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A_1 and A_2 adenosine receptor modulation of contractility in the cauda epididymis of the guinea-pig

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1 The effects of adenosine receptor agonists upon phenylephrine-stimulated contractility and [^{3}H]-cyclic adenosine monophosphate ([^{3}H]-cyclic AMP) accumulation in the cauda epididymis of the guinea-pig were investigated. The α_1-adrenoceptor agonist, phenylephrine elicited concentration dependent contractile responses from preparations of epididymis. In the absence or presence of the L-type Ca^{2+} channel blocker, nifedipine (10 μM) the non-selective adenosine receptor agonist, 5′-N-ethylcarboxamido-adenosine (NECA, 1 μM) shifted phenylephrine concentration-response curves to the left (4 and 5 fold respectively). Following the incubation of preparations with pertussis toxin (200 ng ml^{-1} 24 h) NECA shifted phenylephrine concentration-response curves to the right (5.7 $\pm$ 0.9 fold).

2 In the presence of phenylephrine (1 μM), NECA and the A_1 adenosine receptor selective agonists, N^6-cyclopentyladenosine (CPA) and (2S)-N^6-[2-endo-norbornyl]adenosine ((S)-ENBA) elicited concentration-responses dependent contractions from preparations of epididymis (pEC_{50} values 8.18 $\pm$ 0.19, 7.79 $\pm$ 0.29 and 8.15 $\pm$ 0.43 respectively). The A_3 adenosine receptor agonists N^6-iodobenzyl-5′-N-methyl-carboxamido adenosine (IBMECA) and N^6-2-(4-aminophenyl) ethyladenosine (APNEA) mimicked this effect (but only at concentrations greater than 10 μM). In the presence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 30 nM) CPA concentration-response curves were shifted, in parallel to the right (apparent pK_B 8.75 $\pm$ 0.88) and the maximal response to NECA was reduced.

3 In the presence of DPCPX (100 nM) the adenosine agonist NECA and the A_{2A} adenosine receptor selective agonist, CGS 21680 (2-*p*-(2-carboxyethyl)-phenethylamino-N-ethylcarboxamido adenosine), but not CPA, inhibited phenylephrine (20 μM) stimulated contractions (pIC_{50} 7.15 $\pm$ 0.48). This effect of NECA was blocked by xanthine amine congener (XAC, 1 μM) and the A_{2A} adenosine receptor-selective antagonist 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385; 30 nM).

4 (S)-ENBA (in the absence and presence of ZM 241385, 100 nM), but not NECA or CPA inhibited the forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP in preparations of the epididymis of the guinea-pig (by 17 $\pm$ 6% of control). In the presence of DPCPX (100 nM) NECA and CGS 21680, but not (S)-ENBA, increased the accumulation of [^{3}H]-cyclic AMP in preparations of epididymis (pEC_{50} values 5.35 $\pm$ 0.35 and 6.42 $\pm$ 0.40 respectively), the NECA-induced elevation of [^{3}H]-cyclic AMP was antagonised by XAC (apparent pK_B 6.88 $\pm$ 0.88) and also by the A_{2A} adenosine receptor antagonist, ZM 241385 (apparent pK_B 8.60 $\pm$ 0.76).

5 These studies are consistent with the action of stable adenosine analogues at post-junctional A_1 and A_2 adenosine receptors in the epididymis of the guinea-pig. A_1 Adenosine receptors potentiate α_1-adrenoceptor contractility, an effect blocked by pertussis toxin, but which may not be dependent upon an inhibition of adenylyl cyclase. The epididymis of the guinea-pig also contains A_2 adenosine receptors, possibly of the A_{2A} subtype, which both inhibit contractility and also stimulate adenylyl cyclase.

Keywords: guinea-pig epididymis; A_1 adenosine receptor; A_2 adenosine receptor

Introduction

Previous studies from this laboratory have shown that Gi-protein coupled (pertussis toxin sensitive) α_2-adrenoceptors and NPY Y_1-like receptors potentiate α_1-adrenoceptor-mediated contractility in the epididymis of the guinea-pig (Haynes & Hill, 1996; Haynes *et al*., 1997). We have also recently shown that A_1 adenosine receptor agonists both inhibit the purinergic, and also potentiate the adrenergic components of electrically-evoked contractions in the vas deferens and epididymis of the guinea-pig (Haynes *et al*., 1998). Although this study clearly shows that NECA augments phenylephrine-induced contractions in the vas deferens and epididymis of the guinea-pig, the subtypes of receptor, and mechanism(s) of action are not clear. There is, however, evidence that A_1 adenosine receptors augment both H_1 histamine receptor and α_{1B}-adrenoceptor-stimulated increases in $[Ca^{2+}]_i$, and also stimulate inositol phosphate accumulation in the (hamster) vas deferens derived cell line, DDT_1MF-2 (White *et al*., 1992; Dickenson & Hill, 1993; Dickenson *et al*., 1995; Gerwins & Fredholm, 1995). In contrast with these studies we have also shown that epididymal α_2-adrenoceptors augment Ca^{2+} influx through L-type Ca^{2+} channels and inhibit adenylyl cyclase activity, but do not potentiate [^{3}H]-inositol phosphate accumulation (Haynes & Hill, 1996).

These previous studies raise the possibility that epididymal A_1 adenosine receptors and α_2-adrenoceptors may utilise distinct mechanisms to elicit similar effects. In this study we extend our earlier observation of an adenosine receptor-mediated potentiation of phenylephrine-induced contractility (Haynes *et al*., 1998), classify the post-junctional adenosine receptor mediating this effect and investigate some of the possible mechanisms by which adenosine receptors modulate contractility of the epididymis of the guinea-pig.

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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 use animals were killed by cervical dislocation and the vasa deferentia and testis removed.

Tissue preparation

The cauda epididymis was unravelled (with the aid of a N.U.M.S. HM 1 binocular dissecting microscope), placed into modified Krebs solution (of composition, mM; NaCl 118; KCl 4.7; $MgSO_4$ 0.45; K_2HPO_4 2.5; $NaHCO_3$ 25; $CaCl_2$ 1.9; glucose 11) and used for either contractility or second messenger accumulation studies.

Contractility studies

In sequence, from the vas deferens to the testicle four sections of cauda epididymis, each approximately 1 cm long, were cut. For contractility studies preparations 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. The upper end of each preparation was connected, via another silk thread, to a Grass FTO3 force-displacement transducer. Preparations of cauda epididymis were suspended under 0.30 g resting force in 8 ml tissue baths. Recordings of contractile force were made using Grass FTO3 isometric transducers coupled to a Grass (model 79D) chart recorder. Under these conditions the α_1-adrenoceptor agonist phenylephrine elicits concentration-dependent increases in peak contractile force, as has been shown previously (Haynes & Hill, 1996). Thus, in the present study we also measure the peak maximal increase in contractile force following the addition of phenylephrine (in the absence or presence of adenosine receptor agonists). To minimise possible heterogeneity of the contractile responses along the length of cauda epididymis sections of tissue were randomly assigned within each experimental paradigm.

Following a 40 min equilibration period preparations were stimulated with phenylephrine (for 150 s or until a maximal contractile response was achieved), and then washed (three to five times) with fresh Krebs solution and left for 13 min before the next addition of phenylephrine. Only one concentration-response curve was generated from any single tissue.

Since we have previously shown that adenosine deaminase has no effect upon electrically-evoked contractions in preparations of the epididymis of the guinea-pig (Haynes *et al*., 1998) we have not included adenosine deaminase in these experiments.

Pertussis toxin treatment

To verify our earlier observation of a NECA (1 μM) potentiation of phenylephrine concentration response curve (Haynes *et al*., 1998) and to identify the G-protein coupling of the epididymal adenosine receptors, preparations of cauda epididymis were incubated with pertussis toxin (200 ng ml^{-1}) or vehicle in Dulbecco's modified Eagles Medium (DMEM) at 37°C for 24 h (under an O_2 (95%):CO_2 (5%) atmosphere). Preparations were then set up for contractility studies, as described above and stimulated with NECA (1 μM) or with NECA vehicle for 90 s prior to the addition of phenylephrine (100 nM – 100 μM).

Adenosine agonist concentration-response curves

Preparations were set up as described above, stimulated with a near maximal concentration of phenylephrine (30 μM), washed (three to five times), and then (at 15 min intervals) given repeated threshold concentrations of phenylephrine (1 μM). Adenosine receptor agonists were added to preparations 90 s prior to the addition of phenylephrine (1 μM; 90 s contact period every 12–15 min), tissues were then washed three to five times (bath volume) with fresh buffer. Some preparations were incubated with DPCPX (100 nM) throughout each experiment.

In another series of studies preparations were repeatedly stimulated with phenylephrine (20 μM) in the presence of DPCPX (100 nM). Adenosine receptor agonists were added to preparations 90 s prior to the addition of phenylephrine (20 μM; 60 s contact period every 12–15 min), tissues were then washed three to five times (bath volume) with fresh buffer. Some preparations were also incubated with XAC (100 nM) throughout the experiment. Under these conditions phenylephrine (20 μM) contractions were reproducible for up to 2.5 h.

In no cases did the addition of adenosine receptor antagonists significantly affect the contractile responses to phenylephrine (1 or 20 μM; $n = 5–6$).

Accumulation of [^{3}H]-cyclic AMP

Preparations of cauda epididymis were obtained, as described above and incubated (37°C) in Krebs buffer containing [^{3}H]-adenine (1 μCi ml^{-1}) for 2 h. Preparations were washed once in 10 ml fresh Krebs and incubated for 20 min in fresh Krebs (35–36°C) containing the phosphodiesterase inhibitor, rolipram (30 μM), in the absence or presence of the A_1 adenosine receptor antagonist, DPCPX (100 nM). Some preparations were also incubated with the non-selective antagonist, XAC (1 μM) or the A_{2A} adenosine receptor-selective antagonist, ZM 241385 (30 or 100 nM; Poucher *et al*., 1995). These preparations were then used for either [^{3}H]-cyclic AMP accumulation or inhibition experiments. Following the equilibration period agonists were then added and preparations left for 14 min.

For the inhibition of [^{3}H]-cyclic AMP accumulation adenosine receptor agonists were added 2 min prior to the addition of forskolin (30 μM), preparations were then left for a further 12 min. The reactions were terminated by the addition of concentrated HCl (5% of incubation volume). Tissues were frozen (−20°C) overnight until use. On the day of assay [^{3}H]-cyclic AMP was extracted from the incubation media using acidic alumina columns (Johnson *et al*., 1994). Tritium levels in samples were determined by liquid scintillation counting.

[^{3}H]-inositol phosphate accumulation

Preparations were obtained as described above and incubated (37°C) in Krebs buffer containing (0.45 μCi) [^{3}H]-*myo*-inositol (NEN, Dupont) for 4.5 h (in an O_2 (95): CO_2 (5) atmosphere). All tissues were then rinsed in fresh Krebs solution, blotted dry and allowed to incubate for 25 min in fresh Krebs buffer (35–36°C) containing LiCl (20 mM). Preparations received (S)-ENBA (1 nM – 10 μM) at least 120 s prior to the addition of a submaximal concentration of phenylephrine (1 μM). Preparations were allowed to incubate for a further 13 min prior to transfer to 0.5 ml ice-cold methanol: (0.2 M) HCl (1 : 1) and

storage overnight at −70°C. The incubation buffer was neutralized with NaOH. Total [^{3}H]-inositol phosphates were separated from free [^{3}H]-*myo*-inositol by anion exchange chromatography (Hall & Hill, 1988). Tritium levels in supernatant were determined by liquid scintillation counting.

Statistics

Estimates of pIC_{50} or pEC_{50} (±s.e), slope and maximum contractile response were generated using a four-parameter logistic curve fitting and graphics program PRISM v2.0 (GraphPad Software Inc., San Diego U.S.A.). Concentration-response curves were compared with an iterative curve fitting program, FLEXIFIT (see Guardabasso *et al*., 1988), significant changes were determined with an *F*-test. One-way ANOVA and the 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 using the Gaddum equation:

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

where CR is the mean ratio of concentrations of agonist that cause equal contractile responses in absence and presence of an antagonist.

Drugs

N^6-2-(4-aminophenyl) ethyladenosine (APNEA, synthesized by Dr E.A. Boyd, Department of Pharmaceutical Sciences, University of Nottingham U.K.); 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), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), forskolin and xanthine amine congener (XAC) (were from Research Biochemicals Inc., U.S.A.); 5′- N-ethylcarboxamidoadenosine (NECA), N^6-cyclopentyladenosine (CPA), phenylephrine HCl and nifedipine (were from Sigma Chemical Co., U.K.); 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin - 5 -ylamino] ethyl)phenol (ZM 241385) (Tocris Cookson Ltd, U.K.). Human peptide YY (PYY) was obtained from Calbiochem-Novabiochem Ltd. U.K.). Pertussis toxin (Porton Products/ Speywood, U.K.).

Adenosine receptor agonists and antagonists were made up as a stock solution in DMSO, aliquoted and frozen. Phenylephrine was dissolved in distilled water. PYY was dissolved in 0.01 M acetic acid, aliquoted and frozen, on the day of use PYY was made up in Krebs solution containing BSA (0.1%). On the day of use all other 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

Responses to phenylephrine

As we have described earlier (Haynes & Hill, 1996), preparations of cauda epididymis responded to phenylephrine with contractions. In this study we now demonstrate that these contractions are potentiated by adenosine receptor agonists (see Figure 1 for typical contractile responses).

Mean maximal contractile responses to phenylephrine were reduced (by 80%) by the L-type Ca^{2+} channel blocker, nifedipine (10 μM). As shown in previous studies (Haynes *et al*., 1998) NECA (1 μM) shifted phenylephrine concentration response curves (4.1±0.6 fold) to the left (Figure 2a). In the presence of nifedipine (10 μM) NECA still shifted phenylephrine concentration response curves to the left (5.0±0.6, Figure 2b).

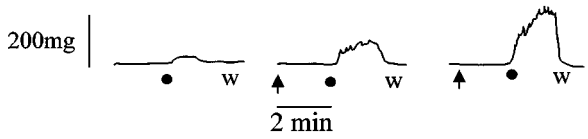

Figure 1 Typical responses to phenylephrine, in the absence and presence of NECA, of the epididymis of the guinea-pig. The first panel shows the response to phenylephrine (1 μM; •). The second panel shows responses to phenylephrine (1 μM; •) in the presence of NECA (1 μM; ↑). The third panel shows responses to phenylephrine (1 μM; •) in the presence of NECA (10 μM; ↑). W - Indicates wash.

Effects of pertussis toxin

Following the pre-incubation of preparations of cauda epididymis with pertussis toxin vehicle NECA (1 μM) shifted phenylephrine concentration response curves 2.7±0.5 fold to the left ($n=6$, Figure 2c). Following the incubation of preparations with pertussis toxin (200 ng ml^{-1} 24 h) NECA (1 μM) shifted phenylephrine concentration response curves significantly ($P<0.05$, *F*-test, df = 1, 67) 5.7±0.9 fold to the right ($n=6$, Figure 2d). Compared to vehicle controls pertussis toxin treatment did not affect phenylephrine concentration-response curves (pEC_{50} values 5.48±0.07 and 5.32±0.08 respectively).

Effects of adenosine receptor agonists and antagonists

In the presence of a threshold concentration of phenylephrine (1 μM), the agonists, NECA, (S)-ENBA and CPA, elicited concentration-dependent increases in contractile force (pEC_{50} values 8.18±0.19, 8.15±0.43 and 7.79±0.29, Figure 3a,b and c). The mean maximal response to NECA was significantly ($P<0.05$, *F*-test, df = 1, 52) reduced in the presence of DPCPX (30 nM; Figure 3a). The contractile responses to CPA were competitively antagonised by DPCPX (30 nM) with an apparent pK_B of 8.75±0.88 (Figure 3b). APNEA and IBMECA also elicited small responses from preparations of phenylephrine-stimulated cauda epididymis (Figure 3c).

In the presence of DPCPX (100 nM) the adenosine agonist NECA significantly ($P<0.05$, Paired *t*-test, d.f. = 5) inhibited phenylephrine (20 μM)-stimulated contractions (pIC_{50} 7.15±0.48). XAC (1 μM; data not shown) and ZM 241385 (30 nM) antagonised this effect (Figure 4a). CGS 21680 was less effective than NECA and CPA did not inhibit contractile responses to phenylephrine (Figure 4b).

Effects of agonists upon [^{3}H]-cyclic AMP accumulation in cauda epididymis

(S)-ENBA, but not NECA or CPA (data not shown), inhibited the forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP in preparations of cauda epididymis (pIC_{50} 7.92±0.55, maximal inhibition 17±8% of control, data not shown). In the presence of the A_{2A} adenosine receptor antagonist ZM 241385 (100 nM) (S)-ENBA was a slightly more potent, though still poor inhibitor (pIC_{50} 8.20±0.43, Figure 5a). Under identical assay conditions peptide YY (PYY) and the α_2-adrenoceptor agonist, xylazine (data from Haynes & Hill, 1996), inhibited the

forskolin (30 μM)-stimulated accumulation of [^{3}H]-cyclic AMP (Figure 5a).

In the presence of DPCPX (100 nM) NECA and CGS 21680 significantly stimulated the accumulation of [^{3}H]-cyclic AMP (pEC_{50} values 5.50$\pm$0.62 and 7.60$\pm$0.49 respectively; Figure 5b). The effect of NECA was antagonized by XAC (apparent pK_B 6.88$\pm$0.88; data not shown) and also by ZM 241385 (apparent pK_B 8.60$\pm$0.76; Figure 5b).

[^{3}H]-inositol phosphate accumulation

We have previously shown that phenylephrine elicits concentration-dependent increases in the accumulation of [^{3}H]-inositol phosphates in preparations of the cauda epididymis of the guinea-pig (Haynes & Hill, 1996). The addition of (S)-ENBA (1 nM – 10 μM) did not affect the [^{3}H]-inositol phosphate production stimulated by a submaximal

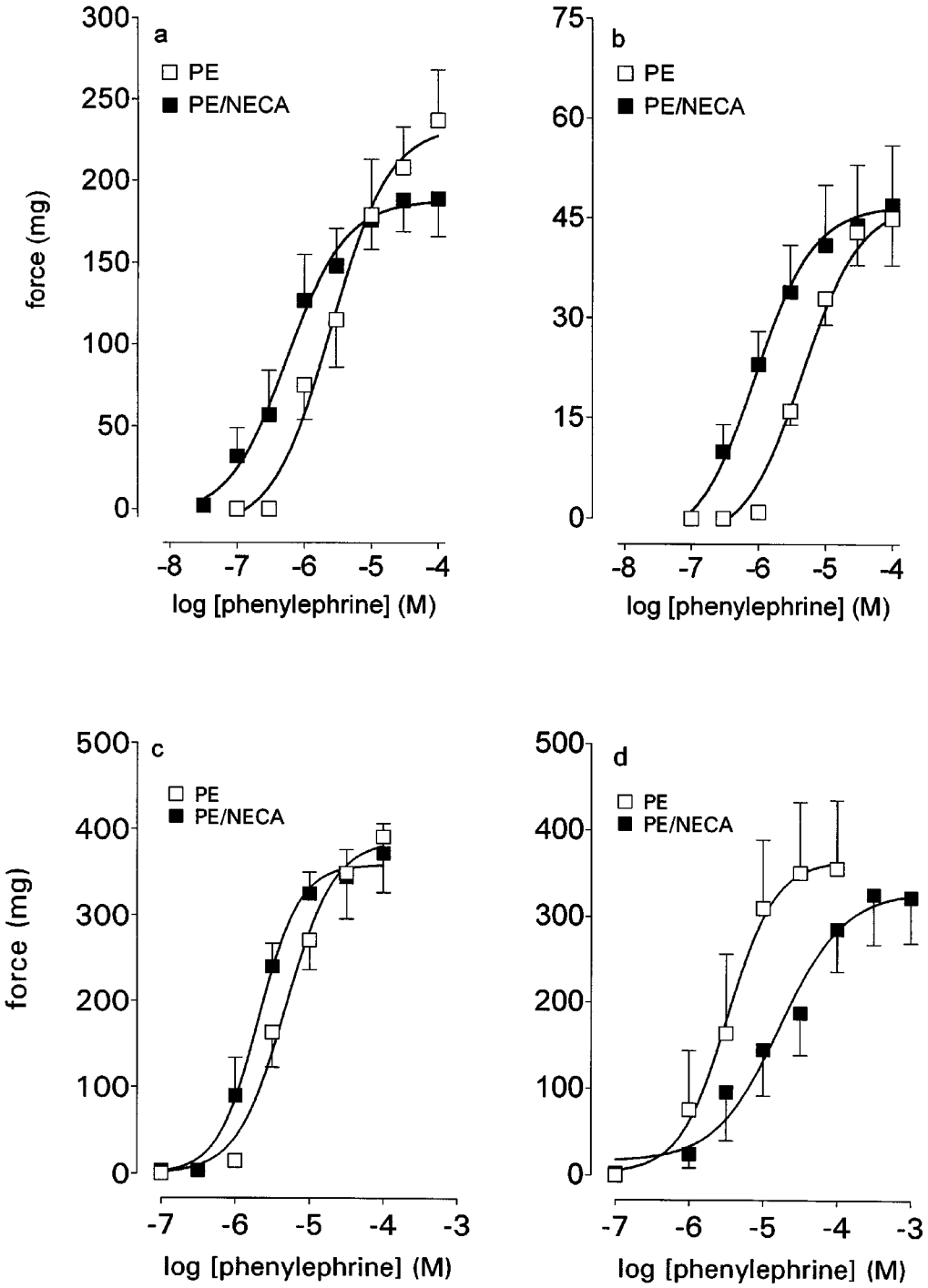

Figure 2 Effects of NECA upon phenylephrine concentration-response curves. (a) Shows the effect of NECA (1 μM) upon phenylephrine concentration-response curves (data from Haynes *et al.*, 1998). (b) Shows the effect of NECA (1 μM) upon phenylephrine concentration-response curves in the presence of nifedipine (10 μM). (c) Shows the effect of NECA (1 μM) upon phenylephrine concentration-response curves following the incubation of tissues in pertussis toxin vehicle (24 h). (d) Shows the effect of NECA (1 μM) upon phenylephrine concentration-response curves following the incubation of tissues with pertussis toxin (200 ng ml^{-1} 24 h). Bars represent s.e.mean (some omitted for clarity) of five to six experiments.

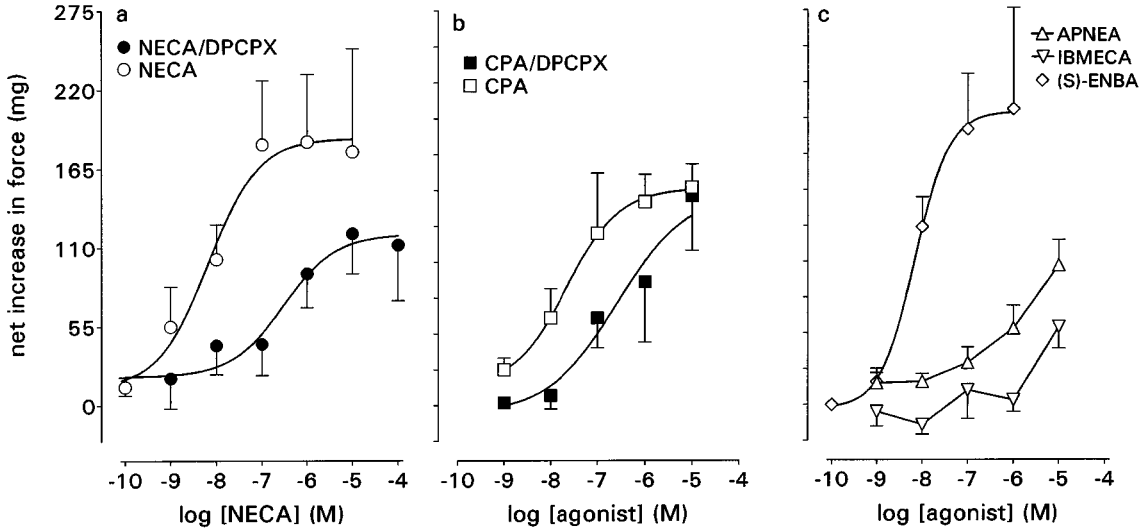

Figure 3 Effects of adenosine receptor agonists upon threshold concentrations of phenylephrine (1 μM). (a) Shows NECA concentration-response curves in the absence and presence of DPCPX (30 nM). (b) Shows CPA concentration-response curves in the absence and presence of DPCPX (30 nM). (c) Shows APNEA, (S)-ENBA and IBMECA responses. Bars represent s.e.mean (some omitted for clarity) of five to six experiments. Net increase in force is the maximal tension in the presence of adenosine receptor agonists minus the maximal tension in the absence of adenosine receptor agonists.

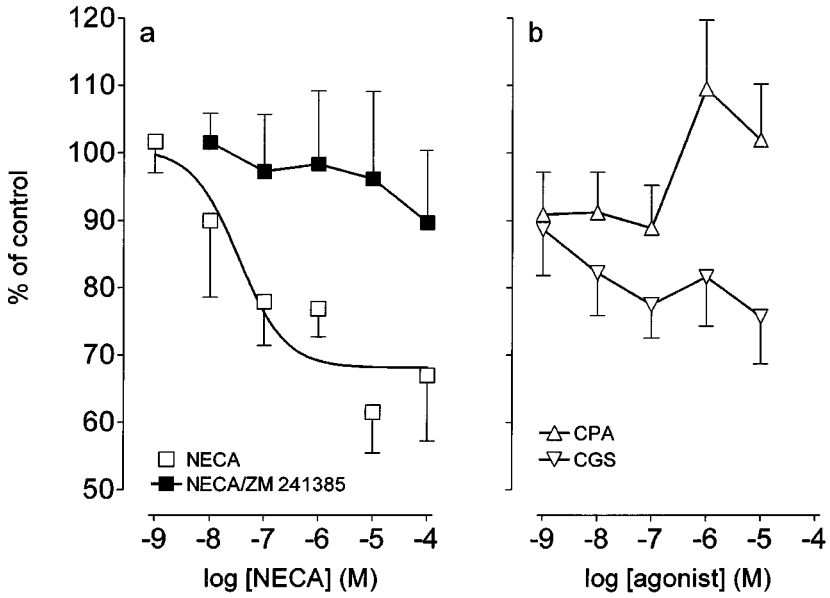

Figure 4 NECA inhibition of responses to phenylephrine. In the presence of DPCPX (100 nM) preparations were stimulated with phenylephrine (20 μM). (a) Shows the effects of NECA upon responses to phenylephrine (20 μM) in the absence and presence of ZM 241385 (30 nM). (b) Shows the effects of CPA and CGS 21680 upon responses to phenylephrine (20 μM). Bars represent s.e.mean (some omitted for clarity) of five to six experiments.

concentration of phenylephrine (1 μM; $n=6$, data not shown).

Discussion

In an earlier study we demonstrated that adenosine receptor agonists potentiated phenylephrine and electrically-evoked contractile responses of preparations of the cauda epididymis of the guinea-pig (Haynes *et al.*, 1998). In this study we characterize the adenosine receptor subtypes modulating phenylephrine-stimulated contractility and also investigate the possible mechanisms of action in the cauda epididymis of the guinea-pig.

The adenosine receptor agonists NECA, (S)-ENBA, CPA, APNEA and IBMECA all potentiated contractile responses to threshold concentrations of phenylephrine (rank order of potency NECA $\geqslant$ (S)-ENBA $\geqslant$ CPA > APNEA > IBMECA). DPCPX competitively antagonized the contractile responses to CPA with an apparent pK_B of 8.75. The high affinity of DPCPX and the rank order of agonist potency are consistent with the action of these adenosine receptor agonists at post-junctional A_1 adenosine receptors. In previous studies we have demonstrated that (Gi/o protein coupled) α_2-adrenoceptors and NPY Y_1 receptors potentiate contractile responses to phenylephrine in the cauda epididymis of the guinea-pig (Haynes & Hill, 1996; Haynes *et al.*, 1997). In this study we show that A_1 adenosine receptor potentiation of phenylephrine

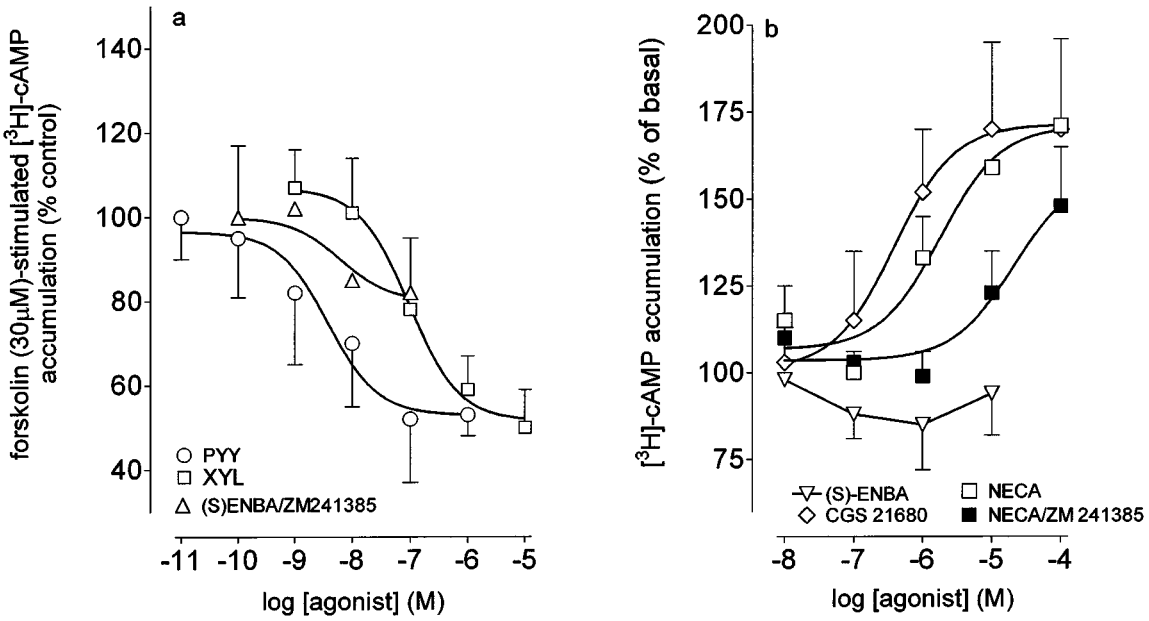

Figure 5 Agonist-stimulated changes in [^{3}H]-cyclic AMP accumulation in preparations of epididymis. (a) Shows the effects of the adenosine receptor agonist (S)-ENBA (in the presence of ZM 241385, 100 nM), the neuropeptide receptor ligand, peptide YY and the α_2-adrenoceptor agonist, xylazine (XYL, from Haynes & Hill, 1996) upon forskolin (30 μM)-stimulated [^{3}H]-cyclic AMP accumulation. (b) Shows the effects of NECA (in the absence and presence of ZM 241385, 30 nM), CGS 21680 and (S)-ENBA upon [^{3}H]-cyclic AMP accumulation in preparations of the epididymis of the guinea-pig. All concentration response curves in (b) were constructed in the presence of DPCPX (100 nM). Bars represent s.e.mean (some omitted for clarity) of five to 11 experiments.

α_1-adrenoceptor-stimulated contractions is pertussis toxin sensitive, a finding consistent with the possibility that the epididymal A_1 adenosine receptors are coupled to effectors through Gi/o protein subunits.

In an earlier report we demonstrated that the α_2-adrenoceptor-mediated potentiation of phenylephrine-induced contractile responses in the cauda epididymis of the guinea-pig was sensitive to the addition of the L-type voltage operated Ca^{2+} channel blocker, nifedipine (Haynes & Hill, 1996). In this study, we report that A_1 adenosine receptor-mediated effects can be demonstrated in the presence of nifedipine. Since the epididymis of the guinea-pig contains both α_{1A}- and α_{1B}-adrenoceptor subtypes (Haynes & Hill, 1996), these data indicate that A_1 adenosine receptors may augment nifedipine insensitive contractile responses. This hypothesis is consistent with data reported by Dickenson (1994) who showed that A_1 adenosine receptor activation increased α_{1B}-adrenoceptor-stimulated accumulation of inositol phosphates in DDT_1 MF-2 cells. We cannot, however, rule out the possibility that A_1 adenosine receptors also augment the nifedipine-sensitive contractile response to phenylephrine. Some of our findings were not consistent with the action of adenosine receptor agonists only at A_1 adenosine receptors. Thus the maximal contractile response to NECA, but not that to CPA was reduced by the A_1 adenosine receptor-selective antagonist, DPCPX. In addition the incubation of preparations with pertussis toxin (which ribosylates the α-subunit of Gi/o proteins) changed the NECA-stimulated potentiation into an inhibition of phenylephrine concentration response curves. This finding is in contrast with a previous finding from this laboratory (Haynes & Hill, 1996) where the potentiation of contractile responses to phenylephrine, by the α_2-adrenoceptor agonist xylazine, was blocked by pertussis toxin – but not shifted to the right.

Since there is evidence of an A_2 adenosine receptor-induced inhibition of contractility in the rat vas deferens (Brownhill *et al*., 1996), we investigated the possibility that A_2 adenosine receptors also modulate contractility in the epididymis of the guinea-pig. In the presence of DPCPX, NECA and CGS 21680 (but not CPA) inhibited contractile responses to phenylephrine. At lower concentrations CGS 21680 was equally (if not more) effective than NECA. At higher concentrations, however, CGS 21680 did not achieve the same level of inhibition as NECA. This finding may be consistent with our previous studies, where we have shown that CGS 21680 (at concentrations greater than 1 μM) potentiates, through some as yet undefined mechanism, electrically-evoked contractions of preparations of epididymis (Haynes *et al*., 1998). In contrast to our functional studies we have shown that CGS 21680 is a little more effective than NECA at stimulating the accumulation of [^{3}H]-cyclic AMP. This finding is consistent with the action of these adenosine analogues at A_{2A} rather than A_{2B} adenosine receptors (Fredholm *et al*., 1994). Additional evidence for an A_{2A} adenosine receptor-mediated effect is indicated by the low apparent pK_B value (8.60) of the A_{2A}-selective antagonist ZM 241385. This pK_B is consistent with the pA_2 values reported at the A_{2A} adenosine receptors in guinea-pig isolated Langendorff hearts (8.57 and 9.02) and is almost two orders of magnitude greater than estimates of affinity at the A_{2B} adenosine receptors of the guinea-pig aorta (7.06; Poucher *et al*., 1995).

We have, in this study, shown that PYY inhibits forskolin-stimulated adenylyl cyclase activity in the cauda epididymis of the guinea-pig; a finding consistent with earlier studies showing that PYY also potentiates phenylephrine-induced contractility in the cauda epididymis of the guinea-pig (Haynes *et al*., 1997). Since we have shown that the Gi-coupled α_2-adrenoceptors also inhibit adenylyl cyclase activation in the cauda epididymis of the guinea-pig (Haynes & Hill, 1996) we were surprised to find that A_1 adenosine receptor agonists are poor inhibitors of adenylyl cyclase in this tissue. One possible explanation for this finding is that

A_2 adenosine receptors oppose the action of A_1 adenosine receptors. Some support for this hypothesis is evident in our studies showing that the inhibition of forskolin-stimulated [^{3}H]-cyclic AMP accumulation by (S)-ENBA is enhanced in the presence of the A_{2A} adenosine receptor antagonist, ZM 241385. However, even in the presence of ZM 241385 the maximal inhibition of forskolin-stimulated [^{3}H]-cyclic AMP accumulation by (S)-ENBA is considerably reduced in comparison to that of either PYY or xylazine (Haynes & Hill 1996). This finding is curious since α_2-adrenoceptors, neuropeptide (NPY) Y_1 receptors and A_1 adenosine receptors all potentiate contractile responses to phenylephrine with similar maximal responses (Haynes & Hill, 1996; Haynes *et al*., 1997). Alternative mechanisms by which A_1 adenosine receptors may potentiate α_1-adrenoceptor-stimulated contractions may include the inhibition of K^+ channel function, protein kinase C or phospholipase C activation (for review see Fredholm *et al*., 1994). It is, however, unlikely that A_1 adenosine receptors stimulate phospholipase C since (S)-ENBA did not potentiate the phenylephrine-stimulated accumulation of inositol phosphates in this tissue.

We have, in the present study, shown that A_1 and A_{2A} adenosine receptors modulate phenylephrine-induced contractions in the cauda epididymis of the guinea-pig. In contrast with the α_2-adrenoceptors of this preparation, epididymal A_1 adenosine receptors potentiate both nifedipine-sensitive and insensitive components of the contractile response to phenylephrine and are not well coupled to adenylyl cyclase. We also report that epididymal A_{2A} adenosine receptors both stimulate adenylyl cyclase and also inhibit phenylephrine-induced contractions. Sperm delivery to the vas deferens is through the epididymis, at present we would speculate that epididymal adenosine receptors modify the smooth muscle activity in response to transmitters such as noradrenaline.

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