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European Journal of Pharmacology 303 (1996) 87–90

ejp

Short communication

Differential distribution of adenosine A_2 receptors in the epididymal and prostatic portions of the rat vas deferens

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Received 27 October 1995; revised 23 January 1996; accepted 26 January 1996

Abstract

In the rat vas deferens there are prejunctional A_1 receptors mediating inhibition of transmitter release and post-junctional A_1 and A_2 receptors mediating enhancement and inhibition of contractions respectively. In this study the distribution of adenosine receptors in the prostatic and epididymal portions of the bisected rat vas deferens was investigated. The pre- and post-junctional A_1 receptors were present on both portions of the bisected tissue. However, post-junctional A_2 receptors appear to be present only in the prostatic region, showing that adenosine receptors are differentially distributed along the length of the rat vas deferens.

Keywords: Vas deferens, rat; Adenosine receptor; Receptor distribution

1. Introduction

Stimulation of the sympathetic nerves of the rat vas deferens produces a biphasic response, which is thought to be due to adenosine 5′-triphosphate (ATP) and noradrenaline released as cotransmitters (White, 1988; Burnstock, 1990; Von Kügelgen and Starke, 1991). It is well documented that there is regional variation in the contractile responses to nerve stimulation along the length of the rat vas deferens, with the prostatic region showing the purinergic component and the epididymal region showing the adrenergic component (Sneddon and Machaly, 1992). Adenosine has been shown to have a neuromodulatory role mediating inhibition of neurotransmitter release acting via pre-junctional A_1 receptors in this tissue (Muller and Paton, 1979; Paton, 1981). ATP has also been shown to have a neuromodulatory role acting via pre-junctional purinoceptors, and this may be due to a direct effect of nucleotides on the A_1 receptor (Forsyth et al., 1991; Kurz et al., 1993). It was therefore of interest to investigate the distribution of adenosine receptors on the prostatic and epididymal portions of the bisected rat vas deferens, to see whether these receptors are also localised in the prostatic region of the tissue where the purinergic component predominates. In the rat vas deferens there are pre-junctional A_1 receptors, mediating inhibition of transmitter release (Muller and Paton, 1979; Paton, 1981), and post-junctional A_2 receptors mediating inhibition of contractions induced by ATP or noradrenaline (Hourani et al., 1993). Recently, the presence of an excitatory post-junctional A_1 receptor mediating enhancement of contractions has also been shown (Hourani and Jones, 1994). In this study, we examined the distribution of adenosine receptors along the length of the rat vas deferens using the A_1-selective agonist N^6-cyclopentyladenosine (CPA), the non-selective agonist 5′-*N*-ethylcarboxamidoadenosine (NECA) and the selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) which shows nanomolar affinity at A_1 receptors compared to micromolar affinity at A_2 receptors (for review, see Collis and Hourani, 1993).

2. Materials and methods

Male Wistar rats (200–250 g) were killed by cervical dislocation. Equal portions (1.0–1.5 cm) of the epididymal and prostatic ends of the vas deferens were removed, freed of any connective tissue, and mounted in 4 ml organ baths containing Mg^{2+}-free Krebs of the following composition, 118 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 4.8 mM KCl, 2.5 mM $CaCl_2$, 11 mM glucose, gassed with 95% O_2 : 5% CO_2, and maintained at 37°C. A resting tension of 0.5 g was applied, and contractile responses

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PII S0014-2999(96)00077-5

were measured isometrically with a Grass FT03 transducer and recorded on a Grass model 79D polygraph. Tissues were equilibrated for 90 min before the addition of drugs and concentration-response curves were obtained non-cumulatively.

To measure the inhibition of nerve-stimulated contractions, tissues were field stimulated (Grass S48 stimulator, twin pulses of 1 ms duration, 75 ms apart, 70 V) via parallel platinum electrodes at a frequency of 0.1 Hz resulting in regular contractions 10 s apart. The purines were added when the response to the nerve stimulation had stabilised, and left in contact with the tissue until maximum inhibition of the contractions were observed. The inhibition was expressed as percent reduction of the nerve-mediated response. To investigate the effect of DPCPX (10 nM or 1 μM), concentration-response curves for agonists were obtained in the same tissue before and after incubation for 30 min with the antagonist. To measure the inhibitory effect of NECA or the enhancement by CPA of KCl-induced (35 mM) contractions, the tissues were pre-incubated with the purines for 1 min before the addition of KCl. Responses were expressed as percent reduction (% inhibition) or increase (% enhancement) of control response in the absence of the purines, calculated as the mean of several control contractions obtained in each tissue over the course of the experiment. To investigate the effect of tetrodotoxin (1 μM) on the nerve-mediated or KCl-induced contractions, responses were obtained to nerve stimulation or KCl (35 mM) in the same tissue before and after incubation for 30 min with tetrodotoxin.

For inhibition of nerve-mediated responses, pD_2 values were calculated as the negative $\log_{10}$ of the molar concentration of the agonist producing 50% inhibition and dose ratios were calculated using these values. Apparent pA_2 values were estimated as the negative $\log_{10}$ of the molar concentration of the antagonist divided by the dose ratio − 1. For inhibition of KCl-induced contractions, a pEC_{20} value was calculated as the negative $\log_{10}$ of the molar concentration of the agonist producing 20% inhibition.

DPCPX and CPA were obtained from Research Biochemicals International (USA) and NECA was obtained from Sigma Chemical Company (UK). The buffer salts were of analytical grade and were obtained from BDH (UK). CPA (10 mM) was dissolved in 20% ethanol and DPCPX (1 mM) was dissolved in 6% aqueous dimethylsulphoxide (DMSO) containing NaOH (6 mM), and after dilution to the final bath concentration the solvents had no effect.

3. Results

Nerve-mediated responses were abolished by tetrodotoxin (1 μM) in the prostatic and epididymal portions of the bisected rat vas deferens, whereas KCl-induced responses were unaffected (results not shown). CPA (0.01–0.3 μM) enhanced contractions induced by KCl (35 mM) equally in both the epididymal and prostatic portions of the bisected tissue, with a maximal enhancement at 0.3 μM CPA of approximately 40% in each case (Fig. 1a). NECA (0.01–30.0 μM) potently inhibited contractions induced by KCl (35 mM) in the prostatic portion ($pEC_{20} = 6.3 \pm 0.3$), but was almost inactive even at 100 μM in the epididymal portion of the bisected tissue (Fig. 1b).

CPA and NECA inhibited nerve-mediated contractions in both the epididymal and prostatic portions of the bisected rat vas deferens (Fig. 2). The pD_2 values for CPA and NECA for the epididymal region were 7.4 ± 0.1 and 6.7 ± 0.2 respectively, whereas the order of potency was reversed at the prostatic end, where the pD_2 values were 6.6 ± 0.1 and 7.3 ± 0.1 respectively. DPCPX (10 nM) antagonised the inhibition by CPA of nerve-mediated contractions on both regions of the bisected tissue (Fig. 2a,b), giving a dose ratio of 20.8 ± 2.1 and an apparent pA_2 value of 9.3 ± 0.1 for the epididymal region, and for the prostatic region a dose ratio of 3.7 ± 0.6 and an apparent pA_2 value of 8.4 ± 0.1. DPCPX (10 nM) antagonised the response to NECA in the epididymal region (Fig. 2c), giving a dose ratio of 5.8 ± 0.6 and an apparent pA_2 value

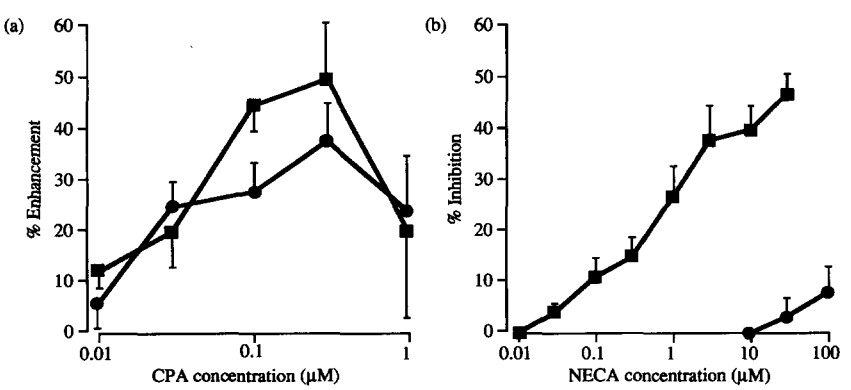

Fig. 1. Effect of (a) CPA and (b) NECA on KCl-induced (35 mM) contractions of the epididymal (●) and prostatic (■) portion of the bisected rat vas deferens. Results are expressed as (a) % enhancement of KCl-induced contraction in the absence of CPA and (b) % inhibition of KCl-induced contraction in the absence of NECA. Results are the mean of three to four determinations and the vertical bars show S.E.M.

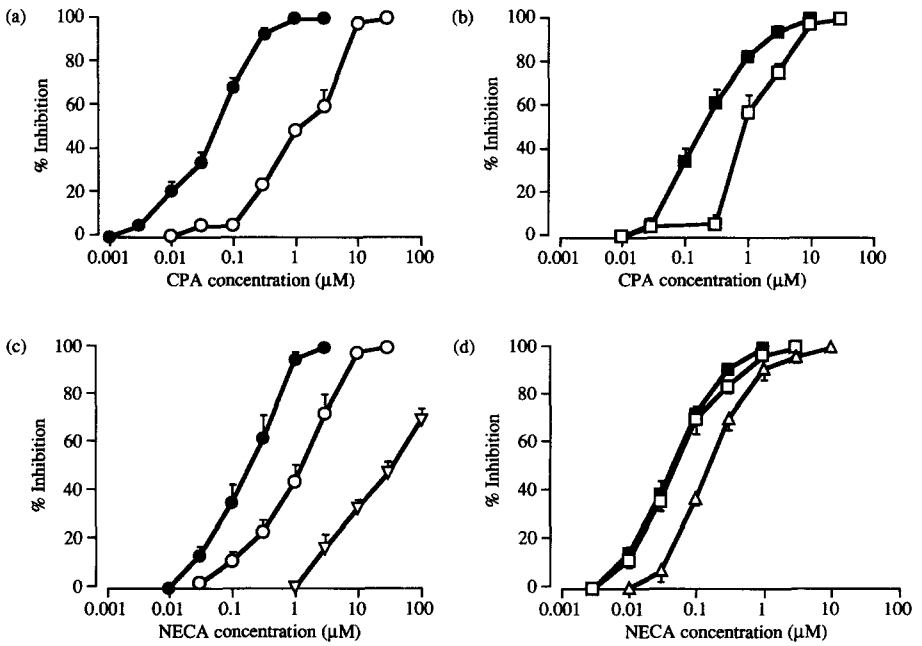

Fig. 2. The effect of DPCPX on the inhibition of nerve-mediated contractions by CPA and NECA. (a) CPA in the absence of DPCPX (●) and in the presence of DPCPX (10 nM) (○) in the epididymal portion; (b) CPA in the absence of DPCPX (■) and in the presence of DPCPX (10 nM) (□) in the prostatic portion; (c) NECA in the absence of DPCPX (●) and in the presence of DPCPX, 10 nM (○) and 1 μM (▽) in the epididymal portion; (d) NECA in the absence of DPCPX (■) and in the presence of DPCPX, 10 nM (□) and 1 μM (△) in the prostatic portion of the rat vas deferens. Results are expressed as % inhibition of nerve-mediated contractions. Results are the mean of four determinations and the vertical bars show S.E.M.

of 8.6 ± 0.1, whereas it did not antagonise the response to NECA in the prostatic region (Fig. 2d). In the epididymal region, 1 μM DPCPX antagonised the response to NECA (Fig. 2c), giving a dose ratio of 155.8 ± 34.2 and an apparent pA_2 value of 8.2 ± 0.1, whereas it was much less effective at the prostatic region (Fig. 2d), giving a dose ratio of 3.5 ± 0.5 and an apparent pA_2 value of 6.4 ± 0.1.

4. Discussion

CPA clearly enhanced contractions induced by KCl in the epididymal and prostatic regions of the bisected rat vas deferens, presumably due to the activation of the previously reported post-junctional A_1 receptor (Hourani and Jones, 1994). In this study KCl was used as the contractile agent instead of ATP, as used in the previous study (Hourani and Jones, 1994), as ATP-induced contractions have been shown to vary along the length of the tissue, predominating at the prostatic region (Sneddon and Machaly, 1992). Responses to KCl were unaffected by tetrodotoxin suggesting that these responses were due to a direct effect on the smooth muscle and not to stimulation of excitatory nerves. These results suggest that post-junctional A_1 receptors are evenly distributed along the length of the rat vas deferens. NECA potently inhibited contractions induced by KCl in the prostatic region, presumably due to the activation of post-junctional A_2 receptors (Hourani et al., 1993). However, the lack of an appreciable response to NECA in the epdidymal region suggests that the A_2 receptors are localised mainly in the prostatic region of the tissue.

In the whole rat vas deferens, CPA inhibited nerve-mediated contractions via pre-junctional A_1 receptors, antagonised by a nanomolar concentration of DPCPX, whereas NECA inhibited nerve-mediated contractions via post-junctional A_2 receptors, antagonised by a micromolar concentration of DPCPX (Hourani et al., 1993). In the bisected tissue nerve-mediated contractions were inhibited by CPA in both regions, and CPA was acting via pre-junctional A_1 receptors as it was antagonised by a nanomolar concentration of DPCPX. However, the potency of CPA in activating the pre-junctional A_1 receptors in the epididymal region ($pD_2 = 7.4 \pm 0.1$) is almost 10-fold higher than that for the prostatic region ($pD_2 = 6.6 \pm 0.1$). This may be due to a larger population of pre-junctional A_1 receptors in the epididymal region when compared to the prostatic region, or may reflect differences in post-receptor events. Like CPA, NECA also inhibited nerve-mediated contractions in both regions of the bisected tissue, but unlike CPA the potency of NECA in inhibiting the nerve-mediated contractions was higher at the prostatic region ($pD_2 = 7.3 \pm 0.1$) than at the epididymal region ($pD_2 = 6.7 \pm 0.2$). The inhibitory effect of NECA on nerve-mediated responses was antagonised by a nanomolar concentration of DPCPX in the epididymal region, and this together with a potency order of CPA > NECA suggests the activation by NECA of pre-junctional A_1 receptors here. There was no antagonism of the effect of NECA by this concentration of DPCPX in the prostatic region, as previously

shown for the whole tissue (Hourani et al., 1993), suggesting that NECA was acting here via A_2 receptors not A_1. A micromolar concentration of DPCPX antagonised the response to NECA in both regions, and yielded an apparent pA_2 in the nanomolar range in the epididymal region, supporting the activation by NECA of pre-junctional A_1 receptors. However, in the prostatic region the apparent pA_2 for DPCPX was in the micromolar range, similar to the situation in the whole tissue, supporting the activation by NECA of post-junctional A_2 receptors here. In the whole tissue NECA activated A_2 receptors rather than pre- or post-junctional A_1 receptors (Hourani et al., 1993; Hourani and Jones, 1994), but the activation by NECA of pre-junctional A_1 receptors in the epididymal region of the bisected tissue can be explained by the lack of post-junctional A_2 receptors in this region of the rat vas deferens.

In conclusion, this work has shown a differential distribution of adenosine receptor subtypes along the length of the rat vas deferens, with pre- and post-junctional A_1 receptors being present over both regions of the bisected tissue, whereas post-junctional A_2 receptors were only present in the prostatic region.

Acknowledgements

We thank the Wellcome Trust (Grant 030318/Z/93/Z/1.5) for financial support. V.R.B. is a University of Surrey research scholar.

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