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A_1 adenosine receptor modulation of electrically-evoked contractions in the bisected vas deferens and cauda epididymis of the guinea-pig

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1 The effects of adenosine receptor agonists upon both electrically-evoked and phenylephrine-induced contractile responses were investigated in the bisected vas deferens and the cauda epididymis of the guinea-pig. Electrical field-stimulation (10 s trains of pulses at 9 Hz, 0.1 ms duration, supramaximal voltage) elicited biphasic and monophasic contractile responses from preparations of bisected vas deferens and cauda epididymis, respectively; these responses were abolished by tetrodotoxin (300 nM).

2 In the prostatic half of the vas deferens the A_1 selective adenosine receptor agonists, N^6-cyclopentyladenosine (CPA) and (2**S**)-N^6-[2-endo-norbornyl]adenosine ((**S**)-ENBA) and the non-selective A_1/A_2 adenosine receptor agonist, 5′-N-ethylcarboxamidoadenosine (NECA) inhibited electrically-evoked contractions ($pIC_{50} \pm$s.e.mean values 6.15 ± 0.24, 5.99 ± 0.26 and 5.51 ± 0.24, respectively). The responses to CPA were blocked by the A_1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine, DPCPX (100 nM).

3 In the epididymal half of the vas deferens NECA potentiated (at $\leqslant 100$ nM) and inhibited (at $\geqslant 1$ μM) electrically-evoked contractions. In the presence of the non-selective α-adrenoceptor antagonist phentolamine (3 μM), the α_1-adrenoceptor antagonist, prazosin (100 nM), or at a reduced train length (3 s) NECA inhibited electrically-evoked contractions (pIC_{50} values 6.05 ± 0.25, 5.97 ± 0.29 and 5.71 ± 0.27, respectively). CPA (at 10 μM) also inhibited electrically-evoked contractions in this half of the vas deferens. In the presence of prazosin (100 nM), CPA also inhibited electrically-evoked contractions (pIC_{50} 6.14 ± 0.67); this effect was antagonized by DPCPX (30 nM, apparent pK_B 8.26 ± 0.88). In the presence of the P2 purinoceptor antagonist, suramin (300 μM), CPA (up to 1 μM) potentiated electrically-evoked contractions.

4 NECA, CPA and APNEA potentiated electrically-evoked contractions in preparations of cauda epididymis (pEC_{50} values 7.49 ± 0.62, 7.65 ± 0.74 and 5.84 ± 0.86, respectively), the response to CPA was competitively antagonized by DPCPX (100 nM) with an apparent pK_B value of 7.64 ± 0.64.

5 The α_1-adrenoceptor agonist phenylephrine elicited concentration-dependent contractile responses from preparations of bisected vas deferens and cauda epididymis. NECA (1 μM) potentiated responses to phenylephrine ($\leqslant 1$ μM) in the epididymal, but not in the prostatic half of the vas deferens. In preparations of epididymis NECA (1 μM) shifted phenylephrine concentration response curves to the left (4.6 fold). In the presence of a fixed concentration of phenylephrine (1 μM), NECA elicited concentration-dependent contractions of preparations of the epididymal half of the vas deferens and of the epididymis (pEC_{50} values 7.57 ± 0.54 and 8.08 ± 0.18, respectively). NECA did not potentiate responses to ATP in either the epididymal half of the vas deferens or the epididymis.

6 These studies are consistent with the action of stable adenosine analogues at prejunctional A_1 and postjunctional A_1-like adenosine receptors. The prejunctional A_1 adenosine receptors only inhibit the electrically-evoked contractions of purinergic origin (an effect predominant in the prostatic half of the vas deferens). At the epididymis, where electrically-evoked contractions are entirely adrenergic, the predominant adenosine receptor agonist effect is a potentiation of α_1-adrenoceptor-, but not of ATP-induced contractility.

Keywords: Guinea-pig vas deferens; guinea-pig epididymis; A_1 adenosine receptor

Introduction

In the vas deferens of both the rat and guinea-pig, trains of electrical field-stimulation induce biphasic contractile responses, consisting of both a fast twitch and a sustained component (Saxena, 1970; Swedin, 1971). The fast twitch and the slow components of this response are (largely) mediated through the actions of ATP and noradrenaline, respectively (McGrath, 1978; Burnstock & Kennedy, 1985; Mallard *et al*., 1992). A_1 adenosine receptors have been shown to inhibit both the electrically-evoked contractions in the rat vas deferens (Lee & Cheung, 1985; Major *et al*., 1989; Hourani *et al*., 1993), and also neurotransmitter (predominantly ATP) release from the guinea-pig vas deferens (Driessen *et al*., 1994). In addition to prejunctional A_1 adenosine receptors, there is also evidence of postjunctional A_1 adenosine receptors which potentiate contractions of the rat deferens (Brownhill *et al*., 1996b).

The cauda epididymis is anatomically contiguous with the vas deferens, and like the vas deferens receives innervation from the hypogastric nerve (Mitchell, 1935). However, there are no comparable studies of the A_1 adenosine receptor modulation of electrically-evoked contractions in this tissue. In this study we determined whether A_1 adenosine receptors

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modulate electrically-evoked contractions in the prostatic and epididymal halves of the vas deferens and also the epididymis of the guinea-pig

Methods

Animals

Male Dunkin-Hartley guinea-pigs (650–1000 g) were housed in open runs (20°C) with a 12 h light dark cycle. Food consisted of BeKay pellets with green vegetables and water *ad libitum*. On the day of the use animals were killed and the vasa deferentia and testis removed.

Tissue preparation

Whole vasa deferentia were stripped of their serous coating and bisected, the cauda epididymis was unravelled (with the aid of a N.U.M.S. HM 1 binocular dissecting microscope) and placed into modified Krebs solution (composition, mM; NaCl 118, KCl 4.7, $MgSO_4$ 0.45, K_2HPO_4 2.5, $NaHCO_3$ 25, $CaCl_2$ 1.9 and glucose 11). In sequence, from the vas deferens to the testicle four sections of epididymis, each approximately one cm long, were cut. For contractility studies preparations of vasa deferentia or of cauda epididymis were attached, with a silk thread (1/0), to a tissue holder, containing platinum electrodes (approximately 6 mm apart). Preparations were equilibrated in modified Krebs solution (gassed with carbogen, O_2: CO_2, 95:5), at 36°C, in 8 ml isolated organ chambers. The upper end of each preparation was connected, via another silk thread, to a Grass FTO3 force-displacement transducer. Preparations of vas deferens were suspended under 1 g and preparations of epididymis under 0.30 g resting force. Recordings of contractile force were made using Grass FTO3 isometric transducers coupled to a Grass (model 79D) chart recorder. To minimize possible heterogeneity of the responses along the length of epididymis sections of tissue were randomly assigned within each experimental paradigm.

Responses to electrical stimulation

Preparations were set up and allowed to equilibrate (50–60 min) before electrical field stimulation (9 Hz, 0.1 ms, supramaximal voltage) for either 3 or 10 s every 17 min (Haynes *et al*., 1997). Following the first electrical stimulation antagonists were added. Once responses had stabilized agonists or vehicle were added 2 min before subsequent stimuli. Following electrical field stimulation preparations were washed with 2–4 times bath volume. Results are calculated as a percentage of the first electrically-evoked contraction before the addition of agonist. In some cases the A_1 adenosine receptor selective antagonist DPCPX (100 nM; Alexander *et al*., 1994; Alexander, 1995), the α_1-adrenoceptor selective antagonist, prazosin (100 nM), the non-selective α-adrenoceptor antagonist, phentolamine (3 μM), or the P2 purinoceptor antagonist, suramin (300 μM) were added to preparations during the equilibration period and remained throughout the experiment. In the presence of these antagonists responses to electrically-evoked contractions remained consistent for up to 3 h ($n=4$, data not shown).

At the end of some experiments tetrodotoxin (300 nM) or phentolamine (3 μM) were added to preparations before electrical stimulation (9 Hz, 0.1 ms, supramaximal voltage; 10 s).

Effect of NECA upon phenylephrine concentration-response curves

Preparations of bisected vasa deferentia and epididymis were obtained as described above, suspended under 1 or 0.30 g resting force (respectively) in modified Krebs solution and allowed at least 40 min to equilibrate. Before the addition of agonists, one concentration of KCl (60 mM) was added to each preparation to ensure tissue viability. Following the washout (3–4x bath volume) of KCl preparations were allowed a further 15 min equilibration time. NECA (1 μM) or vehicle were added 90 s before the addition of phenylephrine, preparations were left for a further 90 s or until a maximal response was obtained; preparations were then washed out and approximately 15 min later the next concentration of phenylephrine was added.

Effect of NECA upon threshold concentrations of phenylephrine or ATP

NECA or CPA (1 nM–100 μM) were added to preparations of cauda epididymis or the epididymal half of the vas deferens two minutes before the addition of either ATP (1 μM) or phenylephrine (1 μM), preparations were allowed to incubate for a further two minutes, or until a maximal response was reached, before agonist washout (3–4x bath volume). Agonist addition was repeated at 15 min intervals. ATP (at 1 μM) elicited submaximal contractions (21 ± 4 mg force) from preparations of epididymis (at 10 μM these contractions were 68 ± 10 mg force).

Statistics

Estimates of $-\log$ molar (p)IC_{50} ($\pm$s.e.), slope and maximum response were generated using a four-parameter logistic curve fitting and graphics programme PRISM v2.0 (GraphPad Software Inc., San Diego). Comparisons between concentration-response curves were determined by the use of an iterative curve fitting programme, FLEXIFIT (see Guardabasso *et al*., 1988), significant changes were determined with a *F* test. One-way ANOVA and Student's *t* test were used to determine changes within data sets. In all cases $P<0.05$ was taken as the level of significance. Apparent pK_B values were determined from the Gaddum equation:

$$pK_B = \log[CR - 1] - \log [\text{antagonist concentration}]$$

Where CR is the concentration ratio of mean response to agonist in the presence of antagonist over the mean equi-effective response to agonist in the absence of antagonist.

Drugs

Adenosine deaminase (Boehringer Mannheim, GmBH). N^6-2-(4-aminophenyl) ethyladenosine (APNEA, synthesized by Dr E.A. Boyd, Department of Pharmaceutical Sciences, University of Nottingham); 2-*p*-(2-carboxyethyl) phenethylamino-5′-N-ethylcarboxamidoadenosine (CGS 21680), (2S)-N^6-[2-endo-norbornyl]adenosine ((S)ENBA), N^6-iodobenzyl 5′-N-methylcarboxamido adenosine (IBMECA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Research Biochemicals Inc., U.S.A.); Adenosine 5′ triphosphate (ATP), 5′-N-ethylcarboxamidoadenosine (NECA), N^6-cyclopentyladenosine (CPA), phenylephrine HCl and tetrodotoxin (were from Sigma Chemical Co., U.K.); phentolamine mesylate (Rogitine, Ciba).

Adenosine receptor agonists and antagonists were made up as a stock solution in DMSO placed in aliquots and frozen.

Phenylephrine, phentolamine and idazoxan were dissolved in distilled water. Tetrodotoxin was stored as a stock solution in acidified ethanol. On the day of use all drugs were made up to volume in buffer. In no cases did the ethanol or DMSO vehicle exceed 0.1 or 0.5% (respectively) of the tissue bath volume.

Results

Electrically-evoked contractions

Preparations of vas deferens responded to electrical field-stimulation (9 Hz for 10 s, 0.1 ms duration, supramaximal voltage) with biphasic contractions. The first phase of the response to electrical field-stimulation was a fast 'twitch' response followed by a slower sustained response (see Figure 1 for typical responses). Both phases of the contractile response were abolished by incubation of preparations with tetrodotoxin (300 nM, $n=4$, data not shown. DPCPX (up to 10 μM) had no effect upon electrically-evoked contractions in either half of the vas deferens ($n=4$, data not shown).

The incubation of preparations of the epididymal half of the vas deferens with phentolamine (3 μM) significantly ($P<0.05$, Paired t test, d.f. = 5) reduced electrically-evoked contractions (by $16\pm5\%$). The incubation of preparations with suramin (300 μM) also significantly ($P<0.05$, paired t test, d.f. = 4) reduced electrically-evoked contractions (by $74\pm5\%$). Adenosine deaminase (18 u ml^{-1}) did not affect electrically-evoked contractions in either half of the vas deferens or the epididymis ($n=3-4$, data not shown).

Preparations of cauda epididymis responded to field-stimulation (9 Hz for 10 s, 0.1 ms duration, supramaximal voltage) with monophasic contractions (see Figure 1 for a typical response) which were completely abolished by both phentolamine (3 μM, $n=5$) and tetrodotoxin (300 nM, $n=4$), but were unaffected by suramin (100 μM) or by DPCPX (100 nM, $n=4$), data not shown.

The adenosine receptor agonists used in this study did not elicit contractions from preparations of unstimulated bisected vas deferens or epididymis.

Effects of adenosine receptor agonists upon electrically-evoked contractions in the prostatic half of the vas deferens

CPA, (S)-ENBA and NECA, but not CGS 21680 or APNEA inhibited electrically-evoked contractions with pIC_{50} ($\pm$s.e.mean) values of 6.15 ± 0.24, 5.99 ± 0.26 and 5.51 ± 0.24, respectively (Figure 2a and 2b). DPCPX (100 nM) attenuated ($P<0.05$; Student's t test, d.f. = 9) the CPA (10 μM)-induced inhibition of electrically-evoked responses (Figure 2a).

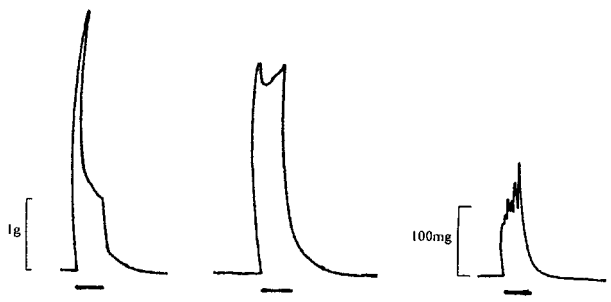

Figure 1 Typical responses to field stimulation in preparations of the bisected vas deferens and epididymis of the guinea-pig. The first, second and third panels show typical responses to field-stimulation (10 s train, 9 Hz, supramaximal voltage, 0.1 ms duration) of preparations of prostatic and epididymal halves of the vas deferens and of epididymis. The solid bar indicates duration of stimulation.

Effects of adenosine receptor agonists upon electrically-evoked contractions in the epididymal half of the vas deferens

NECA elicited a bell-shaped potentiation of electrically-evoked contractions; this response was maximal and significant (Student's t test, $P<0.05$, d.f. = 17) at 100 nM (Figure 3a). DPCPX (100 nM) antagonized the effects of NECA (Figure 3a). In the presence of the α-adrenoceptor antagonist, phentolamine (3 μM) or the α_1-adrenoceptor antagonist, prazosin (100 nM), NECA inhibited electrically-evoked contractions with pIC_{50} values of 5.97 ± 0.29 ($n=6$, Figure 3a) and 6.05 ± 0.25, respectively ($n=6$, data not shown). At a reduced train length (3 s) NECA (up to 100 nM) potentiated electrically-evoked contractions, at higher concentrations, NECA (>100 nM) inhibited these contractions with a pIC_{50} of 5.71 ± 0.27 ($n=5$, Figure 3a).

(S)-ENBA, CGS 21680 and APNEA alone did not significantly affect electrically-evoked contractions in this half of the vas deferens, but CPA did (Figure 3b and c). In the presence of prazosin (100 nM) the inhibition of electrically-evoked contractions by CPA was enhanced (pIC_{50} 6.14 ± 0.67), an effect antagonized by DPCPX (30 nM, $n=6$, apparent pK_B 8.26 ± 0.88, see Figure 3b). In the presence of suramin (300 μM) CPA (up to 1 μM) potentiated ($P<0.05$, Student's t test) electrically-evoked contractions (Figure 3b).

Effects of adenosine receptor agonists upon electrically-evoked contractions in the epididymis

In preparations of epididymis NECA, CPA and APNEA potentiated electrically-evoked contractions ($n=5-6$, pEC_{50}

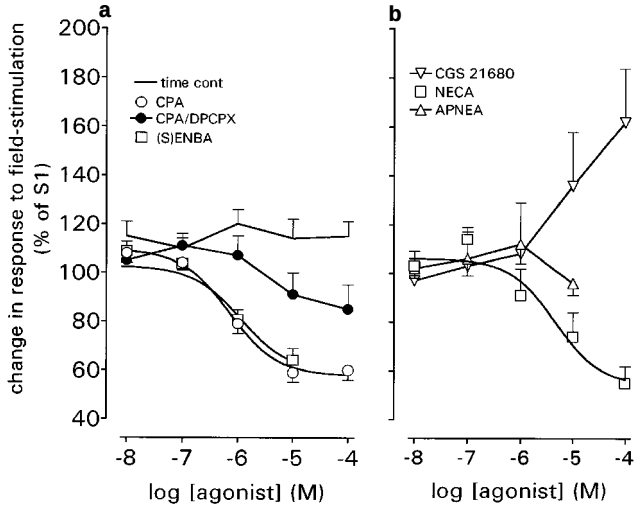

Figure 2 Effects of adenosine receptor agonists upon responses to field stimulation (10 s train, 9 Hz, supramaximal voltage, 0.1 ms duration) in preparations of the prostatic half of the vas deferens. Each symbol represents the mean response to field-stimulation in the presence of agonist. (a) Responses to field-stimulation in the presence of (S)-ENBA, CPA and CPA in the presence of DPCPX (100 nM). Time control responses are shown by the symbol-free line. (b) Responses to field stimulation in the presence of CGS 21680, APNEA and NECA. Vertical lines represent s.e. mean (some omitted for clarity) of 5–6 experiments.

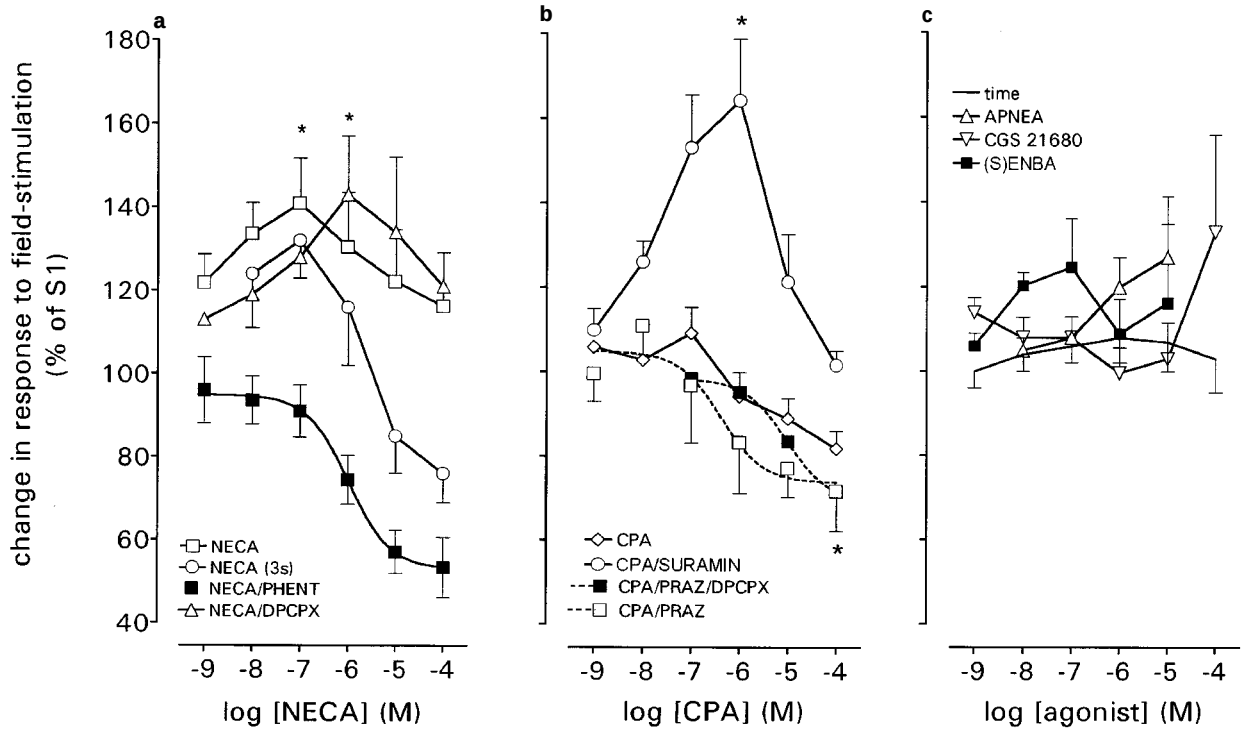

Figure 3 Effects of adenosine receptor agonists upon responses to field stimulation (10 s train, 9 Hz, supramaximal voltage, 0.1 ms duration) in preparations of the epididymal half of the vas deferens. (a) The effects of NECA, alone and in the presence of DPCPX (100 nM) or phentolamine (3 μM) and at a reduced (3 s) stimulation train duration. (b) The effects of CPA, alone and in the presence of either suramin (300 μM), prazosin (100 nM) or both prazosin (100 nM) and also DPCPX (30 nM). (c) The responses to CGS 21680, APNEA and (S)-ENBA. Time control responses are shown by the symbol-free line. Vertical lines represent s.e.mean (some omitted for clarity) of 5–11 experiments. *Indicates a significant (Student's t test, $P<0.05$) difference from agonist-free electrically-evoked contractions.

values 7.49 ± 0.62, 7.65 ± 0.74 and 5.84 ± 0.86, respectively, Figure 4). The CPA-induced potentiation of electrically-evoked contractions was antagonized by DPCPX (100 nM) with an apparent pK_B value of 7.64 ± 0.64 (Figure 4).

Effects of adenosine receptor agonists upon responses to phenylephrine or to ATP

Preparations of bisected vas deferens and of epididymis responded to the addition of phenylephrine with contractions. In the prostatic half of the vas deferens these responses to phenylephrine were unaffected by the addition of NECA (1 μM; Figure 5a). In the epididymal half of the vas deferens NECA (1 μM) significantly potentiated ($P<0.05$; t test, d.f. = 8) responses to low concentrations of phenylephrine ($\leqslant 1$ μM; Figure 5b).

In preparations of epididymis NECA (1 μM) significantly ($P<0.05$; F test, d.f. = 1, 12) shifted mean phenylephrine concentration-response curves to the left (4.6 ± 0.7 fold; Figure 5c). CPA (1 μM) also significantly ($P<0.05$; F-test, d.f. = 1, 17) potentiated mean phenylephrine concentration-response curves (2.4 ± 0.5 fold, $n=6$, data not shown).

To investigate further the effects of adenosine receptor agonists in the epididymal half of the vas deferens and epididymis, we added a low concentration of phenylephrine (1 μM) to preparations stimulated with NECA or CPA. In the epididymal half of the vas deferens NECA and CPA (1 nM–100 μM) potentiated responses to (1 μM) phenylephrine (with pEC_{50} values 7.57 ± 0.54 and 7.72 ± 0.27, respectively; Figure 6a). In the epididymis NECA potentiated responses to phenylephrine (1 μM) with a pEC_{50} value of 8.08 ± 0.18 (Figure 6c).

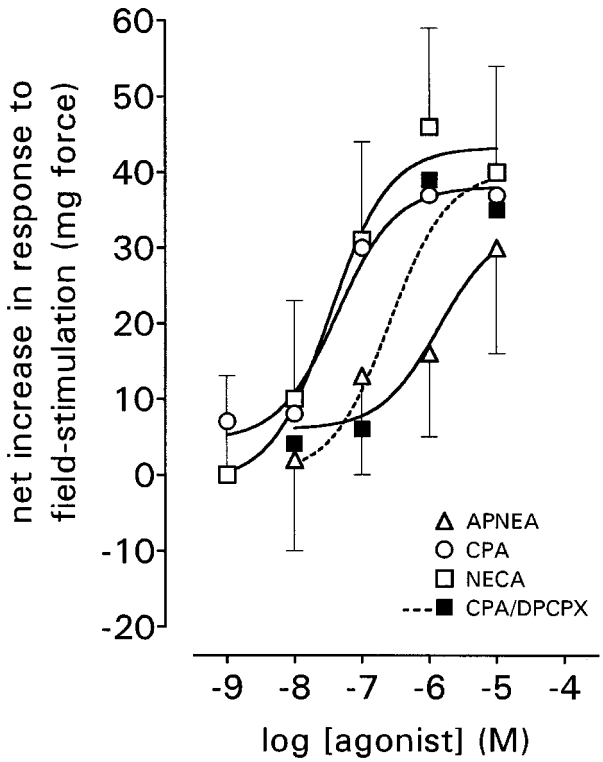

Figure 4 Effects of adenosine receptor agonists upon responses to field stimulation (10 s train, 9 Hz, supramaximal voltage, 0.1 ms duration) in preparations of epididymis. Each symbol represents the mean response to field stimulation in the presence of NECA, CPA ($\pm$DPCPX, 100 nM) and APNEA. Vertical lines represent s.e.mean (some omitted for clarity) of 5–10 experiments.

To determine the effects of the adenosine receptor agonist, NECA upon ATP-induced contractions we added a low concentration of ATP to preparations of the epididymal half of the vas deferens and epididymis previously stimulated with NECA (1 nM – 100 μM). NECA did not potentiate responses to ATP (1 μM) in either the epididymal half of the vas deferens or the epididymis (Figure 6a and b).

Discussion

The results of the present study, showing that the vas deferens of the guinea-pig responds to electrical field-stimulation with biphasic contractile responses, is consistent with earlier findings (Saxena, 1970; Swedin, 1971). That these responses were abolished by the neurotoxin, tetrodotoxin, indicates that these contractions are of neurogenic origin. Since adenosine

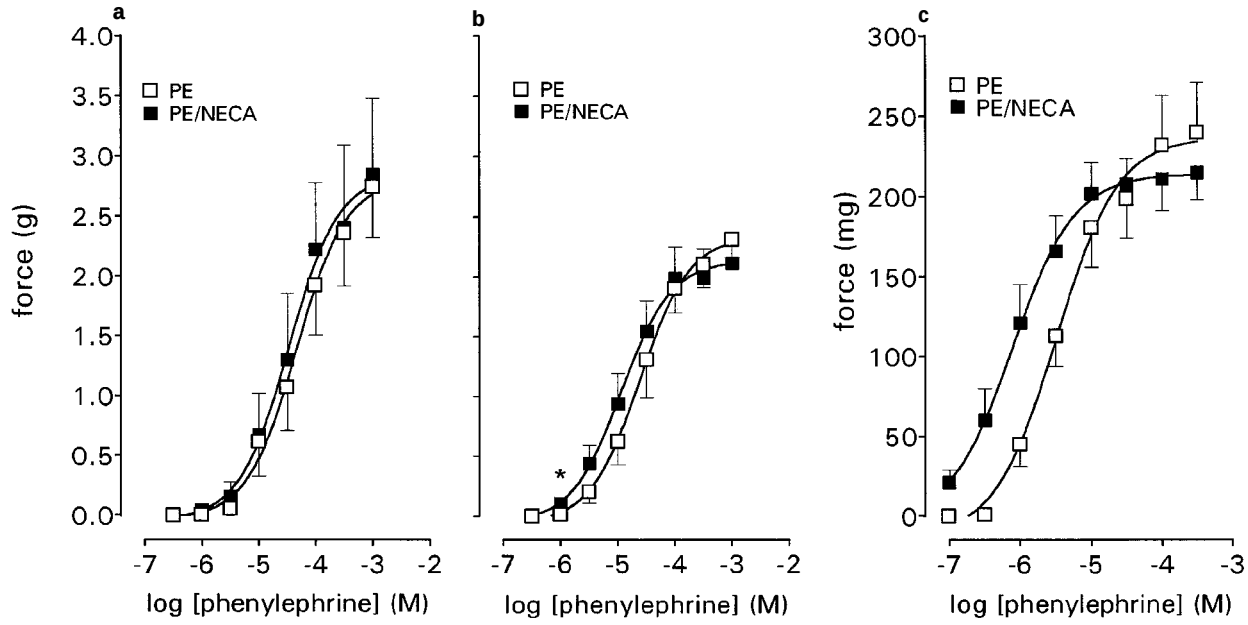

Figure 5 Effects of NECA upon phenylephrine concentration-response curves in preparations of the bisected vas deferens and the epididymis of the guinea-pig. (a), (b) and (c) Show responses to phenylephrine in the absence and presence of NECA (1 μM) in preparations of the prostatic and epididymal halves of the vas deferens and the epididymis, respectively. Vertical lines represent s.e.mean (some omitted for clarity) of 5–6 experiments. *At 1 μM the phenylephrine effects in the absence and presence of NECA (1 μM) are significantly different ($P<0.05$; t test, d.f. = 8).

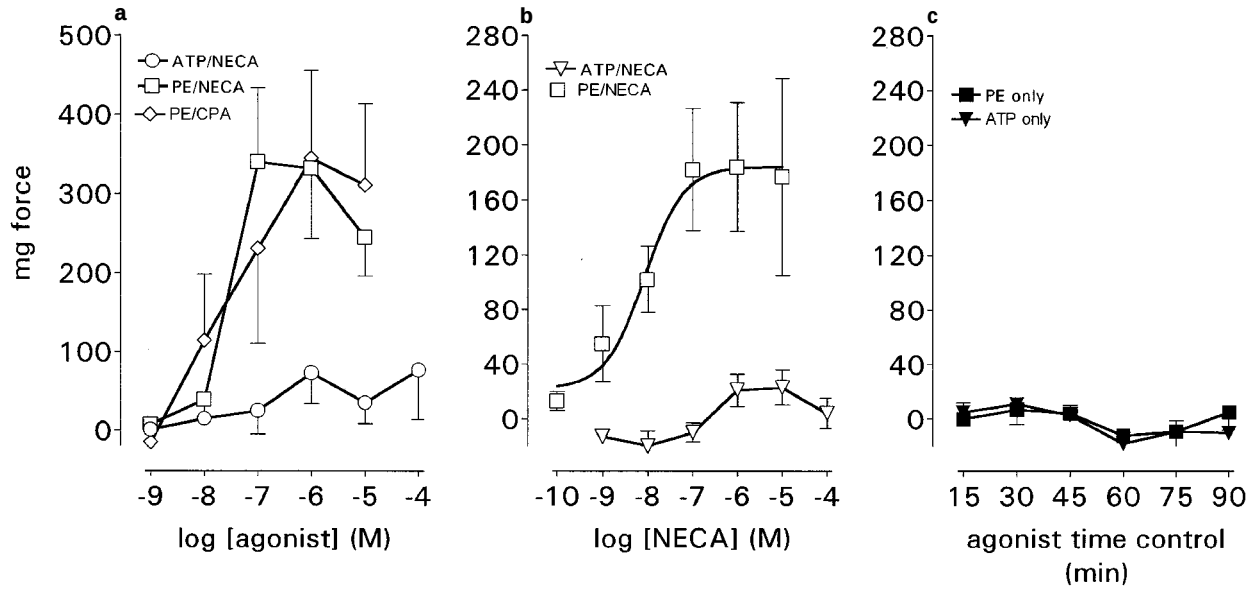

Figure 6 Effects of NECA or CPA upon low concentrations of phenylephrine or of ATP in the epididymal half of the vas deferens and epididymis of the guinea-pig. (a) The effects of NECA or CPA upon low concentrations of phenylephrine (1 μM) or of ATP (1 μM) in the epididymal half of the vas deferens. (b) The effects of NECA upon responses to ATP (1 μM) or phenylephrine (1 μM) in the epididymis of the guinea-pig. (c) Time control data for ATP (1 μM) and phenylephrine (1 μM) addition to preparations of the epididymis of the guinea-pig. Vertical lines represent s.e.mean (some omitted for clarity) of 5–6 experiments.

deaminase did not affect electrically-evoked contractions (in the epididymal half of the vas deferens) we must conclude that, at the parameters used in this study, endogenous adenosine either has no effect upon electrically-evoked contractions or that adenosine deaminase may not penetrate far enough into preparations of vasa deferentia to elicit a significant effect. We feel that the former explanation is more likely since DPCPX, at a sufficiently high concentration to block the effects of CPA, had no effect upon the electrically-evoked contractions of preparations of vas deferens.

We also showed that, in the prostatic half of the vas deferens, electrically-evoked contractions were inhibited by both the A_1 adenosine receptor selective agonists, CPA and (**S**)-ENBA, and also by the non-selective agonist, NECA, but not by the A_3 adenosine receptor selective agonist, APNEA, nor the A_{2A} adenosine receptor agonist, CGS 21680. The agonist rank order of potency of CPA $\geqslant$(**S**)-ENBA$\geqslant$ NECA$>>$CGS 21680$=$APNEA is consistent with the previously described action of these agonists at A_1 adenosine receptors (Alexander *et al*., 1994; Fredholm *et al*., 1994). Since the A_1-selective adenosine receptor antagonist, DPCPX, reduced the effect of CPA, we conclude that the inhibition of electrically-evoked contractions in the prostatic half of the vas deferens is mediated through prejunctional A_1 adenosine receptors. This hypothesis is consistent with previous findings demonstrating an A_1 adenosine receptor-mediated inhibition of electrically-evoked contractions in preparations of vas deferens from both the rat (Lee & Cheung, 1985; Major *et al*., 1989; Brownhill *et al*., 1996a) and guinea-pig (Driessen *et al*., 1994).

In the epididymal half of the vas deferens low concentrations of NECA (up to 100 nM) increased electrically-evoked contractions, at higher concentrations NECA inhibited electrically-evoked contractions. Neither CGS 21680, CPA or APNEA, mimicked this bell-shaped effect, but the A_1 adenosine receptor antagonist, DPCPX, pushed the bell-shaped curve to the right. Given the high potency of DPCPX at antagonizing the effects of NECA upon electrically-evoked contractions, we investigated the possibility that NECA was acting at post-junctional, as well as prejunctional A_1 adenosine receptors. In this half of the vas deferens NECA and CPA both potentiated responses to low concentrations of phenylephrine (1 μM), indicating an action at postjunctional A_1-like adenosine receptors. Since A_1 adenosine receptors inhibit the fast twitch component of electrically-evoked contractions in the prostatic half of the vas deferens we reduced the stimulation train length to preparations of the epididymal half of the vas deferens (3 s) to reduce the adrenergic component of electrically-evoked contraction. Under these conditions we found the NECA inhibited electrically-evoked contractions, indicating that this half of the vas deferens also contains prejunctional adenosine receptors.

To verify that the postjunctional potentiation of adrenergic contractions masked the inhibition of fast-twitch responses we used the α_1- and α-adrenoceptor antagonists, prazosin and phentolamine to block postjunctional α-adrenoceptors. At a train length of 10 s and in the presence of either prazosin or phentolamine NECA inhibited the fast-twitch component of electrically-evoked contractions. This finding indicates that the postjunctional potentiation of the adrenergic component of electrically-evoked contractions by adenosine receptor agonists does mask prejunctional effects. The finding that CPA, in contrast to NECA, inhibited electrically-evoked contractions may indicate that these agonists have different affinities or efficacies at pre- and postjunctional adenosine receptors. We have previously obtained similar findings in the epididymis of the guinea-pig where neuropeptide Y, through an action at pre- and postjunctional peptide receptors, had no effect upon electrically-evoked contractions (Haynes *et al*., 1997).

Our finding that NECA does not potentiate responses to exogenously applied ATP is in contrast with that of Brownhill *et al*. (1996b) who demonstrated an A_1 adenosine receptor-mediated potentiation of ATP-, but not of phenylephrine-induced contractions in the rat vas deferens, at present we have no explanation, other than species differences, for these opposing effects.

The cauda epididymis responds to electrical field-stimulation with monophasic responses (completely blocked by tetrodotoxin and also by phentolamine, Haynes *et al*., 1997). Since this preparation also responds to ATP with contractions we must conclude that ATP is not a significant neurotransmitter in the cauda epididymis of the guinea-pig. Since prejunctional A_1 adenosine receptors only inhibit the fast twitch (α-adrenoceptor antagonist insensitive) component of the response to field-stimulation in the vas deferens of the guinea-pig, and since there is no fast twitch component in the cauda epididymis, it is not surprising that adenosine receptor agonists do not inhibit electrically-evoked contractions in this tissue. The adenosine receptor agonist potentiation of electrically-evoked contractions in preparations of cauda epididymis has a rank order of potency of NECA$\geqslant$CPA$>$APNEA. This order of agonist potency and the finding that DPCPX inhibits the response to CPA with an apparent pK_B value of 7.64 is consistent with an action at a postjunctional A_1 rather than A_2 or A_3 adenosine receptors (see Fredholm *et al*., 1994). That our pK_B value was 10 fold lower than radioligand binding estimates of pK_D (Fredholm *et al*., 1994) may be due to species differences, since our pK_B value is consistent with the estimates of affinity obtained at the A_1 adenosine receptors of the guinea-pig cerebral cortex (8.3 and 7.9; Alexander *et al*., 1994).

Our report of the action of adenosine receptor agonists in the cauda epididymis of the guinea-pig is not consistent with the possibility of prejunctional purinoceptors, as shown in the epididymis of the rat (Ventura & Pennefather, 1992). However, it should be noted that Ventura & Pennefather (1991) also demonstrated a significant purinergic component of the response to field stimulation in preparations of the cauda epididymis of the rat. As in the epididymal half of the vas deferens we also demonstrate that postjunctional A_1-like adenosine receptors do not potentiate responses to ATP in the cauda epididymis of the guinea-pig.

This study has demonstrated that adenosine receptor agonists inhibit the fast twitch (purinergic) component of electrically-evoked contractions in both halves of the vas deferens. The rank orders of agonist potency in inhibiting electrically-evoked contractions, and the high affinity of the A_1 adenosine receptor antagonist, DPCPX, are consistent with effects mediated through prejunctional A_1 adenosine receptors. As the purinergic component of electrically-evoked contractions decreases (from the prostatic half of the vas deferens to the epididymis), the adrenergic component increases along with an increase in postjunctional adenosine receptor-mediated effects. At present we are uncertain of whether the prejunctional adenosine receptors inhibit both noradrenaline and ATP release (as shown by Driessen *et al*., 1994). We have previously shown that prejunctional neuropeptide Y receptors mask postjunctional effects in the epididymis of the guinea-pig (Haynes 1997). It is, therefore, possible that postjunctional adenosine receptor activation also masks the inhibition of noradrenaline release by prejunctional A_1 adenosine receptors. The A_1 adenosine receptor inhibition of electrically-evoked contractions in the prostatic half of the vas deferens may,

therefore, be consistent with the finding that postjunctional adenosine receptors do not potentiate ATP-induced contractility.

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