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Short communication

Breakdown of extracellular ATP by the prostatic and epididymal ends of the guinea pig vas deferens

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Abstract

Adenosine 5′-triphosphate (ATP) has a higher potency at the prostatic than at the epididymal end of the guinea pig vas deferens. We bisected the tissue and measured the breakdown of ATP by each half, and although the half-lives differed, the rate constants per gram of tissue were not significantly different. For a range of tissue portions, a correlation was found between the portion weight and the half-life of ATP. The difference in half-life at the two ends is therefore due to the different weights of the tissues, and the difference in potency of ATP cannot be explained by differences in degradation.

Keywords: Vas deferens; Ectonucleotidase; ATP; Purinergic

1. Introduction

In the vas deferens, there is considerable evidence that adenosine 5′-triphosphate (ATP) acts as a cotransmitter released with noradrenaline from sympathetic nerves to cause contraction of the tissue (Hoyle, 1992; Morris and Gibbins, 1992; Burnstock, 1995; Hourani et al., 1998). ATP causes a rapid transient contraction by acting at ionotropic $P2X_1$ receptors, while noradrenaline causes a slower and more sustained contraction via α_1-adrenoceptors (Morris and Gibbins, 1992; Valera et al., 1994). The non-adrenergic (purinergic) component of the response to sympathetic nerve stimulation is more pronounced at the prostatic end of the bisected vas deferens, while the adrenergic component is more marked at the epididymal end (McGrath, 1978). This difference, rather than reflecting different proportions of the co-transmitters being released at the two ends, probably reflects the fact that noradrenaline is more potent at the epididymal end while ATP is more potent at the prostatic end, and that the responses of the prostatic end are faster and more transient than those of the epididymal end (Sneddon and Machaly, 1992). Responses to adenosine also vary at the two ends, as although prejunctional inhibitory adenosine A_1 receptors and postjunctional stimulatory adenosine A_1 receptors are present at both ends, postjunctional inhibitory A_2 receptors are only present at the prostatic end of the rat vas deferens (Brownhill et al., 1996).

ATP is degraded by ectonucleotidases present in the vas deferens by sequential dephosphorylation to give ADP, AMP and adenosine which can be further metabolized by deamination to inosine or taken up into cells (Bailey and Hourani, 1994; see Plesner 1994; Ziganshin et al., 1994; Zimmermann et al., 1995 for reviews). There is also evidence suggesting that nucleotidases are released from the nerves upon electrical stimulation in the guinea pig vas deferens, with adenosine being the major breakdown product (Todorov et al., 1997). The breakdown of ATP may reduce its observed potency and generate adenosine which overall has inhibitory effects, and indeed inhibition of ecto-ATPase enhances responses to ATP and the purinergic component of nerve stimulation (Westfall et al., 1996). We therefore studied the breakdown of ATP by ectonucleotidases in the bisected guinea pig vas deferens, to see if this, like the receptor population, differed at the two ends. Some of these results have been previously published in abstract form (Tennant et al., 1997).

2. Materials and methods

Male Dunkin–Hartley guinea pigs (350–400 g) were killed by cervical dislocation and both vas deferens were

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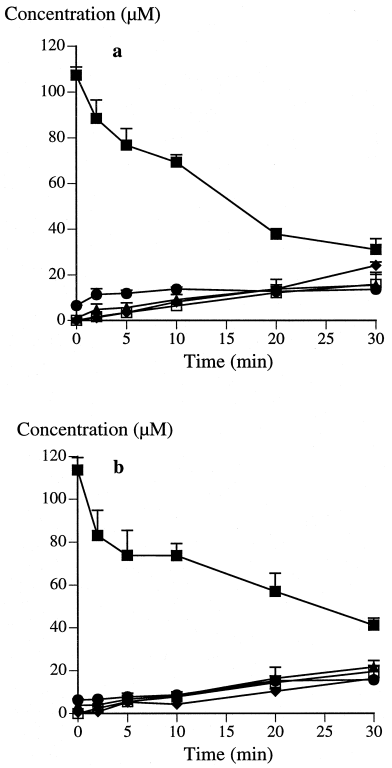

Fig. 1. Degradation of ATP (100 μM) (■) by (a) the prostatic and (b) the epididymal portions of the guinea pig vas deferens to ADP (●), AMP (▲), adenosine (♦) and inosine (□). Each point is the mean of at least three determinations and the vertical bars show S.E.M.

removed, freed from any connective tissue, and placed in warmed (37°C) Krebs solution of the following composition (mM): NaCl 118, KCl 4.8, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, and glucose 11, gassed with 95% O_2/5% CO_2. Each tissue was bisected or cut into portions as required, and the portions were blotted dry, weighed and placed in a separate tube containing 1.5 ml of warmed (37°C) aerated Krebs solution, and then equilibrated for 1 h. After equilibration, the tissue portions were transferred to tubes containing ATP (100 μM), and at various time points (0–30 min) aliquots (50 μl) were taken and placed immediately on ice prior to being frozen at −20°C for later analysis by high performance liquid chromatography (HPLC). This concentration of ATP was chosen to allow comparison with previous results, also because it represents a concentration at which ATP causes a reliable contractile response in this tissue (see, e.g., Sneddon and Machaly, 1992; Bailey and Hourani, 1994). HPLC analysis was carried out using a Shimadzu LC 10AT system with a Techsphere 5 μm ODS C_{18} column (25 cm × 4.6 mm) eluted with 0.1 M KH_2PO_4/8 mM tetrabutylammonium hydrogen sulphate (pH 6.0) (solvent A) and a 60:40 mixture of solvent A and acetonitrile (pH 6.73) (solvent B) using a non-linear gradient (0–2.5 min 0% B, 2.5–5 min 0–20% B, 5–10 min 20–40% B, 10–13 min 40–100% B, 13–18 min 100% B) at a flow rate of 1.3 ml min^{-1}. Nucleotides and nucleosides were detected by UV absorbance (259 nm) and quantified by the area of their absorbance peaks, which was linearly related to their concentration. The results were displayed graphically as the mean and S.E.M. of the concentrations at the various time points. A half-life was calculated for each tissue portion from the linear plot of log concentration vs. time; in each case, the correlation coefficient indicated a significant linear relationship between time and log concentration ($P < 0.01$). A rate constant was calculated for each portion as 0.69/half-life, to allow for comparison of rates of breakdown after adjustment for the weight of the tissue. The mean and S.E.M. of the half-lives and the rate constants for each half of the bisected tissue were compared by Student's unpaired *t*-test.

ATP, ADP, AMP, adenosine, inosine and tetrabutylammonium hydrogen sulphate were obtained from Sigma, Poole, UK. Acetonitrile (HPLC grade) and all buffer salts (analytical grade) were obtained from Fisher Scientific, Loughborough, UK.

3. Results

ATP (100 μM) was dephosphorylated by both the prostatic and epididymal portions of the guinea pig vas deferens, with ADP, AMP, adenosine and inosine being detected (Fig. 1). The half-life of ATP in the presence of the prostatic portion was 16.4 ± 1.9 min, whereas that for the epididymal portion was 24.0 ± 3.8 min ($P < 0.05$). The rate constants for degradation of ATP at the prostatic and epididymal ends were 0.042 ± 0.005 and 0.029 ± 0.004 min^{-1}, respectively ($P < 0.05$). Taking into account the weights of the tissues (35 ± 1 and 18 ± 1 mg, respectively), the rate constants per gram were 1.2 ± 0.15 and

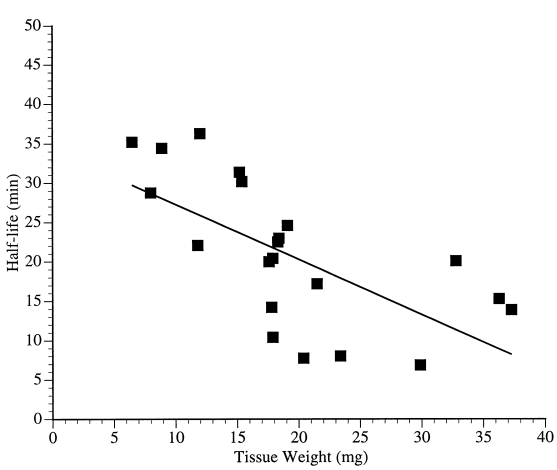

Fig. 2. The relationship between the half-life for the degradation of ATP (100 μM) by different portions of the guinea pig vas deferens and the wet weight of the portion. The line shows the fitted regression line, which has the equation $y = -0.699x + 34.288$ and a correlation coefficient (r) of 0.647.

1.7 ± 0.24 min^{-1} g^{-1}, respectively. These values are not significantly different ($P > 0.05$).

When the vas deferens were cut into unequal lengths and the breakdown by these portions were measured and compared to their weight regardless of whether the portions came from the epididymal or prostatic end, over the range of weights tested a highly significant correlation ($r = 0.647$, $P < 0.01$) was found between the weight of the tissue portion and the half-life of ATP in the presence of that tissue portion (Fig. 2).

4. Discussion

The pattern of breakdown of ATP was very similar in both the prostatic and epididymal portions of the guinea pig vas deferens, with both adenosine and inosine being detected. However, the rate of breakdown of ATP was greater in the prostatic than in the epididymal end, suggesting that different rates of breakdown cannot be responsible for the differences in potency of ATP which have been reported previously (Sneddon and Machaly, 1992). If anything, this difference in breakdown would tend to reduce any differences in potency as the more rapid breakdown of ATP at the prostatic end would be expected to reduce its observed potency there even more than at the epididymal end. The idea that different rates of breakdown are not responsible for the difference in potency was suggested by Sneddon and Machaly (1992) based on their finding that adenosine 5′-(α,β-methylenediphosphonate), a stable analogue of ATP, is also more potent at the prostatic than at the epididymal end, and our findings therefore support this suggestion. The difference in potency may therefore reflect a greater density of $P2X_1$ receptors at the prostatic end of the tissue.

The difference in the rate of breakdown at the two ends of the vas deferens does not appear to be due to any true difference in the levels of enzymes present at the two ends, but rather to reflect the fact that the prostatic end of the tissue is considerably larger than the epididymal end as the differences in the rate constants for breakdown disappeared when these were expressed in terms of the wet weight of the tissue. Indeed, comparison of the half-life for ATP in portions of vas deferens of different sizes showed a good correlation between tissue weight and half-life, suggesting that the difference between the two ends is indeed simply a reflection of the greater size of the prostatic end. A similar relationship between tissue weight and the rate of hydrolysis of ATP was found in segments of the rat mesenteric artery (Plesner, 1997). There does not, therefore, appear to be a differential distribution of ectonucleotidases at the two ends of the tissue, in contrast to the differential distribution of receptors for ATP, noradrenaline and adenosine (Sneddon and Machaly, 1992; Brownhill et al., 1996). The amount of adenosine generated extracellularly from neuronally-released ATP is likely therefore to be constant throughout the length of the vas deferens, although the responses to both the ATP and the adenosine may be different.

Acknowledgements

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