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PRESYNAPTIC P_1-PURINOCEPTORS IN JEJUNAL BRANCHES OF THE RABBIT MESENTERIC ARTERY AND THEIR POSSIBLE FUNCTION

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SUMMARY

1. Excitatory junction potentials (EJPs) evoked by nerve stimulation with fifteen pulses at 1 Hz were recorded from smooth muscle cells of the rabbit isolated mesenteric artery. The effects of P_1-purinoceptor agonists and antagonists, as well as of substances which interfere with the inactivation of endogenous adenosine, were tested.

2. Adenosine and its analogues depressed the EJPs in the train in a concentration-dependent manner. The percentage inhibition of the first EJP and that of the later ones was similar; some early EJPs, however, were inhibited more markedly. The rank order of potency of the agonists was (−)-N^6-(*R*-phenylisopropyl)-adenosine (*R*-PIA) ≃ 5′-*N*-ethylcarboxamidoadenosine (NECA) > (+)-N^6-(*S*-phenylisopropyl)-adenosine (*S*-PIA) > adenosine. The respective IC_{40} values (the concentrations producing 40% inhibition of the first EJP in the train) were 0·018, 0·028, 0·83 and 4·7 μmol/l.

3. Three methylxanthines, namely 8-phenyltheophylline (1, 10 μmol/l), 8-cyclopentyltheophylline (0·1, 1 μmol/l) and 8-(*p*-sulphophenyl)-theophylline (100 μmol/l), antagonized the effect of *R*-PIA (0·1 μmol/l). When given alone they also enhanced the amplitudes of all EJPs in the train. The percentage facilitation of the first EJP and that of the later ones was similar. Some early EJPs, however, were potentiated more markedly. 8-Phenyltheophylline was less potent than 8-cyclopentyltheophylline both in preventing the action of *R*-PIA and in enhancing the EJPs. A concentration (100 μmol/l) of 8-(*p*-sulphophenyl)-theophylline, which strongly antagonized the *R*-PIA effect, produced only a moderate facilitation of EJPs.

4. *S*-(*p*-nitrobenzyl)-6-thioguanosine (10 μmol/l) both depressed the EJPs, and enhanced the inhibitory effect of adenosine. Adenosine deaminase (10 μg/ml) caused some potentiation of EJPs; this action was prevented by a concentration (10 μmol/l) of deoxycoformycin, which had no effect of its own. AH21-132 (10 μmol/l) enhanced all EJPs in the train.

5. None of the above substances influenced the resting membrane potential of the smooth muscle cells. In addition, *R*-PIA (0·1 μmol/l) did not change the depolarization induced by noradrenaline (3 μmol/l).

6. We suggest that the axon terminals of postganglionic sympathetic neurones in the rabbit mesenteric artery possess P_1-purinoceptors of the A_1-type. The activation

of these presynaptic receptors by endogenous adenosine may inhibit the release of the main neuroeffector transmitter, which is probably ATP. Under our conditions this inhibition is only slightly dependent on neuronal activity; adenosine may originate from the smooth muscle rather than the nerve terminals.

INTRODUCTION

Purinoceptors in different peripheral tissues were originally characterized according to their sensitivity against agonists; they were reported to belong either to the P_1- or to the P_2-type (Burnstock, 1978; Satchell, 1984; Burnstock & Kennedy, 1985). The supposed endogenous ligands of the P_2-receptor are adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP), whereas those of the P_1-receptor are adenosine 5′-monophosphate (AMP) and adenosine. This latter receptor has been subclassified into A_1- (or R_i-) and A_2- (or R_a-) types (Van Calker, Müller & Hamprecht, 1979; Londos, Cooper & Woolf, 1980; Burnstock & Buckley, 1985; Stiles, 1986). The A_1-receptor is negatively coupled to adenylate cyclase; (−)-N^6-(*R*-phenylisopropyl)-adenosine (*R*-PIA) has at this site a higher potency than 5′-*N*-ethylcarboxamidoadenosine (NECA). Moreover, in contrast to A_2-effects a marked stereoselectivity for PIA has been described, the (−) isomer being more potent than the (+) one. The A_2-receptor is positively coupled to adenylate cyclase and the order of potency of adenosine analogues is the reverse of that at the A_1-receptor. It is interesting to note that in contrast to this biochemical approach, functional studies revealed the presence of A_1-receptors in smooth muscle and heart with somewhat unusual characteristics (Collis, 1985; Paton & Olsson, 1985). At this receptor NECA and *R*-PIA are about equipotent, in spite of a pronounced stereoselectivity of the PIA effect. P_1-Purinoceptor-mediated reactions can be antagonized by methylxanthines; some of these structures are rather selective for either A_1- or A_2-receptors (Bruns, Lu & Pugsley, 1985; Schwabe, Ukena & Lohse, 1985; Bruns, Fergus, Badger, Bristol, Santay, Hartman, Hays & Huang, 1987).

Nerve stimulation has been reported to release both ATP and its degradation products, including adenosine, from sympathetically innervated tissues. These purines seemed to originate not only from postsynaptic structures, such as adipose tissue (Fredholm & Sollevi, 1981) or smooth muscle (Luchelli-Fortis, Fredholm & Langer, 1979; Fredholm & Hedqvist, 1980; Fredholm, 1981), but also from nerve terminals (Westfall, Stitzell & Rowe, 1978; Levitt & Westfall, 1982; Su, 1983). At this latter site ATP is contained in the same storage vesicles as noradrenaline and probably functions as a co-transmitter (Muramatsu, Fujiwara, Miura & Sakakibara, 1981; Sneddon, Westfall & Fedan, 1982; Meldrum & Burnstock, 1983; Sneddon & Burnstock, 1985; Burnstock & Kennedy, 1986). Thus, ATP released upon nerve stimulation may activate postsynaptic purinoceptors of the P_2-type, and lead thereby to the efflux of purines. Subsequently, both neuronal and non-neuronal purines may depress transmitter release via an action on presynaptic P_1-purinoceptors (Enero & Saidman, 1977; Hedqvist, Fredholm & Ölund, 1978; Fredholm, 1981; Katsuragi & Su, 1982; Su, 1983).

In medium-sized arteries both the nerve stimulation-induced release of purines and the presence of pre- and postsynaptic purinoceptors has been amply documented

(Su, 1985; Burnstock & Kennedy, 1986). In addition, recent evidence indicates that in peripheral branches of the rabbit mesenteric artery excitatory junction potentials (EJPs; Ishikawa, 1985) and vasoconstriction evoked by electrical stimulation (Ramme, Regenold, Starke, Busse & Illes, 1987) are due to the release of ATP or a closely related substance.

The amplitude of EJPs in a train is a measure of transmitter release per impulse (Illes, 1983). Such recordings demonstrated in rabbit jejunal arteries the presence of presynaptic α_2-adrenoceptors which mediate a feed-back inhibition of transmitter release (Mishima, Miyahara & Suzuki, 1984; Illes & Nörenberg, 1987; Ramme *et al.* 1987). It has been reported that adenosine also depresses EJPs in this tissue, but it was not clarified whether its site of action is pre- or postsynaptic (Kuriyama & Makita, 1984). The present experiments were performed to characterize the P_1-purinoceptor by means of subtype selective agonists and antagonists. We also searched for the localization of this receptor, and for a possible function of endogenous adenosine in the modulation of neuroeffector transmission.

METHODS

Preparation and recording

Rabbits of either sex (1·5–2·5 kg) were killed by cervical dislocation. Jejunal branches of the mesenteric artery were dissected out and carefully cleaned from connective tissue. Only small arteries with an external diameter of 0·2–0·4 mm were used; about 5 mm long segments were pinned to the bottom of a 1·5 ml organ bath. The preparation was superfused with medium (in mmol/l: NaCl, 118; KCl, 4·8; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 0·9; $NaHCO_3$, 25 and glucose, 11) saturated with 95% O_2 plus 5% CO_2 and maintained at 37 °C. The flow rate was 1 ml/min. Intracellular potentials were recorded from smooth muscle cells with glass microelectrodes filled with KCl, 3 mol/l (resistances 50–90 MΩ) as previously described (Illes, Ramme & Starke, 1986). The resting membrane potential of single cells was between 60 and 75 mV.

Nerve stimulation-evoked EJPs

The perivascular nerves were stimulated with bipolar platinum electrodes placed perpendicularly to the length of the organ and 0·5 mm apart. The site of recording was between the two stimulating electrodes. Pulses of 1 ms duration and 1–15 V were supplied by the stimulus-isolation unit of a Grass S88 stimulator. The voltage was adjusted so that an EJP of about 7 mV amplitude was evoked by a single pulse. This was possible, because smooth muscle cells in arteries are electrically coupled to each other with only a fraction of cells being directly innervated by the perivascular nerves (Illes, 1983). However, depolarization caused by the transmitter in an innervated cell is decrementally conducted to its non-innervated counterparts. Thus, a change in stimulation voltage will affect the number of nerve fibres excited, and thereby the amplitude of EJPs.

Up to seven trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_7). Agonists were added either immediately after T_1 or, in interaction experiments, immediately after T_4. Antagonists, the phosphodiesterase blocker AH21-132 and drugs which interfere with the inactivation of adenosine, were added immediately after T_1 or, when their mutual interaction was studied, immediately after T_4. The effects of all drugs were evaluated as percentage change. When given alone, EJP amplitudes evoked by the train before addition (T_1) were compared either with the second train in the presence of agonists (T_3; 6 min incubation) or the third train in the presence of all other drugs (T_4; 9 min incubation). In interaction experiments, EJP amplitudes evoked by the train before addition (T_4) were compared either with the second train in the presence of agonists (T_6; 6 min incubation) or the third train in the presence of all other drugs (T_7; 9 min incubation). The IC_{40} value, i.e. the concentration which produced 40% inhibition of the first EJP in the train, was read off the concentration–response curve. 8-Phenyltheophylline increased the IC_{40} of *R*-PIA; the dissociation constant (K_B) of the antagonist receptor complex was calculated according to

eqn (4) of Furchgott (1972). In order to determine whether EJPs changed with time, recordings were performed in the absence of drugs. If a cell was lost, the same or a closely neighbouring cell was re-impaled; this procedure did not influence the EJP amplitudes (Ramme, Illes, Späth & Starke, 1986).

Noradrenaline-induced depolarization

When the effect of *R*-PIA on resting membrane potential and noradrenaline-induced depolarization was tested, the medium contained ascorbic acid (0·3 mmol/l) and Na_2EDTA (0·03 mmol/l). Measurements were carried out while the tissue was superfused in the following order: drug-free medium, medium containing noradrenaline (3 μmol/l) for 12 min; and medium containing noradrenaline (3 μmol/l) plus *R*-PIA (0·1 μmol/l) for an additional 12 min. The membrane potential of three cells was measured both before addition of drugs and after 5 min incubation in each solution. In order to determine whether noradrenaline-induced depolarization changed with time, in analogous experiments superfusion with medium containing only noradrenaline (3 μmol/l) was maintained for 24 min.

Materials

The following drugs were used: (−)-N^6-(*R*-phenylisopropyl)-adenosine, (+)-N^6-(*S*-phenylisopropyl)-adenosine (Boehringer, Mannheim, F.R.G.); (−)-noradrenaline hydrochloride (Hoechst, Frankfurt, F.R.G.); 2′-deoxycoformycin (M. Suffness, National Institutes of Health, Bethesda, MD, U.S.A.); 8-(*p*-sulphophenyl)-theophylline (Research Biochemicals, MA, U.S.A.); *cis*-6-(*p*-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo[c][1,6]-naphthydrine-bis(hydrogenmaleinate) (AH21-132; R. Markstein, Sandoz, Basel, Switzerland); adenosine, adenosine deaminase type VII, 8-phenyltheophylline, *S*-(*p*-nitrobenzyl)-6-thioguanosine (Sigma, München, F.R.G.); 8-cyclopentyltheophylline, 5′-*N*-ethylcarboxamidoadenosine (J. A. Bristol, Warner–Lambert, Ann Arbor, MI, U.S.A.).

Stock solutions (1–10 mmol/l) of all substances were prepared with distilled water, except those of *R*-PIA, *S*-PIA and *S*-(*p*-nitrobenzyl)-6-thioguanosine, which were dissolved in dimethyl sulphoxide (Sigma, München, F.R.G.). Further dilutions were made with medium. 8-Phenyltheophylline and 8-cyclopentyltheophylline were added to medium to a concentration of 10 μmol/l and diluted further if needed. Equivalent quantities of the solvent had no effect.

Statistics

Results are expressed as means ± S.E.M. Differences between means were tested for significance by the non-parametric Kruskal–Wallis analysis followed by the Mann–Whitney test (Hollander & Wolfe, 1973). A probability level of 0·05 or less was considered to be statistically significant.

RESULTS

Stimulation condition

Stimulation of the perivascular nerves with fifteen pulses at 1 Hz elicited EJPs that increased up to the third response and then declined to an approximately constant level (Fig. 1). Because of this variability in amplitudes we selected for statistical evaluation the first, second, third and fifteenth EJP. None of these responses changed significantly when the trains were repeated up to seven times at 3 min intervals in the absence of drugs (Tables 1, 2 and 3). With the exception of noradrenaline, concentrations of the substances used in this study did not influence the resting membrane potential of the smooth muscle cells.

Effects of P_1-purinoceptor agonists

Adenosine (10 μmol/l) depressed all EJPs in the train (Fig. 1*A*). The percentage blockade of the first EJP was less marked than that of the next four. Then, the degree of inhibition slowly declined; it reached its original level with the sixth EJP

(Fig. 1*B*). *R*-PIA, 0·01 (Fig. 2*A*) and 0·1 (Fig. 2*B*) μmol/l, also depressed the EJPs; this effect depended on the concentration applied. Table 1 shows the inhibitory action of different agonists, namely adenosine, *R*-PIA, *S*-PIA and NECA on the four selected EJP amplitudes. The effects of all these substances were concentration

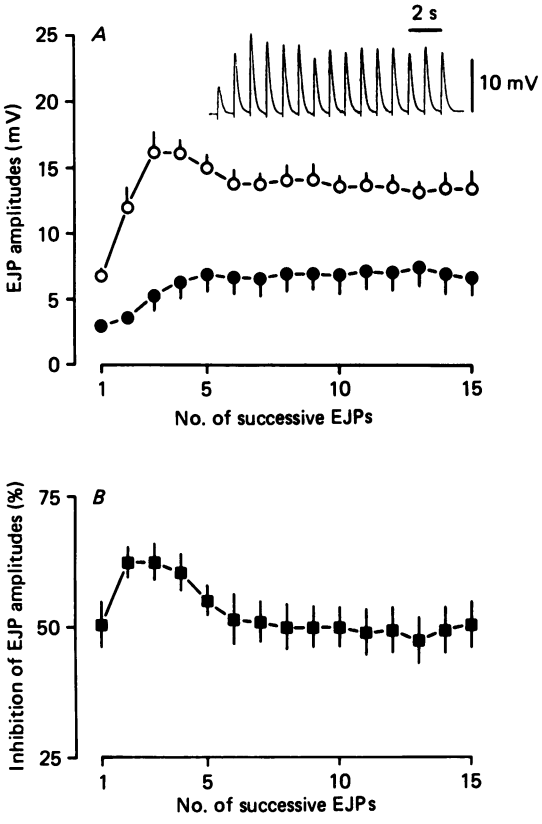

Fig. 1. Effect of adenosine on the amplitude of EJPs in jejunal branches of the rabbit mesenteric artery. Three trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_3). *A*, ○, before drug addition (T_1); ●, after 6 min of exposure to adenosine (10 μmol/l; T_3). *B*, percentage inhibition of EJP amplitudes by adenosine (10 μmol/l) calculated from the data of ○ and ● in *A*. Means ± S.E.M. of seven experiments. The inset shows a representative tracing of a first EJP series.

dependent and related in a similar manner to the number of pulses as demonstrated previously for adenosine (10 μmol/l). Concentration–response curves of the agonists were constructed by plotting the percentage inhibition of the first EJP amplitudes against the logarithm of the concentrations (Fig. 3). The maximum effects of *R*-PIA and *S*-PIA were about 70% and 50%, respectively; in the case of NECA and adenosine no clear maximum was reached. The IC_{40} values derived from the curves were 0·018 μmol/l (*R*-PIA), 0·028 μmol/l (NECA), 0·83 μmol/l (*S*-PIA) and 4·7 μmol/l (adenosine). Thus, the rank order of potency of the substances was *R*-PIA ≃ NECA > *S*-PIA > adenosine.

Effects of P_1-purinoceptor antagonists and their interaction with R-PIA

8-Phenyltheophylline (10 μmol/l) enhanced all EJPs in the train (Fig. 4*A*). This effect did not decline over a considerable period of time. The percentage potentiation of the first EJP was less pronounced than that of the second and third. Then,

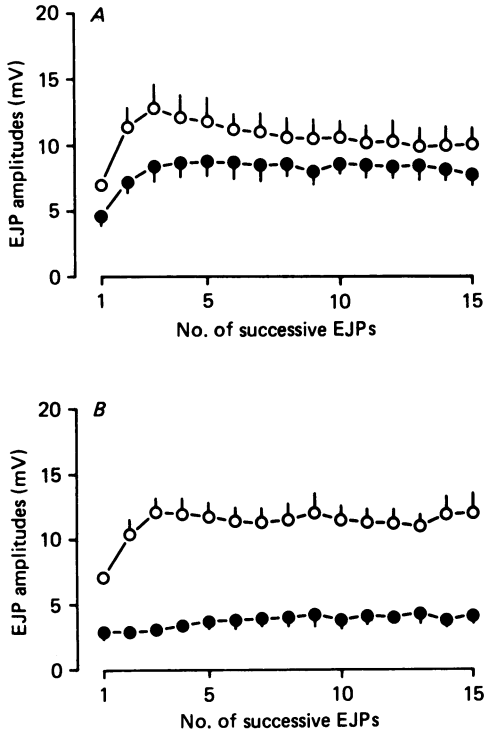

Fig. 2. Concentration-dependent effect of *R*-PIA on the amplitude of EJPs. Three trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_3). *A*, ○, before drug addition (T_1); ●, after 6 min of exposure to *R*-PIA (0·01 μmol/l; T_3). *B*, ○, before drug addition (T_1); ●, after 6 min exposure to *R*-PIA (0·1 μmol/l; T_3). Means ± S.E.M. of five experiments both in *A* and *B*.

however, the increase in facilitation rapidly vanished, and from the fourth EJP onwards stabilized at its original level (Fig. 4*B*). A similar relationship between the amplitude of the first few EJPs in a train and the number of pulses could be observed also with two additional antagonists, namely with 8-cyclopentyltheophylline and 8-(*p*-sulphophenyl)-theophylline (Table 2). Actually the transient enhancement of an otherwise constant potentiation of EJPs by antagonists is the mirror image of the transient increase of inhibition of EJPs by agonists (Fig. 1; Table 1). As documented for a few selected EJPs in Table 2, all antagonists enhanced these potentials in a concentration-dependent manner.

The following experiments were designed to find out whether there is any correlation between the facilitatory effect and the antagonistic potency of a given drug. Such a correlation became evident when a constant concentration of *R*-PIA

TABLE 1. Effects of purinoceptor agonists on EJP amplitudes

Agonist (μmol/l)		Inhibition (%) 1st EJP amplitude	2nd EJP amplitude	3rd EJP amplitude	15th EJP amplitude	n
—	—	0·7 ± 5·7	−7·6 ± 5·6	−10·5 ± 7·2	−5·2 ± 3·5	16
Adenosine	1	17·4 ± 7·5	29·9 ± 4·5**	30·4 ± 5·0**	20·5 ± 3·2**	7
	10	50·8 ± 8·8**	64·6 ± 6·4**	65·1 ± 7·0**	50·8 ± 8·7**	7
R-PIA	0·001	−4·9 ± 10·0	−1·0 ± 5·8	−12·7 ± 9·1	0·6 ± 5·2	5
	0·01	34·3 ± 4·9**	35·9 ± 5·0**	33·5 ± 1·0**	22·0 ± 5·5**	5
	0·1	57·7 ± 9·3**	69·5 ± 5·9**	74·6 ± 3·2**	66·0 ± 3·0**	5
	1	68·9 ± 3·8**	75·8 ± 3·9**	74·2 ± 4·8**	74·6 ± 3·5**	7
S-PIA	0·1	20·9 ± 6·6*	27·0 ± 6·7**	15·7 ± 5·9	8·9 ± 2·4*	5
	1	41·7 ± 5·8**	56·6 ± 4·8**	56·3 ± 4·6**	50·0 ± 5·4**	7
	10	51·2 ± 11·8**	62·5 ± 11·5**	60·5 ± 10·2**	49·8 ± 7·1**	5
NECA	0·01	24·8 ± 8·3*	33·3 ± 8·3**	14·3 ± 7·2	6·0 ± 3·4	6
	0·1	58·6 ± 5·0**	69·0 ± 3·5**	67·2 ± 4·7**	50·2 ± 5·9**	6
	1	82·9 ± 1·3**	90·5 ± 0·6**	90·6 ± 0·8**	80·9 ± 1·3**	5

Three trains of fifteen pulses were delivered at 1 Hz (T_1–T_3). The agonists were applied immediately after T_1. Values are changes of EJP amplitudes 6 min after agonist addition (T_3), expressed as a percentage of corresponding amplitudes in the last EJP series before treatment with these drugs (T_1). Means ± S.E.M. of n experiments.

* $P < 0.05$; ** $P < 0.01$; significant differences from the corresponding experiments without drugs.

(0·1 μmol/l) was tested in the presence of increasing concentrations of 8-phenyltheophylline (1 and 10 μmol/l; Fig. 5; Table 3) or 8-cyclopentyltheophylline (0·1 and 1 μmol/l; Table 3). Since the interaction of 8-phenyltheophylline (10 μmol/l) with *R*-PIA (1 μmol/l) was also measured, it was possible to construct a concentration–response curve of the agonist with respect to the first EJP. From this curve and from that obtained under control conditions (Fig. 3) the K_B value of 8-phenyltheophylline could be calculated; it was 1·1 μmol/l. Although the K_B values of the other antagonists were not determined, it is clear from the results presented in Table 3 that the antagonistic potency of 8-cyclopentyltheophylline is higher than that of 8-phenyltheophylline. Thus, approximately equiactive antagonistic concentrations of the two drugs (Table 3) also produced similar facilitation of the EJPs, when given alone (Table 2). By contrast, 8-(*p*-sulphophenyl)-theophylline, in a concentration (100 μmol/l) which almost abolished the effect of *R*-PIA (0·1 μmol/l), caused only a moderate facilitation of EJPs.

Effects of drugs which interfere with the inactivation of adenosine, and their interaction with P_1-purinoceptor agonists and antagonists

Uptake blockade by *S*-(*p*-nitrobenzyl)-6-thioguanosine (10 μmol/l) both depressed the EJPs (Fig. 6*B*; Table 2), and enhanced the inhibitory action of subsequently applied adenosine (0·1 μmol/l; compare Fig. 6*A* with Fig. 6*B* and Table 1 with Table 3). Adenosine deaminase (10 μg/ml) produced by itself a moderate facilitation of EJPs (Table 2), which could be prevented by the previous application of the selective enzyme inhibitor deoxycoformycin (10 μmol/l; Table 3). This concentration

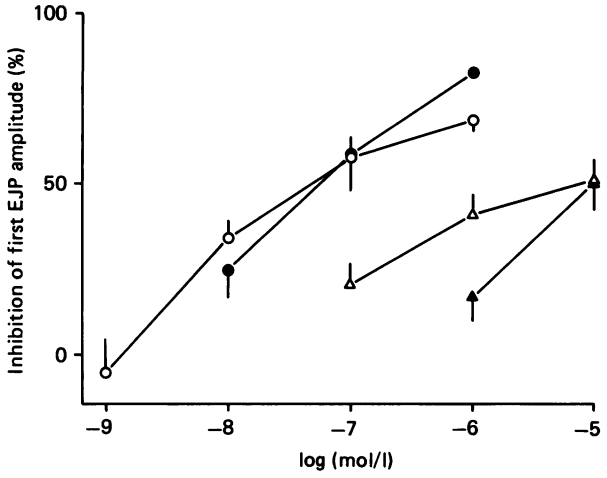

Fig. 3. Concentration–response curves of adenosine and its structural analogues. Drug effects are expressed as percentage inhibition of the first EJP amplitude evoked by a train of fifteen pulses at 1 Hz. Evaluation was after 6 min of exposure to the respective agonist (T_3). ○, *R*-PIA; ●, NECA; △, *S*-PIA; ▲, adenosine. Means ± S.E.M. of five to seven experiments (see Table 1).

of deoxycoformycin had no effect of its own on the EJPs (Table 2). Finally, adenosine deaminase (10 μg/ml) did not change considerably the potentiation of EJPs by 8-phenyltheophylline (10 μmol/l; Table 3).

Effect of the phosphodiesterase inhibitor AH21-132

The effect of the phosphodiesterase inhibitor AH21-132 (10 μmol/l) was also tested; it enhanced all EJPs in the train (Table 2).

Effect of R-PIA on noradrenaline-induced depolarization

Noradrenaline (3 μmol/l) depolarized the smooth muscle cells by 6·1 ± 0·3 mV (five preparations). When, thereafter, *R*-PIA (0·1 μmol/l) was added to the noradrenaline-containing medium, the effect of the amine decreased by 16·0 ± 7·0 %. In another five preparations noradrenaline (3 μmol/l) produced 5·5 ± 0·2 mV depolarization, and there was a 14·3 ± 2·8 % fall over the same period of time as in the tissues which were also in contact with *R*-PIA. Thus, the adenosine analogue had no effect on the noradrenaline-induced change in membrane potential ($P > 0·05$).

DISCUSSION

An important finding of the present study is that adenosine and some of its structural analogues depress neuroeffector transmission in rabbit mesenteric arteries. Recent experiments demonstrated the presence of a P_1-receptor situated at the smooth muscle and mediating relaxation of this tissue, when pre-contracted by noradrenaline (Mathieson & Burnstock, 1985). However, the inhibition of EJPs may be due to a presynaptic action as suggested by three observations. Firstly, the

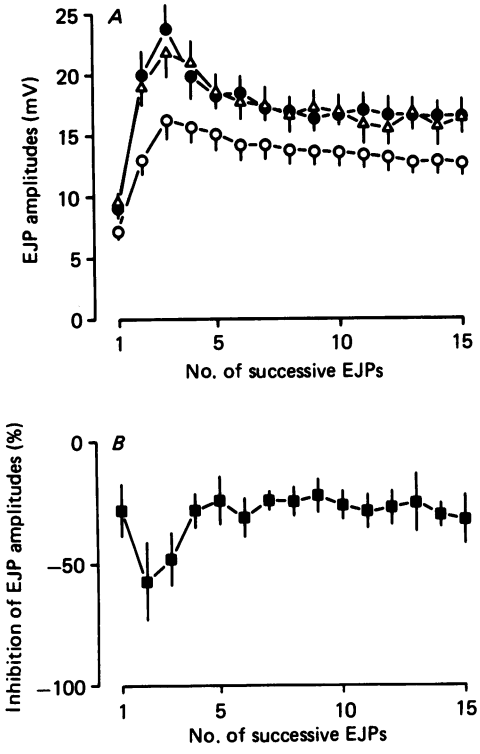

Fig. 4. Effect of 8-phenyltheophylline on the amplitude of EJPs. Six trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_6). *A*, ○, before drug addition (T_1); ●, after 9 min of exposure to 8-phenyltheophylline (10 μmol/l; T_4); △, after 15 min of exposure to 8-phenyltheophylline (10 μmol/l; T_6). *B*, percentage inhibition of EJP amplitudes by 8-phenyltheophylline (10 μmol/l) calculated from the data of ○ and ● in *A*. Means ± S.E.M. of six experiments.

agonists inhibited the early EJPs in a train to a larger extent than the subsequent ones. An exception was the first EJP which was depressed as much as the late potentials. If the effects were postsynaptic, all EJPs should be reduced to the same degree. Secondly, none of the agonists changed the resting membrane potential of the smooth muscle cells. Thirdly, *R*-PIA failed to influence the noradrenaline-induced depolarization.

We have shown previously in rabbit jejunal arteries that the inhibition of EJPs by α_2- (Illes & Nörenberg, 1987) or opioid agonists (Illes *et al.* 1986) was inversely related to the number of pulses in a train. We concluded that the receptors of these substances are presynaptically localized. This suggestion was supported also by the direct determination of noradrenaline release, evoked by electrical stimulation (Mishima *et al.* 1984; Ramme *et al.* 1986). Presynaptic adenosine receptors are present in various sympathetically innervated smooth muscle organs (Paton, 1981; Stone, 1983; Burnstock & Buckley, 1985) including blood vessels, such as the portal vein of rabbits (Brown & Collis, 1983) or rats (Kennedy & Burnstock, 1984). The receptors in most of these tissues were characterized by selective agonists and were suggested

TABLE 2. Effects of purinoceptor antagonists, the phosphodiesterase blocker AH21-132 and drugs which interfere with the inactivation of adenosine, on EJP amplitudes

Treatment (μmol/l)†		Inhibition (%) 1st EJP amplitude	2nd EJP amplitude	3rd EJP amplitude	15th EJP amplitude	n
—	—	−1·3±2·9	0·2±4·8	−7·6±5·2	−6·3±2·3	16
8-Phenyltheophylline	1	−16·5±6·7*	−25·0±6·2**	−36·1±7·0**	−10·3±4·3	12
	10	−31·7±5·2**	−45·8±6·4**	−41·6±5·7**	−22·2±4·3**	22
8-Cyclopentyltheophylline	0·1	−27·4±9·4**	−43·9±13·3**	−38·0±9·9**	−24·6±5·4**	10
	1	−33·0±9·4**	−45·3±11·5**	−42·7±8·5**	−26·2±4·4**	11
8-(*p*-Sulphophenyl)-theophylline	100	−8·7±6·2	−22·8±3·9**	−33·4±9·0*	−17·4±5·8	12
S-(*p*-Nitrobenzyl)-6-thioguanosine	10	40·9±4·8**	−35·0±4·5**	27·5±4·9**	17·5±3·8**	13
Adenosine deaminase	10	−17·2±5·6*	−32·6±9·7**	−32·5±7·0**	−15·5±5·1	14
Deoxycoformycin	10	8·6±5·5	4·5±5·4	0·6±5·6	−7·5±4·6	14
AH21-132	10	−33·5±5·0**	−21·9±9·1*	−37·7±9·1*	−39·6±4·0**	6

Up to seven trains of fifteen pulses were delivered at 1 Hz (T_1–T_7). All substances were applied immediately after T_1. Values are changes of EJP amplitudes 9 min after agonist addition (T_4), expressed as a percentage of corresponding amplitudes in the last series before treatment with these drugs (T_1). Means±S.E.M. of *n* experiments.

* $P < 0.05$; ** $P < 0.01$; significant differences from the corresponding experiments without drugs.

† The concentration of adenosine deaminase is given in μg/ml.

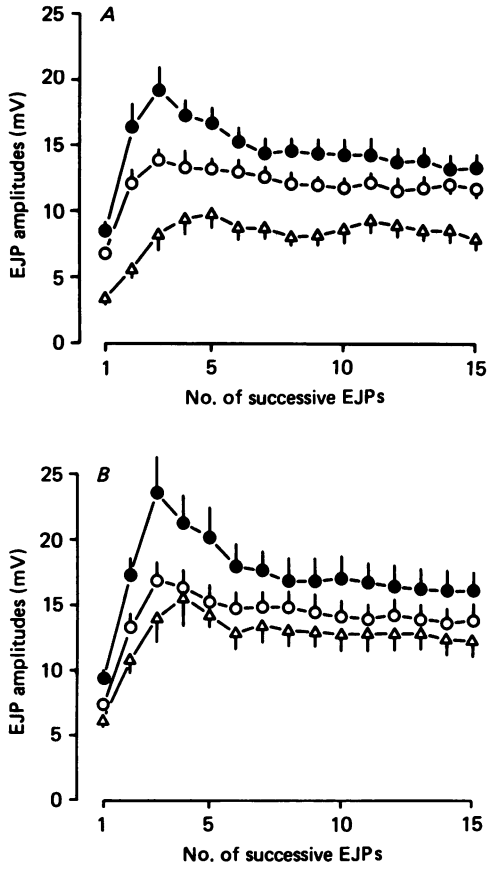

Fig. 5. Effects of 8-phenyltheophylline and 8-phenyltheophylline plus *R*-PIA on the amplitude of EJPs. Six trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_6). *A*, ○, before drug addition (T_1); ●, after 9 min of exposure to 8-phenyltheophylline (1 μmol/l; T_4); △, after 6 min of additional exposure to *R*-PIA (0·1 μmol/l; T_6). Means ± S.E.M. of five experiments. *B*, ○, before drug addition (T_1); ●, after 9 min of exposure to 8-phenyltheophylline (10 μmol/l; T_4); △, after 6 min of additional exposure to *R*-PIA (0·1 μmol/l; T_6). Means ± S.E.M. of nine experiments.

to belong to the A_1-type. An interesting exception is the rat portal vein, which contains an A_2-receptor population.

The rank order of potency of various agonists in the present study was *R*-PIA ≃ NECA > *S*-PIA > adenosine; *R*-PIA was about fifty times more active than *S*-PIA. The results indicate that the P_1-receptor in jejunal arteries belongs to the A_1-type, and that it has the same unusual characteristics as reported for other smooth muscle organs (Collis, 1985; Paton & Olsson, 1985). Experiments with antagonists also favour the existence of an A_1-receptor, although fail to prove it unequivocally. 8-Phenyltheophylline has some preference for A_1-receptors (Schwabe *et al.* 1985; Ukena, Daly, Kirk & Jacobson, 1986) and 8-cyclopentyltheophylline is a rather selective A_1-antagonist (Bruns *et al.* 1985, 1987). Both methylxanthines prevented the effect of *R*-PIA on the EJPs. However, the K_B value (1·1 μmol/l) of

TABLE 3. Interaction of purinoceptor agonists, antagonists and drugs which interfere with the inactivation of adenosine on EJP amplitudes

Pretreatment (μmol/l)†		Treatment (μmol/l)		Inhibition (%) 1st EJP amplitude	2nd EJP amplitude	3rd EJP amplitude	15th EJP amplitude	n
8-Phenyltheophylline	1	—	—	13·6±7·8	13·7±6·5	7·5±4·1	2·0±4·0	6
		R-PIA	0·1	58·7±7·0**	64·3±5·1**	55·8±7·0**	39·6±7·1**§	5
8-Phenyltheophylline	10	—	—	−4·2±2·8	3·4±4·4	7·5±3·4	−0·1±4·1	6
		R-PIA	0·1	32·9±4·1**§	37·3±3·9**§§	38·4±5·7**§§	23·2±5·4**§§	9
		R-PIA	1	60·6±5·6**	73·8±4·7**	72·4±4·0**	58·8±5·0**	7
8-Cyclopentyl-theophylline	0·1	—	—	12·0±5·4	5·7±3·2	1·9±2·9	4·8±3·0	5
		R-PIA	0·1	35·6±4·0*	39·4±7·5**§§	30·2±10·0*§§	23·9±6·2**§§	5
8-Cyclopentyl-theophylline	1	—	—	−13·4±11·8	−2·0±6·5	−1·0±4·8	−0·5±6·9	6
		R-PIA	0·1	3·9±11·5§§	8·6±8·8§§	−2·7±14·2§§	10·5±7·1§§	5
8-(*p*-Sulphophenyl)-theophylline	100	—	—	10·9±5·3	4·3±7·3	6·7±3·7	−1·5±10·9	5
		R-PIA	0·1	19·7±8·2§	17·3±7·2§§	12·5±9·0§§	13·4±8·3§§	7
S-(*p*-Nitrobenzyl)-6-thioguanosine	10	—	—	−14·0±23·0	−9·4±8·5	−7·4±5·1	−1·5±6·7	7
		Adenosine	1	68·8±2·3**§§	78·0±3·1**§§	77·1±3·4**§§	61·5±3·2**§§	6
Deoxycoformycin	10	—	—	20·7±8·8	15·0±11·3	15·6±9·5	14·8±10·5	7
		Adenosine deaminase	10 —	1·8±10·1§	5·3±8·1§§	−6·8±7·1§	5·1±5·6§§	7
Adenosine deaminase	10	—	—	13·1±13·4	13·7±14·6	10·7±11·0	4·1±6·4	6
		8-Phenyl-theophylline	10	−22·5±5·0*	−31·7±3·7*	−30·2±9·4*	−15·8±6·1*	8

Up to seven trains of fifteen pulses were delivered at 1 Hz (T_1–T_7). Pre-treatment was started immediately after T_1, and a further treatment followed immediately after T_4. Values are changes of EJP amplitudes 6 min after agonist addition (T_6), or 9 min after the addition of adenosine deaminase or 8-phenyltheophylline (T_7), expressed as a percentage of corresponding amplitudes in the last EJP series before treatment with these drugs (T_4). Means ± S.E.M. of n experiments.

* $P < 0.05$; ** $P < 0.01$; significant differences from the corresponding experiments without treatment with purinoceptor agonists, adenosine deaminase or 8-phenyltheophylline.

† The concentration of adenosine deaminase is given in μg/ml.

§ $P < 0.05$; §§ $P < 0.01$; significant differences from the corresponding experiments without pre-treatment with drugs (see Tables 1 and 2).

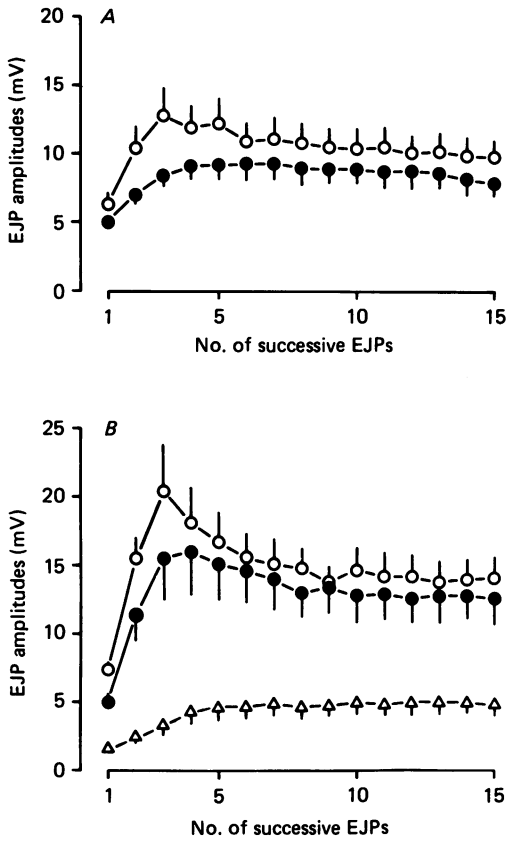

Fig. 6. Effects of adenosine and *S*-(*p*-nitrobenzyl)-thioguanosine plus adenosine on the amplitude of EJPs. Six trains of fifteen pulses at 1 Hz were delivered every 3 min (T_1–T_6). *A*, ○, before drug addition (T_1); ●, after 6 min of exposure to adenosine (1 μmol/l; T_3). Means ± S.E.M. of seven experiments. *B*, ○, before drug addition (T_1); ●, after 9 min of exposure to *S*-(*p*-nitrobenzyl)-6-thioguanosine (10 μmol/l; T_4); △, after 6 min of additional exposure to adenosine (1 μmol/l; T_6). Means ± S.E.M. of six experiments.

8-phenyltheophylline was considerably higher than that reported previously in biochemical experiments measuring either the binding of labelled ligands or the activity of adenylate cyclase (100–350 nmol/l; Schwabe *et al.* 1985; Ukena *et al.* 1986). 8-(*p*-Sulphophenyl)-theophylline, a polar substance which should penetrate cells only to a limited extent (Bruns, Daly & Snyder, 1980) also antagonized the effect of *R*-PIA. Consistent with its lower potency at A_1-receptors, the concentrations used had to be considerably higher than those of 8-phenyltheophylline. In addition to their antagonistic potency, all methylxanthines enhanced the EJP amplitudes.

A potent inhibitor of the adenosine uptake system, *S*-(*p*-nitrobenzyl)-6-thioguanosine (Paterson, 1979), greatly increased the inhibitory effect of adenosine. It also depressed the EJPs when given alone. By contrast, adenosine deaminase which converts the purine to the inactive nucleoside inosine (Daly, 1982), potentiated the EJPs; this effect could be prevented by the selective enzyme inhibitor deoxy-

coformycin (Agarwal, Spector & Parks, 1977). Deoxycoformycin itself had no influence on the EJPs, indicating that endogenous adenosine deaminase may be less important in decreasing extracellular adenosine concentrations than the uptake system (Nimit, Skolnick & Daly, 1981). The methylxanthines, *S*-(*p*-nitrobenzyl)-6-thioguanosine and adenosine deaminase, affected even the first EJP amplitude in the train; this change remained almost constant thereafter, except for some variability within the first few EJPs. However, a similar variability can be observed in the action of different exogenous agonists. Thus, different procedures which might influence the level of endogenous adenosine or antagonize its effect at the receptor, lead to corresponding changes in EJP amplitudes. Comparable approaches have been used recently in rabbit hippocampal slices to demonstrate a local purinergic control of noradrenaline release (Jackisch, Fehr & Hertting, 1985).

It seems to be largely accepted that the release of noradrenaline in blood vessels is regulated by an autoinhibitory mechanism which involves presynaptic α_2-adrenoceptors (Starke, 1977; Langer, 1981). More recently the existence of an additional purinergic feed-back loop has been postulated (Su, 1983). It has been shown that the biochemically determined overflow of noradrenaline is enhanced by methylxanthines (Enero & Saidman, 1977), whereas that of purine compounds (originating probably from the enzymic degradation of ATP) is depressed when uptake and metabolism of adenosine were inhibited (Katsuragi & Su, 1982). However, in these studies methodological limitations did not allow the measurement of the per-pulse release of transmitter. Such information may be obtained if EJPs are recorded by intracellular microelectrodes, as was the case in the present study.

The enhancement of EJPs by 8-phenyltheophylline and 8-cyclopentyltheophylline may be partly due to an effect not related to antagonistic properties at the A_1-receptor. 8-(*p*-Sulphophenyl)-theophylline, which acts only at extracellular sites, was much less potent in enhancing EJPs than the two other methylxanthines. Thus, an additional inhibitory effect involving the cellular enzyme phosphodiesterase cannot be excluded, although at the concentrations used such an action should be negligible (Smellie, Davis, Daly & Wells, 1979). In fact, AH21-132, a phosphodiesterase inhibitor which has no major affinity for adenosine receptors (cf. Markstein, Digges, Marshall & Starke, 1984), considerably increased all EJP amplitudes in the train. A further argument in favour of a non-receptor-mediated action of 8-phenyltheophylline is that the application of adenosine deaminase failed to abolish its effect.

Finally it remains to be clarified why the exclusion of a supposed purinergic feed-back mechanism enhances the first EJP in the train, and why the amplitudes of the subsequent EJPs do not increase gradually with the number of impulses over the whole train. If the pulse-to-pulse output of noradrenaline and ATP is set by the perineuronal concentration of previously released transmitter, this concentration, and in consequence the degree of autoinhibition, should increase with the length of the train. These expectations are fulfilled, for example, after the addition of α_2-adrenoceptor antagonists, which do not enhance the first few EJPs, but do so with the later ones (Mishima *et al.* 1984; Illes & Nörenberg, 1987; Ramme *et al.* 1987). By contrast, P_1-antagonists seem to abolish a tonic inhibitory control of transmitter release; their effects were only slightly dependent on the number of pulses. Thus, the

concentration of adenosine at presynaptic P_1-receptors probably does not increase appreciably with the neuronal activity. Since adenosine may be released from the smooth muscle also in the absence of nerve stimulation (e.g. by hypoxia or cellular damage; Burnstock & Kennedy, 1986), we suggest that under our conditions the purine is of postsynaptic rather than presynaptic origin.

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