release. This suggestion was further supported by a study in

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Elsevier Science B.V.
SSDI 0014-2999(95)00654-0

which the overflow of noradrenaline and ATP elicited by electrical stimulation at 7 Hz in the guinea-pig vas deferens was measured (Driessen et al., 1993). These findings suggest that the release mechanism of purinergic transmitters is different from that of adrenergic transmitters in some respects.

The view that ATP is a co-transmitter with noradrenaline in the vasoconstrictor response to perivascular sympathetic nerve stimulation is based mainly on observations for various arteries from lagomorpha and rodents: rabbit, rat and guinea-pig (Burnstock, 1990; Evans and Cunnane, 1992). Even in the same tissue of the same species (the rat tail artery), considerable variation in the proportion of ATP- and noradrenaline-induced vasoconstrictions has been reported (Holman and Surprenant, 1980; Vidal et al., 1986; Bao and Stjärne, 1993). As for mammals other than lagomorpha and rodents, a vasoconstriction consisting of adrenergic and purinergic components has been identified in the canine mesenteric artery (Muramatsu, 1987; Muramatsu et al., 1989). De Mey and Vanhoutte (1982) reported that phentolamine abolished the vasoconstrictions evoked by electrical stimulation at 8 Hz in the canine splenic, pulmonary, saphenous and femoral arteries. In contrast, our preliminary experiments showed that phentolamine potentiated or partially inhibited the vasoconstriction induced by electrical stimulation at 1 Hz or 10 Hz in the canine splenic artery, respectively. Recently, we reported that only postsynaptic α_1-adrenoceptors were responsible for the exogenous noradrenaline-evoked vasoconstriction, and that the vasoconstrictor response to 10 Hz stimulation was abolished by ω-conotoxin GVIA and tetrodotoxin in the splenic artery (Ren et al., 1994a,b). The property of electrically evoked and phentolamine-resistant vasoconstrictions is not clear. Therefore, the aim of the present study was to investigate whether the vasoconstrictions produced by electrical stimulation have a purinergic component and whether this component can be modulated presynaptically in the isolated perfused splenic artery of the dog.

2. Materials and methods

2.1. Arterial preparations

Mongrel dogs (8–19 kg) of either sex were overdosed with sodium pentobarbital (65 mg/kg, i.v.) and sodium heparin (200 units/kg, i.v.). The spleen with the splenic artery was isolated and immersed in cold Krebs-Henseleit solution (4°C). The arterial branches of the splenic artery running into the spleen were used in the present experiments. The arteries were carefully isolated and cleaned of loose adipose and connective tissues. All side branches of the artery were tied with silk threads. The arteries (0.8–1.8 mm in outer diameter) were cut into segments 15–20 mm in length, and each segment was cannulated and set up for perfusion as described previously (Chiba and Tsukada, 1985; Ren et al., 1994a). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal portion of the needle with silk threads. The cannula was 3–4 cm long and 0.6–1.6 mm in outer diameter and had small side holes 5 mm from the distal sealed end. The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a peristaltic pump (Tokyo Rikakikai) with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 10 mM glucose. The flow rate was kept at 1 ml/min. Perfusion pressure was measured with an electric manometer (Nihon Kohden, MPU-0.5A) and recorded by a rectigraph (Nihon Kohden, WT-685G). After an initial increase in perfusion pressure at the beginning of perfusion, the perfusion pressure gradually decreased and reached a stable baseline (20–50 mmHg); then the preparation was removed from the bath solution and fixed in a vertical position. The preparation was superfused during the experiment. A pair of stimulating electrodes was gently placed on the extraluminal side of the artery wall to stimulate the periarterial nerve fibres. The organ bath was sealed with plastic film to maintain the preparation at 37°C by a thermostat pump (Ren et al., 1994a). A stabilization period of 60 min was needed before the experiments were started.

The effects of electrical stimulation (10 V amplitude and 1 ms pulse duration) were analysed at 1 Hz (1, 3, 10 and 30 pulses) and at 10 Hz (1, 3, 10 and 30 pulses). Intervals between electrical stimulation periods or between the doses of α,β-methylene ATP and ATP were at least 15 min to get reproducible responses. The next dose of noradrenaline was given when the response to the preceding dose had disappeared completely. Intervals between pulse number-response curves or between dose-response curves were 60 min. The preparations were pretreated with one or more of the above antagonists, atropine, propranolol, indomethacin, ω-conotoxin GVIA and guanethidine 60 min before (but tetrodotoxin 10 min before) the second or the third response curves were made for electrical stimulations and agonists. Antagonists and inhibitors were dissolved in perfusate. Each agonist was administered by means of a microsyringe into the rubber tube connecting the cannula in 0.01–0.03 ml for 4 s throughout the experiment. Except for the initial period after α,β-methylene ATP treatment, the other treatments with antagonists and/or inhibitors did not significantly affect the baseline perfusion pressure in the present experiments. The

preparations were treated with α,β-methylene ATP (1 μM) 60 min before the second or the third response curve was made. The perfusion with α,β-methylene ATP (1 μM) initially induced a great increase in perfusion pressure. The increased perfusion pressure gradually decreased and reached the previous baseline in 60 min.

2.2. Drugs

Drugs used were: *dl*-noradrenaline hydrochloride (Sankyo, Japan); disodium adenosine 5′-triphosphate (ATP, Sigma, USA); adenosine hemisulfate salt (Sigma); α,β-methylene adenosine 5′-triphosphate lithium salt (Sigma); atropine sulfate (Wako Pure Chemical Ind., Japan); ω-conotoxin GVIA (Sigma); guanethidine sulfate (Sigma); indomethacin crystalline (Sigma); phentolamine mesylate (Research Biochemicals, USA); *dl*-propranolol hydrochloride (Sumitomo Chemical, Japan); prazosin hydrochloride (Sigma); rauwolscine hydrochloride (Extrasynthese, France); tetrodotoxin (Sigma). Suramin sodium salt was kindly supplied by Dr. K. Saida (Bayer, Kobe, Japan). Stock solution of ω-conotoxin GVIA was made up in 0.5% (w/v) bovine serum albumin in distilled water; that of indomethacin in ethanol and prazosin in distilled water. Other drugs were dissolved in physiological saline before the start of the experiment. The stock solutions were kept at −80°C until used. In the indomethacin experiments, an identical concentration of ethanol (0.01%, v/v) was added to the control solution and did not significantly affect the vascular responses to electrical stimulation.

2.3. Statistical analysis

Vascular responses to drugs and electrical stimulation were expressed as the maximal changes in perfusion pressure (mm Hg) from their control levels. Values presented here are the means ± S.E.M. An analysis of variance was used to evaluate the data. If the F statistic was significant, we compared the individual data with the respective control data by simultaneous multiple comparisons, Dunnett's method (Wallenstein et al., 1980). P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of tetrodotoxin, guanethidine and ω-conotoxin GVIA on the vasoconstrictor responses to electrical stimulation

Electrical stimulation (1 and 10 Hz) constricted the isolated perfused canine splenic artery in a pulse number-dependent manner (Fig. 1). The second and third response curves for electrical stimulation were not significantly different from the first one (data not shown). Tetrodotoxin (0.1 μM) or ω-conotoxin GVIA (0.1 μM) abolished the vasoconstrictor responses to 1 and 10 Hz stimulation, as reported previously (Ren et al., 1994a, b). An adrenergic neuron blocker, guanethidine (100 μM), abolished the responses to stimulation at 1 Hz, but it did not abolish those to 10 Hz. Guanethidine-resistant responses were abolished by additional treatment with phentolamine (10 μM; data

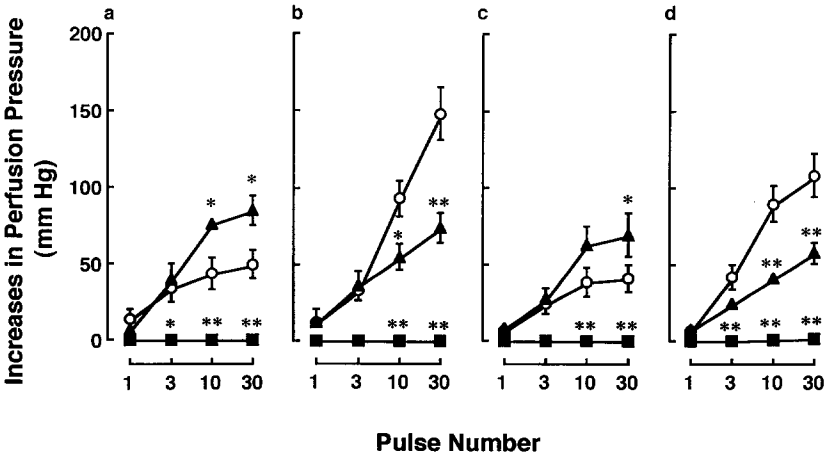

Fig. 1. Effects of phentolamine and the combined treatment with phentolamine and α,β-methylene ATP or suramin on the vasoconstriction induced by electrical stimulation. (a) 1 Hz stimulation; ○, control; ▲, phentolamine 10 μM; ■, phentolamine 10 μM and α,β-methylene ATP 1 μM. (b) 10 Hz stimulation; ○, control; ▲, phentolamine 10 μM; ■, phentolamine 10 μM and α,β-methylene ATP 1 μM. (c) 1 Hz stimulation; ○, control; ▲, phentolamine 10 μM; ■, phentolamine 10 μM and suramin 100 μM. (d) 10 Hz stimulation; ○, control; ▲, phentolamine 10 μM; ■, phentolamine 10 μM and suramin 100 μM. Points represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: $^{*}P < 0.05$; $^{**}P < 0.01$, $n = 4$–5.

not shown). The responses to noradrenaline (0.01–0.3 nmol) and to ATP (0.1–100 nmol) were not affected by the same treatment with tetrodotoxin or guanethidine (data not shown). ω-Conotoxin GVIA, an N-type voltage-operated calcium channel inhibitor, did not significantly affect the responses to noradrenaline (data not shown).

3.2. Effects of phentolamine, propranolol, atropine and indomethacin on the vasoconstrictor responses to electrical stimulation

Phentolamine (10 μM) inhibited the vasoconstrictor responses to 10 Hz stimulation (10 and 30 pulses) by only 46.7% and 48.4%, respectively (Fig. 1b). Phentolamine significantly potentiated the responses to 1 Hz stimulation (10 and 30 pulses) by 65.0% and 70.2% (Fig. 1a). The phentolamine-potentiated or -resistant vasoconstrictor responses to 1 or 10 Hz stimulation were not significantly affected by propranolol (1 μM), atropine (1 μM) and indomethacin (1 μM; data not shown). Phentolamine (10 μM) blocked the vasoconstrictions produced by noradrenaline (0.01–0.3 nmol). The ATP (0.1–100 nmol)-induced vasoconstriction was not significantly changed by the four antagonists (data not shown).

3.3. Effects of P_{2x} purinoceptor desensitization and suramin on the vasoconstrictor responses to electrical stimulation, noradrenaline, α,β-methylene ATP and ATP

Phentolamine (10 μM) and α,β-methylene ATP (1 μM) together or with phentolamine (10 μM) and suramin (100 μM) together abolished the vasoconstrictor responses to 1 or 10 Hz stimulation (Fig. 1). P_{2x} purinoceptor desensitization with α,β-methylene ATP inhibited the vasoconstrictor responses to 1 Hz stimulation by 51.4–65% (Fig. 2a), but it did not inhibit those to 10 Hz stimulation (Fig. 2b). Suramin inhibited the vasoconstrictions elicited by 1 Hz stimulation (10 and 30 pulses) by 84.6% and 77.9% (Fig. 2c) and those elicited by 10 Hz by 41.1% and 37.8%, respectively (Fig. 2d). The suramin- or α,β-methylene ATP-resistant vasoconstrictor responses to 1 or 10 Hz electrical stimulation were inhibited by phentolamine (Fig. 2).

A bolus injection of α,β-methylene ATP or ATP induced a dose-dependent vasoconstriction, but adenosine did not cause any responses (Fig. 3a). The order of potency was α,β-methylene ATP > ATP. Tachyphylaxis to α,β-methylene ATP was not observed even if α,β-methylene ATP was administered to the same preparations after 60 min. Suramin (100 μM) significantly shifted the dose-response curve for α,β-methylene ATP to the right in a parallel manner (Fig. 3b), but it did not significantly affect the vascular responses to ATP (Fig. 3c) and noradrenaline (data not shown). P_{2x} purinoceptor desensitization with α,β-methylene ATP (1 μM) completely inhibited the vasoconstriction elicited by exogenous ATP, but it did not significantly affect the vascular responses to exogenous noradrenaline (Fig. 4).

3.4. Vasoconstrictor responses to electrical stimulation at different frequencies before and after treatment with α-adrenoceptor antagonists

Perfusion with prazosin (0.1 μM) did not affect the vasoconstrictor responses to 1 Hz stimulation, but it

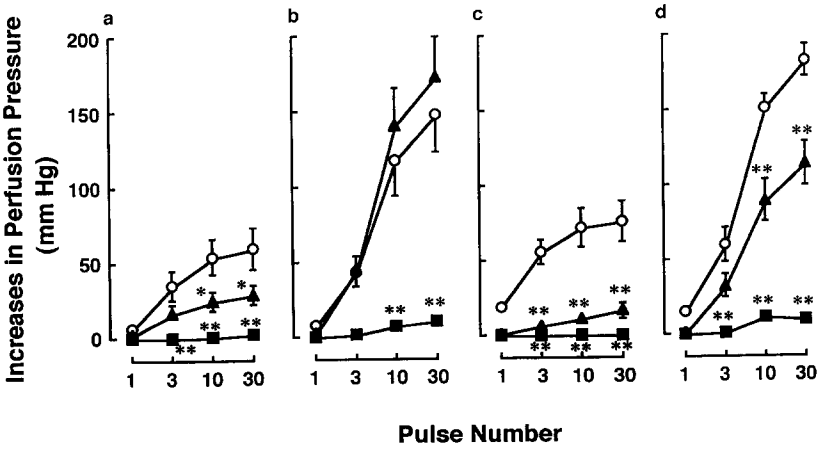

Fig. 2. Effects of α,β-methylene ATP or suramin and the combined treatment with phentolamine on the vasoconstriction induced by electrical stimulation. (a) 1 Hz stimulation; ○, control; ▲, α,β-methylene ATP 1 μM; ■, phentolamine 10 μM and α,β-methylene ATP 1 μM. (b) 10 Hz stimulation; ○, control; ▲, α,β-methylene ATP 1 μM; ■, phentolamine 10 μM and α,β-methylene ATP 1 μM. (c) 1 Hz stimulation; ○, control; ▲, suramin 100 μM; ■, phentolamine 10 μM and suramin 100 μM. (d) 10 Hz stimulation; ○, control; ▲, suramin 100 μM; ■, phentolamine 10 μM and suramin 100 μM. Points represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: $^{*}P < 0.05$; $^{**}P < 0.01$, $n = 5$–6.

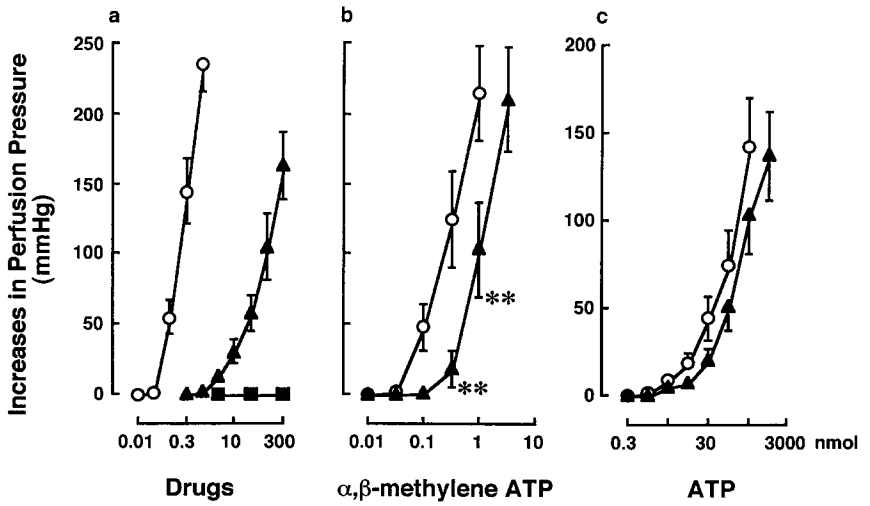

Fig. 3. (a) Dose-response curves for α,β-methylene ATP (○), ATP (▲) and adenosine (■) in canine splenic arteries. (b) (c) Effects of suramin on the vascular responses to α,β-methylene ATP and ATP in the canine splenic arteries. ○, Control; ▲, suramin 100 μM. Points represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: $^{**}P < 0.01$, $n = 8$–16.

reduced those to 10 Hz stimulation (10 and 30 pulses) by 72.1% and 69.6%, respectively (Fig. 5). Prazosin-resistant responses to 1 or 10 Hz stimulation (10 and 30 pulses) were significantly potentiated in the presence of rauwolscine (1 μM, Fig. 5).

4. Discussion

The results of this study suggest that ATP or a closely related compound is a co-transmitter with noradrenaline in the sympathetic nerves of the canine splenic artery. Although we did not measure the neural release of noradrenaline and ATP directly, the pharmacological analysis of the vasoconstrictor responses to electrical stimulation, ATP and noradrenaline suggests that electrical stimulation at 1 Hz of the splenic sympathetic nerves releases purinergic transmitters (ATP or a closely related compound) and that co-released noradrenaline may inhibit the release of purinergic transmitters through presynaptic α_2-adrenoceptors.

Tetrodotoxin (0.1 μM) or ω-conotoxin GVIA (0.1 μM) abolished the vasoconstrictions evoked by electrical stimulation at 1 or 10 Hz, but neither agent affected the vasoconstrictions induced by exogenously administered noradrenaline and ATP in the canine

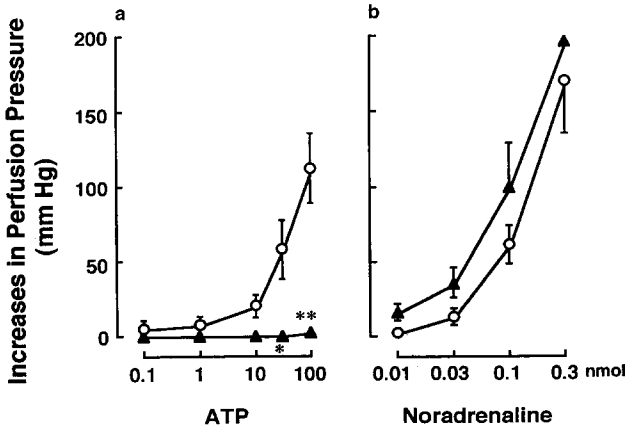

Fig. 4. Effects of desensitization with α,β-methylene ATP on the vascular responses to ATP and noradrenaline in canine splenic arteries. ○, Control; ▲, α,β-methylene ATP 1 μM. Points represent the mean values with S.E.M. Asterisks represent statistical significance vs. the respective control value: $^{*}P < 0.05$; $^{**}P < 0.01$, $n = 5$–7.

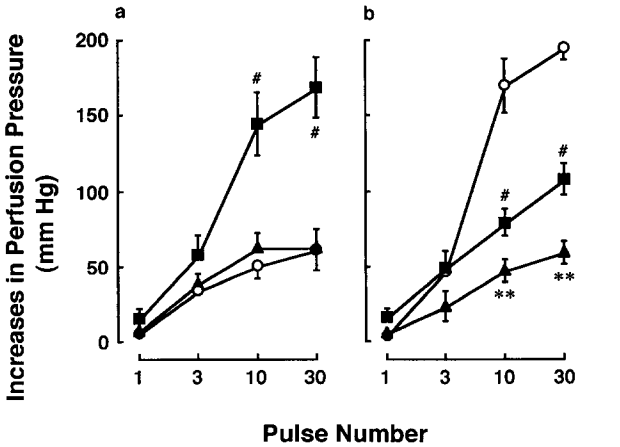

Fig. 5. Effects of prazosin and the combined treatment with rauwolscine on the vascular responses to electrical stimulation in canine splenic arteries. (a) 1 Hz stimulation. (b) 10 Hz stimulation. ○, Control; ▲, prazosin 0.1 μM; ■, rauwolscine 1 μM. Points represent the mean values with S.E.M. $^{**}P < 0.01$ as compared with the control group, and $^{\#}P < 0.05$ as compared with the prazosin group, $n = 5$.

splenic artery. Hence, the vasoconstrictions evoked by electrical stimulation can be attributed to neurotransmitter release from nerve fibres. Guanethidine (100 μM), a sympathetic neuron blocking agent (Burnstock and Warland, 1987; Muramatsu, 1987), inhibited the vasoconstrictor responses to electrical stimulation. Therefore, it is reasonable to reach the conclusion that the vasoconstriction evoked by electrical stimulation is due to the activation of periarterial sympathetic nerves.

The vasoconstrictions induced by 1 Hz stimulation were significantly inhibited by the treatment with α,β-methylene ATP (a P_{2x}-purinoceptor desensitizing agent, Sneddon and Burnstock, 1985) and abolished by the combined treatment with phentolamine and α,β-methylene ATP. Desensitization with α,β-methylene ATP blocked the responses to exogenous ATP, but it did not change those to exogenous noradrenaline. Moreover, in the postsynaptic sites, bolus injections of α,β-methylene ATP (as an agonist) and ATP induced dose-dependent vasoconstrictions, but adenosine did not cause any response. The potency order was α,β-methylene ATP > ATP. The vasoconstrictor responses to 1 Hz stimulation were not affected by prazosin but were potentiated by phentolamine. The same concentration of both antagonists readily abolished the responses to exogenous noradrenaline. We showed that noradrenaline induces vasoconstriction through α_1-adrenoceptors in the canine splenic artery (Ren et al., 1994a). Atropine, propranolol and indomethacin did not affect the electrical stimulation-evoked and phentolamine-potentiated responses. The evidence strongly suggests that the responses to 1 Hz stimulation are primarily produced by neurally released ATP or a closely related compound, and that postsynaptic P_{2x} purinoceptors are involved in the vasoconstriction. Both phentolamine and prazosin significantly inhibited the vasoconstrictor responses to 10 Hz stimulation, suggesting that 10 Hz stimulation evoked noradrenaline release. Furthermore, the combined treatment with phentolamine and α,β-methylene ATP or suramin abolished the vasoconstrictor responses to 10 Hz stimulation. Therefore, it can be concluded that ATP is a co-transmitter with noradrenaline in the sympathetic nerves of the canine splenic artery.

Activation or inactivation of presynaptic α_2-adrenoceptors can reduce or enhance the release of noradrenaline from sympathetic nerves in many tissues including blood vessels (Starke, 1987). It has been also reported that presynaptic α_2-adrenoceptors modulate the release of ATP induced by electrical stimulation (von Kügelgen and Starke, 1985; MacDonald et al., 1992; von Kügelgen et al., 1994b). In the present study, prazosin (0.1 μM, a selective α_1-adrenoceptor antagonist) did not affect the vasoconstriction evoked by 1 Hz electrical stimulation but significantly inhibited that evoked by 10 Hz electrical stimulation. Rauwolscine (1 μM, a selective α_2-adrenoceptor antagonist; Tanaka et al., 1978) potentiated significantly the prazosin-resistant vasoconstriction elicited by stimulation at both 1 and 10 Hz. Phentolamine (1 μM) potentiated the responses to 1 Hz stimulation, and the combined treatment with phentolamine and suramin or α,β-methylene ATP inhibited the enhanced vasoconstriction. These results suggest that released noradrenaline acts on presynaptic α_2-adrenoceptors to inhibit the release of purinergic transmitters, at least that elicited by stimulation at 1 Hz.

Phentolamine or rauwolscine did not enhance the vasoconstrictor responses elicited by stimulation at 1 and 10 Hz with 1 and 3 pulses, but enhanced the responses with 10 and 30 pulses in this study. Bulloch and Starke (1990) reported that yohimbine (an α_2-adrenoceptor antagonist) did not change responses evoked by very short pulse trains (< 2 s) but enhanced responses to longer pulse trains in the rabbit ileocolic artery. Electrical stimulation parameters significantly affect the ratio of neurally released noradrenaline and ATP. Short pulse trains of low-frequency stimulation (Ramme et al., 1987; Bulloch and Starke, 1990; Evans and Cunnane, 1992; this study) and high-frequency stimulation (von Kügelgen and Starke, 1985; Bulloch and Starke, 1990) cause a vasoconstriction that is predominantly purinergic. The adrenergic component comes into play at longer duration (Bulloch and Starke, 1990) and high-frequency stimulation (this study). Therefore, the period of latency for the development of α_2 autoinhibition might be changed by the amount of noradrenaline released from the nerves. Rauwolscine potentiated the vasoconstriction induced by electrical stimulation for 0.9 s (10 pulses at 10 Hz) in this study.

In the present study, guanethidine (100 μM) abolished the vasoconstrictor responses to 1 Hz stimulation, but it did not abolish those to 10 Hz stimulation. Phentolamine (10 μM) abolished the guanethidine-resistant vasoconstriction elicited at 10 Hz. As discussed above, the purinergic component is mainly involved in the vasoconstriction elicited at 1 Hz and both adrenergic and purinergic components are involved at 10 Hz. Therefore, the purinergic component may be more sensitive to guanethidine than the adrenergic component in the canine splenic artery. The electrical stimulation-induced contraction of the canine basilar artery was abolished after desensitization with α,β-methylene ATP or by guanethidine (Muramatsu and Kigoshi, 1987). However, the adrenergic component of neurogenic responses was significantly more sensitive to guanethidine than was the purinergic component in the rat vas deferens (Trachte et al., 1989). This discrepancy may be due to differences in experimental conditions (organ, species, etc.).

Suramin (a competitive P_2 purinoceptor antagonist)

also significantly inhibited the vasoconstrictor responses to 10 Hz stimulation, but desensitization with α,β-methylene ATP did not affect them (Fig. 2). It has been reported that α,β-methylene ATP enhances the contractile responses to noradrenaline-mediated neurogenic contractions in arteries and vas deferens through pre- or postsynaptic sites (Stjärne and Åstrand, 1985; Shinozuka et al., 1990; Bao and Stjärne, 1993). Since the desensitization with α,β-methylene ATP did not significantly affect the responses to exogenous noradrenaline, α,β-methylene ATP might increase the neural release of noradrenaline through some unknown mechanisms (Stjärne and Åstrand, 1985; Shinozuka et al., 1990).

Suramin is a genuine competitive P_2 purinoceptor antagonist in the mouse vas deferens and rabbit ear artery (Dunn and Blakeley, 1988; Leff et al., 1990), but a suramin-insensitive effect of ATP has been reported in the rat, guinea-pig and mouse vas deferens (von Kügelgen et al., 1990; Bailey and Hourani, 1994; Bültmann and Starke, 1994) and cat colon circular muscle (Venkova and Krier, 1993). In the present study, perfusion with 100 μM suramin for more than 1 h did not significantly inhibit the vasoconstrictor responses to exogenous noradrenaline and ATP. However, suramin significantly shifted the dose-response curve for α,β-methylene ATP to the right in a parallel manner, and it inhibited the vascular responses elicited at 1 Hz greatly and those elicited at 10 Hz partially. Suramin selectively inhibited the purinergic responses to electrical stimulation but not those elicited by exogenous ATP. Our results have two implications. One is that neurally released ATP, unlike exogenous ATP, stimulates only suramin-sensitive P_{2x} purinoceptors but not suramin-insensitive sites as suggested for the mouse vas deferens (Von Kügelgen et al., 1990). The other is that suramin did not antagonize the effects of exogenous ATP, because its ecto-ATPase inhibiting activity cancels the purinoceptor antagonizing effect (Crack et al., 1994). In the present study, we administered ATP to the intraluminal side of the arterial preparation by bolus injection and we did not remove the endothelium. Therefore, the latter explanation may make sense with regard to our results. However, further studies are needed to resolve this question.

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