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A study of the actions of P_1-purinoceptor agonists and antagonists in the mouse vas deferens *in vitro*

A.G.H. Blakeley, P.M. Dunn & S.A. Petersen

Department of Physiology, University of Leicester, Leicester LE1 7RH

1 We have examined the effects of purinoceptor agonists and antagonists on the mechanical 'twitch' response, excitatory junction potential (e.j.p.) amplitude and [^{3}H]-noradrenaline overflow in the mouse vas deferens.

2 The agonist profile for inhibition of the mechanical response was N^6-([R]-2-phenylisopropyl)adenosine (L-PIA) $\simeq$ N^6-cyclohexyladenosine (CHA) > 5′ N-ethylcarboxamidoadenosine (NECA) > 2-chloroadenosine (2ClA) $\simeq$ N^6-([S]-2-phenylisopropyl)adenosine (D-PIA).

3 The P_1-purinoceptor agonists inhibited the e.j.p. with an agonist profile of CHA > L-PIA $\simeq$ NECA > 2ClA.

4 2ClA inhibited [^{3}H]-noradrenaline overflow with an EC_{50} of 1.2 μM which was not significantly different from the values for inhibition of the e.j.p. and the mechanical response.

5 The inhibitory action of 2ClA on the mechanical response was antagonized by 5 μM 8-phenyltheophylline (8-PT). However, neither blockade of P_1-purinoceptors by 8-PT nor increasing the rate of degradation of endogenous adenosine by addition of adenosine deaminase had any effect on the mechanical response *per se*. 8-PT (5 μM) also failed to alter the e.j.p. amplitude or [^{3}H]-noradrenaline overflow.

6 These results indicate that there are P_1-purinoceptors present on sympathetic nerve terminals of the mouse vas deferens which are more like A_1- than A_2-receptors, but may be better classified as being of the A_3-subtype (Ribeiro & Sebastiao, 1986). These receptors are not normally involved in the feedback regulation of transmitter release in this tissue.

Introduction

Adenosine and its analogues can inhibit neuroeffector transmission at many central and peripheral sites, including the vas deferens. These actions of adenosine can be antagonized by methylxanthines such as theophylline, and are due to the activation of prejunctional P_1-purinoceptors (Ginsborg & Hirst, 1972; Clanachan *et al.*, 1977; Fredholm & Hedqvist, 1980; Sneddon *et al.*, 1984).

In this study, we have addressed two main questions, (1), which subtype of P_1-purinoceptor is present on sympathetic nerve terminals in the mouse vas deferens, and (2) is there any evidence for a physiological role of these receptors in the regulation of neuroeffector transmission.

In the mouse vas deferens, adenosine 5′-triphosphate (ATP) may act as a co-transmitter with noradrenaline (Stjärne & Åstrand, 1984), and neuronally released ATP may be metabolized to adenosine by ectoATPases (Manery & Dryden, 1979). In addition, adenosine is released from smooth muscle cells during contraction (Westfall *et al.*, 1978). Consequently, there are two possible sources of extracellular adenosine which could act on prejunctional P_1-purinoceptors to provide feedback regulation of transmitter release. An analogous feedback system has been proposed for sympathetic neuroeffector transmission in blood vessels (Enero & Saidman, 1977; Su, 1978). Since there may be at least two neurotransmitters involved in neuroeffector transmission in the vas deferens (Meldrum & Burnstock, 1983; Sneddon & Westfall, 1984; Stjärne & Åstrand, 1984), we have examined the effects of purinoceptor agonists and antagonists on three nerve evoked responses, (1) the fast mechanical 'twitch' response, (2) the excitatory junction potential (e.j.p.) and (3) the overflow of [^{3}H]-noradrenaline. The first two responses are thought to give a measure of ATP release. If, as has been proposed (Sneddon & Westfall, 1984), noradrenaline and ATP are released from the same synaptic vesicles, then their release should

be modified in the same way by any drugs acting on prejunctional receptors.

P_1-purinoceptors have been subclassified into A_1- and A_2-subtypes depending on whether their activation causes stimulation or inhibition of adenylate cyclase (Van Calker *et al.*, 1979). More recently, P_1-purinoceptors have been subclassified into A_1-, A_2- and even A_3-subtypes, based on the relative potencies of different adenosine analogues, and the difference in potency between the two stereoisomers of N^6-(2-phenylisopropyl)adenosine (PIA) (Bruns *et al.*, 1980; Londos *et al.*, 1980; Ribeiro & Sebastiao, 1985; 1986).

In this study, we have examined the effects of different purinoceptor agonists in order to characterize the type of receptor involved. We have then compared the actions of purinoceptor agonists with those of clonidine which inhibits neuroeffector transmission in this tissue by activation of prejunctional α_2-adrenoceptors (Marshall *et al.*, 1978). Finally we have examined the actions of drugs which should prevent any endogenous activation of the prejunctional purinoceptors, to test the hypothesis that these receptors are involved in the physiological control of neuroeffector transmission in this tissue.

Methods

General

Male C57BL6 mice aged 8–14 weeks were killed by cervical dislocation, and the vasa deferentia rapidly dissected, and set up as described below.

All experiments were carried out in Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, $CaCl_2$ 2.1, $MgCl_2$ 1.1, glucose 11, equilibrated with 95% oxygen, 5% carbon dioxide at a temperature of 37°C.

Mechanical recording

Vasa deferentia were suspended in a 50 ml organ bath under a resting tension of 200 mg, between silver/silver chloride stimulating electrodes which were parallel to the tissue. Mechanical responses elicited by supramaximal stimulation (5 pulses, 0.5 ms, 5 Hz every 60 s) were measured isometrically. Drugs were added directly to the bath in volumes of less than 0.5 ml, and were left in contact for 3 min (agonists) or 20 min (antagonists), by which time near maximal effects were observed.

Electrical recording (e.j.ps)

Vasa deferentia were pinned out at approximately resting length in a 2 ml bath, with the prostatic end through a pair of platinum ring electrodes (separation 0.5 mm). The bath was perfused with Krebs solution at 5 ml min^{-1}.

In some experiments, in an attempt to obtain more stable recordings, the vasa deferentia were split longitudinally and the mucosal lining removed. The tissue was then pinned out luminal side down, and the intramural nerves stimulated by means of a pair of bipolar platinum electrodes. There were no obvious differences in results obtained using either approach.

Intracellular recordings were made using glass microelectrodes filled with 3 M KCl (resistance 20–40 MΩ). Cell penetrations were accepted if they satisfied previously defined criteria (Blakeley & Cunnane, 1979). Potentials were amplified using a Dagan 8100 single electrode voltage clamp amplifier in bridge mode, and recorded on a Racal Store 4DS tape recorder (d.c.–3 KHz). E.j.p amplitudes were subsequently measured using a Research Machines 380Z microcomputer (Blakeley *et al.*, 1986).

In most experiments, trains of 10 e.j.ps were recorded from 6 to 10 cells in normal Krebs solution, and then in a further 6 to 10 cells in the presence of drug. A stimulus of constant intensity was used throughout the experiment, and the amplitudes of the fully facilitated e.j.ps in the presence of drug were normalized with respect to the mean fully facilitated control e.j.p. amplitude. In addition, some single cell studies were carried out. In these experiments, on obtaining a stable penetration, the stimulus intensity was adjusted to give an unfacilitated e.j.p. amplitude of approximately 10 mV, which was unaffected by small changes in stimulus intensity. Three trains of 5 or 6 e.j.ps at 2 Hz repeated every 20 s were then recorded before switching the perfusing solution to one containing drug at the required concentration. After 3 min (a time found to give near maximal drug effects) 3 further trains of e.j.ps were recorded. If the impalement remained stable the experiment was repeated with up to three cumulative increases in drug concentration.

[^3H]-noradrenaline overflow

Vasa deferentia were incubated in 1 ml of Krebs solution containing approximately 1 μM [^{3}H]-noradrenaline (44Ci mM^{-1}) at 37°C for 30 min, then mounted in a piece of 1.5 mm diameter tubing containing ring stimulating electrodes, and perfused with Krebs solution (0.8 ml min^{-1}). To maintain as near physiological conditions as possible uptake blockers and antioxidants were not used. After a 65 min washout period, the tissue was given five periods of electrical stimulation (50 pulses, 0.5 ms, 5 Hz of supramaximal voltage for the mechanical response) at 15 min intervals. One minute samples of perfusate

were collected beginning one minute before, and then for 3 min after each period of stimulation, and mid way between periods of stimulation. Radioactivity was determined by liquid scintillation spectrometry. Excess overflow was calculated using a computer programme which constructed a washout curve from the inter-stimulus values, and subtracted this background from the counts in the post-stimulus samples. Drugs were present during the 3rd and 4th periods of stimulation, and drug effects were expressed as percentage change over the mean of pre- and post-drug controls.

Analysis of results

Concentration-response data were analysed by use of an iterative curve fitting routine ('Allfit', De Lean *et al.*, 1978), with a minimum of four observations at each concentration. 'Allfit' was used to test for differences in the parameters between concentration-response curves, by inspecting the effect on the residual variance, of forcing the parameters to be equal (De Lean *et al.*, 1978). Other data were assessed by unpaired t test. P values less than 0.05 were considered significant.

Drugs used

Clonidine HCl, N^6-([S]-2-phenylisopropyl) adenosine (D-PIA; Boehringer Manheim); 2-chloroadenosine (2ClA), N^6-([R]-2-phenylisopropyl)-adenosine (L-PIA), 5'-N-ethylcarboxamidoadenosine (NECA), N^6-cyclohexyladenosine (CHA), dipyridamole, 8-phenyltheophylline (8-PT), yohimbine HCl, tetrodotoxin, α,β-methyleneadenosine 5'-triphosphate (lithium salt) (Sigma); prazosin (Pfizer); (−)-[7,8-^{3}H]-noradrenaline (Amersham U.K.).

Stock solutions were made up in distilled water, except phenylisopropyladenosine, dipyridamole and prazosin which were dissolved in ethanol, and 8-phenyltheophylline which was dissolved in 10 ml of ethanol made alkaline with 1 drop of 0.1 M NaOH. The final concentration of ethanol in the bath (< 1%) had no observable effect on the responses.

Results

Mechanical responses

Stimulation of the vas deferens with 5 supramaximal pulses of 0.5 ms at 5 Hz evoked a 'twitch' response (Figure 1a). These responses were abolished by 1 μM tetrodotoxin, unaffected by prazosin (0.5 μM) but were abolished by α,β-methylene ATP (1 μM), indicating that the responses were neurogenic, and medi-

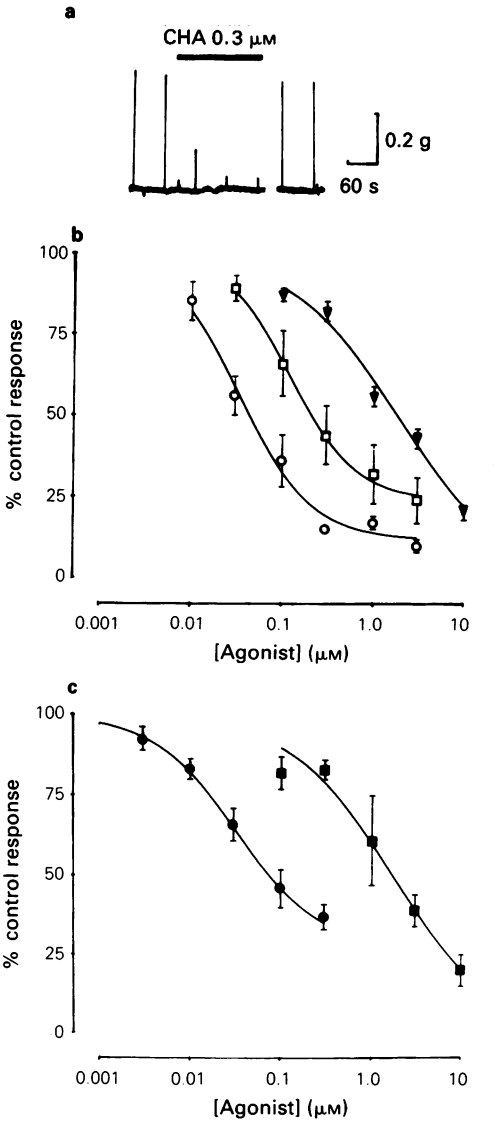

Figure 1 Inhibition of the mechanical response of the mouse vas deferens by adenosine analogues. (a) Responses evoked by supramaximal stimulation (5 pulses 0.5 ms, 5 Hz, every 60 s) before, in the presence and 15 min after the washout of 0.3 μM cyclohexyladenosine (CHA). (b) Log concentration-response curves for inhibition of the mechanical response by CHA (○), N-ethylcarboxamidoadenosine (NECA, □) and 2-chloroadenosine (2ClA, ▼). (c) Log concentration-response curves for inhibition of the mechanical response by ([R]-2-phenylisopropyl)-adenosine (L-PIA, ●) and ([S]-2-phenylisopropyl)-adenosine (D-PIA, ■). In (b) and (c), each point represents the mean of 4 to 12 experiments; vertical lines indicate s.e. mean. The computer generated sigmoid curves were derived by 'Allfit'.

Table 1 EC_{50} values derived by 'Allfit' for inhibition of the mechanical response and excitatory junction potential (e.j.p.) of the mouse vas deferens by purinoceptor and adrenoceptor agonists

Drug	EC_{50} (M) Mechanical	E.j.p.
Clonidine	$2.0 \pm 0.2 \times 10^{-9}$	$3.9 \pm 0.3 \times 10^{-9}$
L-PIA	$3.3 \pm 1.0 \times 10^{-8}$	$3.8 \pm 2.7 \times 10^{-7}$*
CHA	$3.4 \pm 0.6 \times 10^{-8}$	$3.2 \pm 2.0 \times 10^{-9}$
NECA	$1.2 \pm 0.4 \times 10^{-7}$	$2.0 \pm 1.0 \times 10^{-7}$
2ClA	$1.7 \pm 0.2 \times 10^{-6}$	$2.1 \pm 1.7 \times 10^{-6}$
D-PIA	$1.7 \pm 0.3 \times 10^{-6}$	

Values are mean ± s.e. mean, with at least 4 observations (mechanical) or 16 observations (e.j.p.) at each concentration.
* Fitted excluding data for 10 μM L-PIA. For abbreviations used see text.

ated by a non-adrenergic neurotransmitter, possibly ATP (Meldrum & Burnstock 1983; Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984).

The adenosine analogues tested all produced a rapid, reversible, concentration-dependent inhibition of the mechanical response (Figure 1). The order of potency for these agonists was L-PIA ≃ CHA > NECA > 2ClA ≃ D-PIA with L-PIA being approximately 50 times more potent than the D-isomer (Figure 1c). The EC_{50} values estimated by 'Allfit' are given in Table 1.

For comparison, we also examined the effects of the α_2-adrenoceptor agonist clonidine on the mechanical response. The effects of clonidine were similar to those produced by the adenosine analogues, but clonidine was considerably more potent, and produced a slightly steeper log concentration-response curve (Figure 6).

Excitatory junction potentials

In agreement with Sneddon *et al.* (1984), the purinoceptor agonists produced a reversible decrease in e.j.p. amplitude (Figure 2a). However, the degree of inhibition varied greatly from cell to cell. The variability of the effect of 2ClA on e.j.p. amplitude is shown in Figure 2c. While in some cells, 30% inhibition was observed at 0.1 μM, in other cells only 25–30% inhibition was observed at concentrations of 10 to 30 μM. Any e.j.ps remaining in the presence of 30 μM 2ClA were readily abolished by 30 nM clonidine. For comparison, we also examined the effect of the α_2-adrenoceptor agonist clonidine on e.j.p. amplitude. Clonidine produced inhibition of the e.j.p. with a clear concentration-dependence, and an estimated maximum inhibition of 95% (Figure 2b).

Log concentration-response curves for inhibition of the e.j.p. by different P_1-purinoceptor agonists are

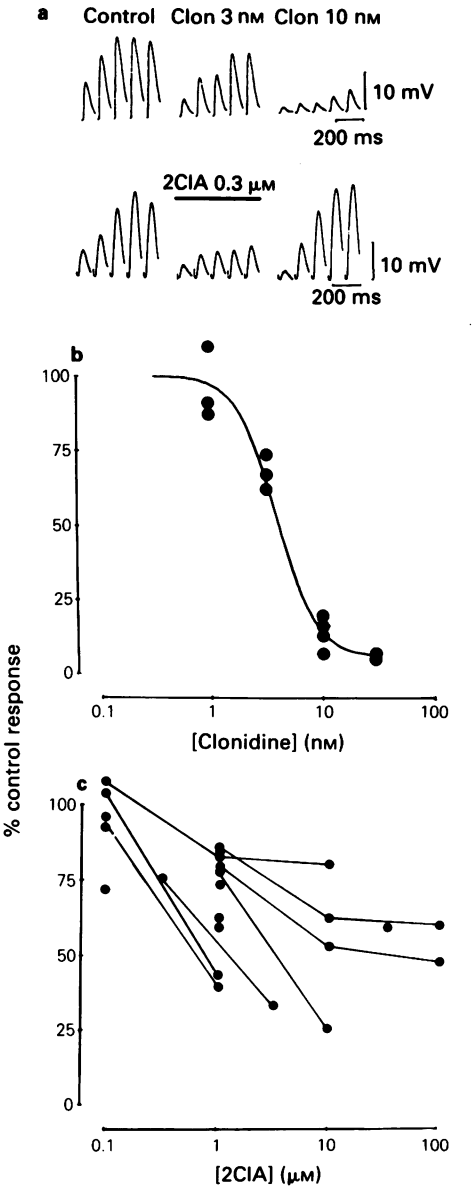

Figure 2 Inhibition of the excitatory junction potential (e.j.p.) by clonidine (Clon) and 2-chloroadenosine (2ClA). (a) Traces showing trains of 5 e.j.ps evoked by 2 Hz stimulation, recorded from the same cell, in the presence of increasing concentrations of clonidine, and before, during and after superfusion with 0.3 μM 2ClA. (b) Concentration-response curve for inhibition of the e.j.p. by clonidine, each point represents a single observation, and the line is a sigmoid curve derived by 'Allfit'. (c) Concentration-response data for inhibition of the e.j.p. by 2ClA. Each point represents a single observation, and the lines indicate where 2 or more concentrations were examined on a single cell.

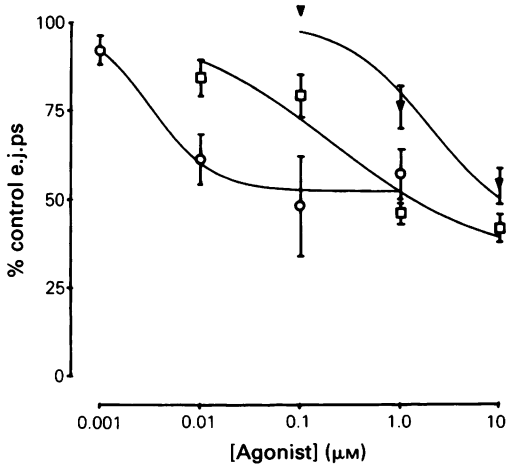

Figure 3 Log concentration-response curves for inhibition of the excitatory junction potential (e.j.p.) by cyclohexyladenosine (○), N-ethylcarboxamidoadenosine (□) and 2-chloroadenosine (▼). Each point represents the mean normalized e.j.p. amplitude (see text) from 16 to 28 cells; vertical lines indicate s.e. mean. The computer generated sigmoid curves were derived by 'Allfit'.

shown in Figures 3 and 4, and the EC_{50} values determined by 'Allfit' are given in Table 1. In contrast to clonidine, the P_1-purinoceptor agonists produced a maximum inhibition of only 50 to 70%. Furthermore, with L-PIA, the inhibition observed at 10 μM was significantly less than that observed at 1 μM giving rise to a 'bell-shaped' concentration-response curve (Figure 4b). In addition, in single cell experiments with L-PIA at concentrations greater than 1 μM, in 2 of 10 cells, e.j.ps with a shorter latency than the shortest that occurred in the absence of drug were observed once or twice in each train of 6 stimuli (Figure 4a).

In the presence of the highest concentrations of 2ClA, NECA, L-PIA and CHA studied, the mean resting potentials were 73.8 ± 0.8 (n = 37), 72.7 ± 1.1 (n = 19), 72.3 ± 1.5 (n = 19) and 71.6 ± 1.1 (n = 16) mV, respectively. These values were not significantly different ($P > 0.05$) from the mean control value of 74.8 ± 0.6 mV (n = 137).

[^3H]-noradrenaline overflow

The evoked release of tritium from the vas deferens after loading with [^{3}H]-noradrenaline reflects the release of noradrenaline (Marshall, 1983). 2ClA produced a reversible concentration-dependent inhibition of the evoked ^{3}H overflow, with an EC_{50} value of 1.2 ± 0.3 μM (Figure 5), a value not significantly different ($P > 0.05$) from the EC_{50} values of

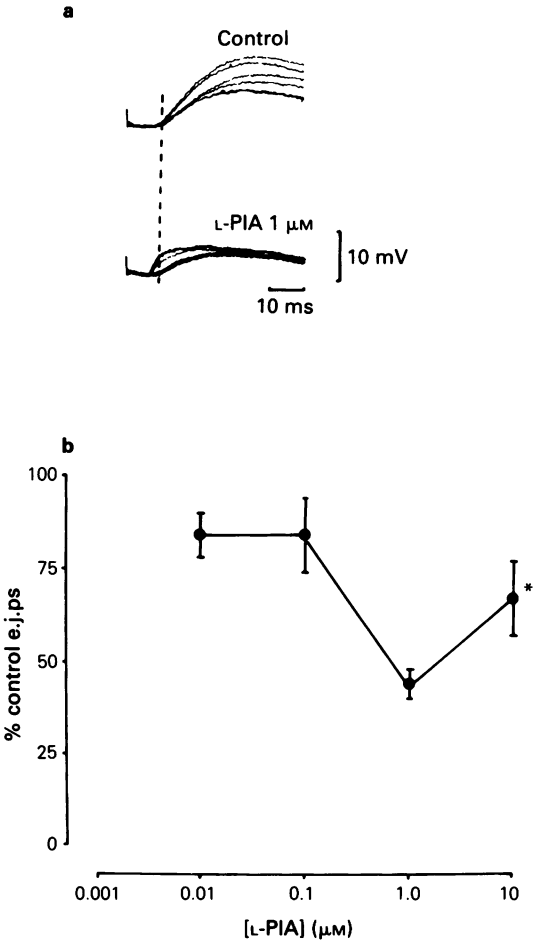

Figure 4 Inhibition of the excitatory junction potential (e.j.p.) by ([R]-2-phenylisopropylyladenosine (L-PIA). (a) Trains of 6 superimposed e.j.ps, evoked by 2 Hz stimulation, recorded from the same cell, before and during superfusion with 1 μM L-PIA. The broken line indicates a fixed latency from the stimulus artifact. (b) Log concentration-response curve for inhibition of the e.j.p. by L-PIA. Each point represents the mean normalized e.j.p. amplitude (see text) from at least 19 cells; vertical lines indicate s.e. mean*. Significantly different from the response at 1 μM $P < 0.05$ by Student's t test.

1.7 ± 0.2 μM and 2.1 ± 1.7 μM for inhibition of the mechanical response and the e.j.p., respectively.

Effects of antagonists

In agreement with previous work (Marshall *et al.*, 1978) yohimbine 0.1 μM antagonized the inhibition of the mechanical response by clonidine, producing a parallel shift in the clonidine log concentration-

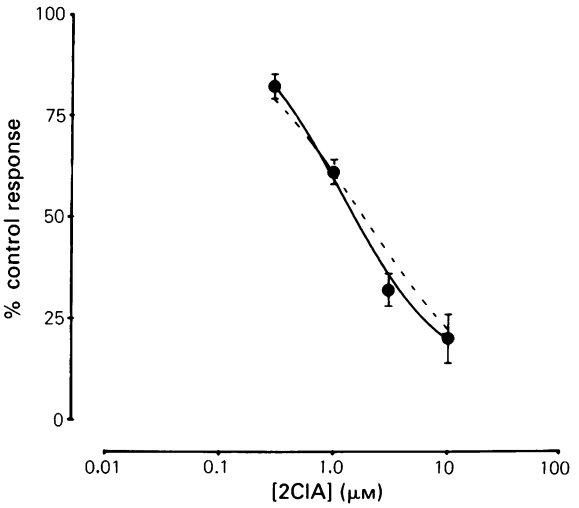

Figure 5 Log concentration-response curve for the inhibition of [^{3}H]-noradrenaline overflow from the mouse vas deferens by 2-chloroadenosine (2ClA). Each point represents the mean from 4 to 6 experiments; vertical lines indicate s.e. mean. The broken line shows for comparison the log concentration-response curve for inhibition of the mechanical response by 2ClA. The slopes and EC_{50} values for the two curves were not significantly different ($P > 0.05$).

response curve, with a dose-ratio of 7.5 (Figure 6). In addition, yohimbine 0.01–0.1 μM produced a significant increase in the mechanical response (Figure 7).

The inhibitory action of 2ClA on the mechanical response was antagonized by 8-PT (5 μM), producing a parallel shift in the log concentration-response curve, with a dose-ratio of 7.2 (Figure 6). However, 8-PT at concentrations up to 10 μM failed to alter the mechanical response of the vas deferens, even when 0.5 μM dipyridamole was present to block the uptake of any endogenous adenosine and increase its concentration at the receptor.

An alternative way of reducing the action of endogeneous adenosine is to increase its rate of degradation by the addition of adenosine deaminase. At a concentration of approximately 1 u ml^{-1}, this enzyme enhances the overflow of noradrenaline from hippocampal slices (Jackish *et al.*, 1985). However, 15 min incubation with adenosine deaminase (0.01 to 3 u ml^{-1}) produced no significant change in the mechanical response of the vas deferens (Figure 7).

While yohimbine (0.1 μM) produced a 42.6% increase in the e.j.p. amplitude, and at 1 μM increased [^{3}H]-noradrenaline overflow approximately four fold, these responses were not significantly altered by 8-PT (5 μM) (Figure 7).

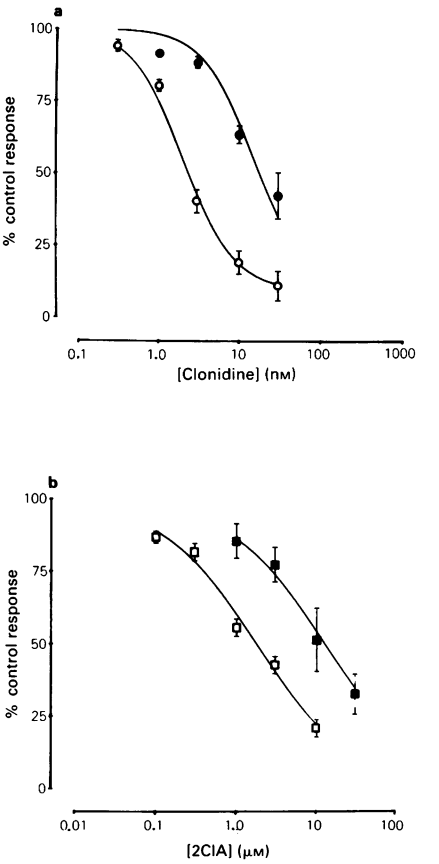

Figure 6 Effect of purinoceptor and adrenoceptor antagonists. (a) Log concentration-response curves for inhibition of the mechanical response by clonidine in the absence (○) and presence (●) of 0.1 μM yohimbine. (b) Log concentration-response curves for inhibition of the mechanical response by 2-chloroadenosine (2ClA) in the absence (□) and presence (■) of 5 μM 8-phenyltheophylline. Each point represents the mean from 4 experiments; vertical lines indicate s.e. mean. The 'Allfit' generated curves were constrained to have the same slope in the absence and presence of antagonist.

Discussion

Adenosine and its analogues have been shown to inhibit neuroeffector transmission in the vas deferens of several mammalian species by activation of prejunctional P_1-purinoceptors (Clanachan *et al.*, 1977; Paton, 1981; Sneddon *et al.*, 1984). In this study, we have attempted to characterize the purinoceptors present in the mouse vas deferens, and to look for evidence of activation of these receptors by endogenous neuromodulators.

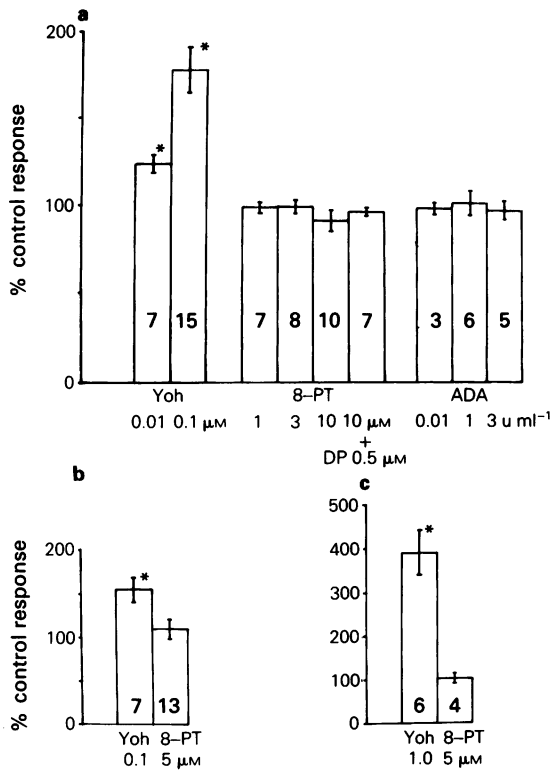

Figure 7 The effects of purinoceptor and adrenoceptor antagonists on responses of the mouse vas deferens. (a) Shows the effects of yohimbine (Yoh), adenosine deaminase (ADA), 8-phenyltheophylline (8-PT), and 8-PT in the presence of dipyridamole (DP) on the mechanical response. The effects of yohimbine and 8-phenyltheophylline on excitatory junction potential amplitude and [^{3}H]-noradrenaline overflow are shown in (b) and (c), respectively. Vertical lines indicate s.e. mean, and the number within each column indicates the number of experiments. *Significantly different from control, $P < 0.05$ by Student's t text.

P_1-purinoceptors have been divided into A_1- and A_2-subtypes based on the rank order of potency of different adenosine analogues (Van Calker *et al.*, 1979; Londos *et al.*, 1980), and on the relative potencies of the stereoisomers of PIA (Bruns *et al.*, 1980). These criteria work well for adenosine receptors coupled to adenylate cyclase, where A_1-purinoceptors with an agonist profile of L-PIA $\simeq$ CHA > 2ClA > D-PIA $\simeq$ NECA, and high stereoselectivity for the PIA isomers, inhibit the enzyme. A_2-purinoceptors with low stereoselectivity and an agonists profile of NECA > 2ClA > L-PIA $\simeq$ CHA > D-PIA stimulate adenylate cyclase. When other responses are studied, there is considerable variation in the agonist profiles observed. This has led to the proposal of a third (A_3) subtype of purinoceptor which is not coupled to adenylate cyclase but is involved in the regulation of calcium-dependent processes (Ribeiro & Sebastiao, 1985; 1986). The rank order of potency proposed for this A_3-purinoceptor is less strict, requiring only that L-PIA, NECA and CHA are more potent than 2ClA, thus accommodating receptors which have an agonist profile not compatible with either the A_1- or A_2-subtypes. However, there are a number of shortcomings of characterizing receptors on the rank order of agonist potency (see Burnstock & Buckley, 1985).

At concentrations similar to those required for inhibiting the mechanical response in the rat vas deferens (Paton, 1981), and for inhibiting calcium currents in sympathetic and sensory neurones (Henon & McAfee, 1983; MacDonald *et al.*, 1986), adenosine analogues inhibited the mechanical response and e.j.p. of the mouse vas deferens. The rank order of agonist potency observed for inhibition of the mechanical response was L-PIA $\simeq$ CHA > NECA > 2ClA $\simeq$ D-PIA. This order of potency resembles more closely that for A_1- than A_2-purinoceptors a view supported by the finding that L-PIA was approximately 50 times more potent than the D-isomer. Alternatively, on the basis of the agonist profile, and since P_1-purinoceptors may inhibit neuroeffector transmission by inhibiting calcium currents in the nerve terminal, these receptors could be classified as being of the A_3-subtype.

The differences in the slopes of the log concentration-response curves and in the maximum inhibition of the mechanical response produced by the different P_1-purinoceptor agonists were not statistically significant. However, since there is evidence for two populations of purinoceptors on enteric nerve terminals (Cook & Christofi, 1986), and since purinoceptor agonists can facilitate neuroeffector transmission in rabbit bronchi (Gustafsson *et al.*, 1986), non-parallel concentration-response curves with different maxima are not inconceivable.

When we examined the action of clonidine on the e.j.p., we found the effect to be consistent from cell to cell, with high concentrations of clonidine (30–100 nM) almost totally abolishing the e.j.p. In contrast, the P_1-purinoceptor agonist 2ClA produced a very variable inhibition, and induced a maximum effect of only 20–60% inhibition. Variation in the inhibitory effects of some opiates and neuropeptide-Y in the vas deferens has also been found (Ramme & Illes, 1986; Stjärne *et al.*, 1986). Since clonidine was able to produce consistent effects on the e.j.p., it seems unlikely that the variable effect of 2ClA resulted from inconsistent tissue penetration of the drug. The variability in effect of these three types of inhibitory agonists may indicate the presence of a

heterogeneous population of sympathetic neurones, or a variable distribution of receptors along the terminal region of the sympathetic axons. It would be interesting to see whether there is any correlation between the sensitivities of release sites to these three classes of agonist, or whether different nerves or release sites are more effectively modulated by different receptor systems.

Although there was good agreement between the EC_{50} values for inhibition of the mechanical response and the e.j.p. by clonidine, 2ClA and NECA, there were marked discrepancies between the EC_{50} values for inhibition of the two responses by L-PIA and CHA. The reason for this is at present unclear, but may be due to the presence of either pre- or post-junctional facilitatory purinoceptors with a different agonist profile. P_1-purinoceptor agonists have been shown to enhance neuroeffector transmission in rabbit bronchi (Gustafsson *et al.*, 1986) and to facilitate the postjunctional response to neutrotransmitter in the vas deferens (Sneddon *et al.*, 1984; Long & Stone, 1986).

Although it is unclear whether the appearance of shorter latency e.j.ps in the presence of high concentrations of L-PIA results from the stimulation of prejunctional facilitatory P_1-purinoceptors, or from some form of disinhibition, the 'bell-shaped' log concentration-response curve for inhibition of the e.j.p. by this agonist could be explained by the presence of a population of facilitatory purinoceptors with a lower affinity for L-PIA than that of the inhibitory receptors.

Since there is evidence that noradrenaline and ATP may act as co-transmitters in the vas deferens (Sneddon & Westfall, 1984; Stjärne & Åstrand, 1984), in this study, we have compared the effects of purinoceptor agonists and antagonists on three measures of neuroeffector transmission, the mechanical response, the junction potential, and [^{3}H]-noradrenaline overflow. The first two responses are thought to give a measure of ATP release, while the third gives a measure of noradrenaline release. The EC_{50} values for inhibition of these three responses by 2ClA were very similar, suggesting that if there are two transmitters involved, their release is modulated in the same way by prejunctional P_1- purinoceptors. However, the maximum inhibition of [^{3}H]-noradrenaline overflow by 2ClA was greater than the maximum inhibition of the e.j.p. by the P_1-purinoceptor agonists. This might also result from the ability of purinoceptor agonists to facilitate the postjunctional response to neurotransmitters (Sneddon *et al.*, 1984; Long & Stone, 1986).

It has been suggested that the P_1-purinoceptors present on sympathetic neurones might be involved in feedback inhibition of transmitter release (Enero & Saidman, 1977; Su, 1978). 8-PT effectively antagonized the actions of P_1-purinoceptor agonists with a potency similar to that observed in guinea-pig atrium and guinea-pig ileum (Griffiths *et al.*, 1981). However, even in the presence of dipyridamole to block the uptake of any endogenous adenosine, 8-PT failed to facilitate neuroeffector transmission. Similarly, incubation with adenosine deaminase even at concentrations greater than those which enhance noradrenaline release in the hippocampus (Jackish *et al.*, 1985) and modify glucose metabolism in adipocytes and skeletal muscle (Gliemann *et al.*, 1985; Espinal *et al.*, 1983) failed to facilitate neuromuscular transmission, suggesting the absence of any purinergic feedback inhibition. In contrast, a concentration of yohimbine which produced comparable antagonism of the prejunctional α_2-adrenoceptors facilitated the mechanical response, e.j.p. amplitude and [^{3}H]-noradrenaline overflow.

These findings are in agreement with the observations that P_1-purinoceptor antagonists did not enhance the mechanical response of the rat and guinea-pig vas deferens (Clanachan *et al.*, 1977; Sneddon *et al.*, 1984), and that following reserpine treatment, which abolishes adrenergic neuroeffector transmission while leaving purinergic transmission unaffected, autofeedback in the vas deferens cannot be demonstrated (French & Scott, 1983).

In conclusion, our results indicate that the P_1-purinoceptors present on sympathetic neurones of the mouse vas deferens, though similar to A_1-purinoceptors, may be better classified as being of the A_3-subtype. The same receptors affect the release of both noradrenaline and the non-adrenergic transmitter and, although they can be activated by exogeneous agonists, our results indicate that these receptors are not normally activated by endogeneous neuromodulators.

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