*Br. J. Pharmacol.* (1993), **110**, 1465–1472 

# Prejunctional modulation of noradrenaline release in mouse and rat vas deferens: contribution of $P_1$- and $P_2$-purinoceptors

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**1** Prejunctional purinoceptors modulating the release of noradrenaline were compared in mouse and rat vas deferens. Tissue slices were preincubated with [$^3$H]-noradrenaline and then superfused and stimulated electrically, in most experiments by trains of 60 pulses, 1 Hz.

**2** In mouse vas deferens, 2-chloroadenosine ($IC_{50}$ 0.24 μM), β,γ-methylene-ATP ($IC_{50}$ 3.8 μM), α,β-methylene-ATP ($IC_{50}$ 2.9 μM) and 2-methylthio-ATP (only 30 μM tested) reduced the evoked overflow of tritium. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), 10 nM, antagonized the effect of 2-chloroadenosine (apparent $pK_B$ 10.2) as well as of β,γ-methylene-ATP (apparent $pK_B$ 9.6) and α,β-methylene-ATP. Suramin, 300 μM, attenuated the effect of 2-chloroadenosine at best very slightly, antagonized the effect of β,γ-methylene-ATP (apparent $pK_B$ 4.5) and, when combined with DPCPX 10 nM, caused a further marked shift to the right of the concentration-response curve of β,γ-methylene-ATP beyond the shift produced by DPCPX alone.

**3** In rat vas deferens, 2-chloroadenosine ($IC_{50}$ 0.20 μM), β,γ-methylene-ATP ($IC_{50}$ 4.8 μM), α,β-methylene-ATP ($IC_{50}$ 3.0 μM) and 2-methylthio-ATP (only 30 μM tested) also reduced the evoked overflow of tritium. DPCPX, 10 nM, antagonized the effect of 2-chloroadenosine (apparent $pK_B$ 9.7) as well as of β,γ-methylene-ATP (apparent $pK_B$ 9.6) and α,β-methylene-ATP. Suramin, 300 μM, did not change the effect of 2-chloroadenosine, attenuated the effect of β,γ-methylene-ATP at best very slightly and, when combined with DPCPX, caused at best a very small shift to the right of the concentration-response curve of β,γ-methylene-ATP beyond the shift produced by DPCPX alone.

**4** It is concluded that prejunctional purinoceptor mechanisms in mouse and rat vas deferens are similar. In either species, both nucleosides such as adenosine and nucleotides such as β,γ-methylene-ATP activate a common release-inhibiting receptor which is a $P_1$- or, more specifically, $A_1$-purinoceptor. There seems to be no need to postulate the existence of a novel prejunctional $P_3$-purinoceptor. Moreover, the sympathetic terminal axons possess an additional $P_2$-purinoceptor in both species which is activated by some nucleotides such as β,γ-methylene-ATP and 2-methylthio-ATP, although the activation of the $P_2$-purinoceptor by β,γ-methylene-ATP is difficult to demonstrate in the rat.

**Keywords:** Mouse vas deferens; rat vas deferens; $P_1$-purinoceptor; $P_2$-purinoceptor; $P_3$-purinoceptor; prejunctional receptors; noradrenaline release; adenine nucleotides; 2-chloroadenosine; β,γ-methylene-ATP

## Introduction

Adenosine inhibits action potential-evoked neuronal release of noradrenaline (Hedqvist & Fredholm, 1976; reviewed by Fredholm & Hedqvist, 1980; Stone, 1981; Olsson & Pearson, 1990). The same holds true for nucleotides such as ATP (Fredholm, 1974; Clanachan *et al.*, 1977; Enero & Saidman, 1977). It was thought for some time that the nucleotides act only indirectly, by way of their degradation product adenosine (e.g. Clanachan *et al.*, 1977; Stone, 1985). However, direct effects on noradrenergic axons are now established (Lukacsko & Blumberg, 1982; Shinozuka *et al.*, 1988; von Kügelgen *et al.*, 1989; 1992a; Forsyth *et al.*, 1991; Fuder *et al.*, 1992). Where do adenosine and the nucleotides act?

It has been suggested that two separate prejunctional, release-inhibiting purinoceptors operate in the vas deferens of the mouse: a classical $P_1$-purinoceptor, activated mainly but not exclusively by the nucleotide adenosine, and a $P_2$-purinoceptor, activated only by nucleotides (von Kügelgen *et al.*, 1989). The main evidence was that adenosine, ATP, its metabolically more stable derivative adenosine 5'-O-(3-thio)-triphosphate (ATPγS) and UTP all inhibited the release of noradrenaline; that the $P_1$-purinoceptor antagonist 8-(*p*-sulphophenyl)theophylline attenuated the effect of adenosine much more than the effects of ATP and ATPγS; that the $P_2$-purinoceptor antagonist suramin (Dunn & Blakeley, 1988) attenuated only the effect of ATPγS but not that of adenosine; and that the effect of the nucleotide UTP was attenuated by both 8-(*p*-sulphophenyl)theophylline and suramin. In contrast, in rat tail artery and vas deferens, it has been suggested that adenosine and nucleotides act through a common 'hybrid' prejunctional purinoceptor of a novel type, called $P_3$ (Shinozuka *et al.*, 1988; 1990; Forsyth *et al.*, 1991). The main evidence was that adenosine and 2-chloroadenosine as well as ATP, its metabolically more stable derivative β,γ-methylene-ATP and UTP all reduced the release of noradrenaline; and that 8-(*p*-sulphophenyl)theophylline and α,β-methylene-ATP, known as a $P_{2X}$-purinoceptor agonist, both acted as antagonists against nucleosides as well as nucleotides. In the experiments of Shinozuka *et al.* (1988, 1990), von Kügelgen *et al.* (1989) and Forsyth *et al.* (1991), release of noradrenaline was measured as overflow. Similar previous suggestions, either of a common prejunctional receptor for nucleosides and nucleotides (Lukacsko & Blumberg, 1982) or of separate prejunctional $P_1$- and $P_2$-purinoceptors (Taylor *et al.*, 1983), were based on postjunctional response measurements which are ambiguous, mainly due to possible desensitization by nucleotides of postjunctional $P_2$-purinoceptors (p. 437 of Burnstock & Kennedy, 1985).

The suggestion of different prejunctional purinoceptor mechanisms in the same tissue – vas deferens – of the rat and the mouse is intriguing. Is there in fact a species difference? Or are the prejunctional purinoceptors in the two species more similar than proposed? In order to answer the question, we compared the effects of 2-chloroadenosine, β,γ-methylene-ATP, α,β-methylene-ATP, the selective $P_{2Y}$-purinoceptor agonist 2-methylthio-ATP, the $P_1$ antagonist 8-cyclopentyl-

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1,3-dipropylxanthine (DPCPX) and suramin on the release of [$^3$H]-noradrenaline in slices of mouse and rat vas deferens under identical conditions. Some of these results have been reported to the Deutsche Gesellschaft für Pharmakologie und Toxikologie (Kurz *et al.*, 1993).

## Methods

Male NMRI mice weighing 35–40 g or male Wistar rats weighing 250–300 g (Savo, Kisslegg, Germany) were killed by cervical dislocation and exsanguination. The vasa deferentia were dissected out and desheathed. Slices, 5–6 mg, were prepared from the prostatic portions. Four slices were preincubated at 37°C for 30 min in each of two vials containing 2 ml medium with (−)-[$^3$H]-noradrenaline 0.1 μM, specific activity 1.62–2.11 TBq mmol$^{-1}$. Following incubation with [$^3$H]-noradrenaline, the slices were washed three times with 30 ml [$^3$H]-noradrenaline-free medium. One slice was transferred to each of eight glass superfusion chambers where it was held by a polypropylene mesh between platinum plate electrodes, 18 mm apart. The slices were superfused with [$^3$H]-noradrenaline-free medium at a rate of 1.25 ml min$^{-1}$ at 37°C. A Stimulator I (Hugo Sachs Elektronik, March-Hugstetten, Germany) operating in the constant mode was used for electrical field stimulation. Six periods of stimulation were applied (rectangular pulses of 1 ms width and 50 mA current strength). The first, delivered after 30 min of superfusion (18 pulses, 1 Hz), was not used for determination of tritium outflow. The following stimulation periods ($S_1$–$S_5$) began after 96, 117, 138, 159 and 180 min of superfusion; each consisted of 60 pulses delivered at 1 or 8 Hz (identical parameters at $S_1$–$S_5$ in each single experiment); unless stated otherwise, the frequency was 1 Hz. The collection of successive 3-min superfusate samples began 6 min before $S_1$. Adenosine deaminase, DPCPX, suramin and (when used for interaction experiments) α,β-methylene-ATP were added to the medium 36 min before $S_1$ and maintained for the remainder of the experiment. Other drugs (including α,β-methylene-ATP in some experiments) were added either at a constant concentration from 3 or 6 min before $S_3$ for the remainder of the experiment, or at increasing concentrations from 6 min before to 15 min after the onset of $S_3$, $S_4$ and $S_5$; the increasing concentrations differed between slices so that concentration-response curves in Figures 3–7 are based on up to seven concentrations. After superfusion, each slice was solubilized in 0.5 ml Soluene-350 (Canberra Packard, Frankfurt am Main, Germany). Tritium was measured in superfusate samples and solubilized slices by liquid scintillation counting.

The superfusion medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, $KH_2PO_4$ 0.9, glucose 11, ascorbic acid 0.3 and disodium EDTA 0.03. The medium used for preincubation contained $CaCl_2$ 0.2 mM instead of $CaCl_2$ 2.5 mM because otherwise the basal efflux of tritium increased during the course of the experiment (see also Limberger *et al.*, 1992). Media were saturated with 5% $CO_2$ in $O_2$. The superfusion but not the preincubation medium also contained desipramine 1 μM, corticosterone 10 μM and yohimbine 1 μM in order to block the cellular uptake of [$^3$H]-noradrenaline and $\alpha_2$-autoreceptors, respectively.

The outflow of tritium was expressed as fractional rate (min$^{-1}$), i.e., (tritium outflow per 3 min)/[3(tritium present in the slice at the onset of the respective collection period)]. The electrically evoked overflow was calculated as the difference between the total overflow during the 12 min after onset of stimulation, and the estimated basal outflow; the basal outflow was assumed to decline linearly from the 3-min interval before, to the interval 12–15 min after, the start of stimulation. The difference (total minus basal; Bq) was expressed as a percentage of the tritium content (Bq) of the slice at the onset of stimulation. $S_2$ served as the reference stimulation period in each tissue slice. The ratio of the overflow of tritium evoked by $S_1$ to the overflow evoked by $S_2$ was calculated as an internal control. Only experiments with an $S_1/S_2$ ratio of 0.9 to 1.1 were evaluated further. Effects of drugs that were added before $S_3$ to $S_5$ on basal tritium efflux were evaluated as ratios of the fractional rate of outflow immediately before $S_3$, $S_4$ and $S_5$ and immediately before $S_2$ ($b_n/b_2$). Effects of drugs that were added before $S_3$ to $S_5$ on electrically evoked overflow were evaluated as ratios of the overflow elicited by $S_3$, $S_4$ and $S_5$ and the overflow elicited by $S_2$ ($S_n/S_2$). $S_n/S_2$ ratios obtained in individual tissue slices were calculated as a percentage of the respective mean ratio in the appropriate control group (solvent added before $S_3$ to $S_5$; '% of control' in Figure 1 and Figures 3–7).

For calculation of maximal inhibitions produced by 2-chloroadenosine, β,γ-methylene-ATP and α,β-methylene-ATP and of their $IC_{50}$ values (concentrations that caused 50% of the maximal inhibition), the sigmoid-shaped function No. 25 of Waud (1976) was fitted to the averaged concentration-response data (Figures 3–7). This function was also fitted to averaged agonist concentration-response data from experiments carried out in the presence of antagonists (DPCPX or suramin); since maximal effects of agonists often were not reached in the presence of antagonists (e.g. Figure 6a), the maximal inhibition was taken as that obtained in the absence of antagonist. DPCPX and suramin $pK_B$ ($-\log K_B$) values were calculated from the increase in $IC_{50}$ values (equation No. 16 of Waud, 1976); since only one antagonist concentration was tested and a competitive character of the antagonism was not verified, the values are apparent $pK_B$ values.

The following drugs were used: suramin hexasodium salt (Bayer, Wuppertal, Germany), (−)-[ring-2,5,6-$^3$H]-noradrenaline (Du Pont, Dreieich, Germany), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 2-methylthioadenosine 5′-triphosphate tetrasodium salt (2-methylthio-ATP) (Research Biochemicals, Natick, MA, U.S.A.), yohimbine HCl (Roth, Karlsruhe, Germany), adenosine deaminase type VII from calf intestinal mucosa (EC 3.5.4.4), 2-chloroadenosine, corticosterone, desipramine HCl, α,β-methylene adenosine 5′-triphosphate lithium salt (α,β-methylene-ATP, APCPP), β,γ-methylene adenosine 5′-triphosphate sodium salt (β,γ-methylene-ATP, APPCP), tetrodotoxin (Sigma, Deisenhofen, Germany). Solutions of drugs were prepared with either distilled water or (corticosterone) 1,2-propanediol (Sigma; final concentration about 8.7 mM) or (DPCPX) dimethyl sulphoxide (Roth; final concentration about 0.4 mM) or (tetrodotoxin) sodium acetate buffer (0.1 M, pH 4.8). Dimethyl sulphoxide was added to superfusion media 36 min before $S_1$ (when administration of DPCPX began) in all experiments except those of Figure 1; since it caused no change, $b_2$ and $S_2$ values with and without dimethyl sulphoxide were pooled.

Means ± s.e.mean are given throughout. Differences between means were tested for significance by the Mann-Whitney test. $P < 0.05$ or lower was taken as the criterion of statistical significance. For multiple comparisons with the same control, $P$ levels were adjusted according to Bonferroni. $n$ is the number of tissue slices.

## Results

Slices of mouse or rat vas deferens were preincubated with [$^3$H]-noradrenaline and then superfused with medium containing desipramine 1 μM, corticosterone 10 μM and yohimbine 1 μM. Electrical stimulation by 60 pulses, 1 Hz markedly increased the outflow of tritium (see Figure 2 below). Values for the overflow elicited by the reference stimulation period $S_2$ are summarized in Table 1. When no drug was present (except desipramine, corticosterone and yohimbine), the overflow at $S_2$ averaged 1.009% of tissue tritium in the mouse and 0.774% in the rat. Adenosine deaminase (not tested in the mouse), DPCPX and suramin, as well as DPCPX and suramin combined, increased the evoked overflow of tritium (except DPCPX in the mouse), whereas α,β-methylene-ATP caused a decrease. The fractional rate of

**Table 1** Electrically evoked overflow of tritium from slices of mouse and rat vas deferens

| *Drugs added 36 min before $S_1$* | *Mouse* $S_2$ (% of tissue tritium) | *n* | *Rat* $S_2$ (% of tissue tritium) | *n* |
|---|---|---|---|---|
| – | 1.009 ± 0.032[a] | 99 | 0.774 ± 0.019[b] | 120 |
| Adenosine-deaminase 0.3 u ml$^{-1}$ | | | 0.979 ± 0.019** | 15 |
| DPCPX 10 nM | 0.999 ± 0.028 | 59 | 0.845 ± 0.026* | 43 |
| Suramin 300 μM | 1.234 ± 0.047** | 33 | 0.963 ± 0.028** | 53 |
| DPCPX 10 nM + Suramin 300 μM | 1.366 ± 0.086** | 15 | 0.959 ± 0.062** | 17 |
| α,β-Methylene-ATP 30 μM | 0.786 ± 0.030** | 39 | 0.657 ± 0.024** | 33 |

$S_2$ is the second period of electrical stimulation (60 pulses, 1 Hz). DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. Means ± s.e.mean of *n* experiments.
[a]Corresponding to 30.4 ± 1.4 Bq.
[b]Corresponding to 41.1 ± 1.6 Bq.
Significant differences from corresponding experiments without drugs added before $S_1$: $*P<0.05$ and $**P<0.01$.

tritium efflux immediately before $S_2$ ($b_2$) was 0.00211 ± 0.00006 min$^{-1}$ ($n = 99$) in the mouse and 0.00162 ± 0.00003 min$^{-1}$ ($n = 120$) in the rat. It was slightly reduced by the compounds listed in Table 1 except adenosine deaminase and DPCPX (rat; not shown).

In experiments without drugs (except desipramine, corticosterone and yohimbine), the basal outflow of tritium as well as the overflow response to electrical stimulation remained fairly constant (see Figure 2a and 2d below). In mouse vas deferens, the $b_3/b_2$, $b_4/b_2$, and $b_5/b_2$ ratios were 0.95 ± 0.02, 0.92 ± 0.02 and 0.95 ± 0.04, and $S_3/S_2$, $S_4/S_2$, and $S_5/S_2$ ratios were 0.99 ± 0.02, 0.97 ± 0.05 and 0.94 ± 0.06, respectively ($n = 8$). Average $b_n/b_2$ ratios also were slightly below unity, and $S_n/S_2$ ratios close to unity, when the compounds listed in Table 1 were present in the medium from 36 min before $S_1$ but no second drug was added later (not shown). The same was true for rat vas deferens.

In initial experiments, 2-chloroadenosine, 2-methylthio-ATP, α,β-methylene-ATP and β,γ-methylene-ATP were added 6 min before $S_3$ at a concentration of 30 μM and maintained throughout. All reduced the evoked overflow of tritium in both mouse and rat vas deferens, and for all the effect was approximately constant from $S_3$, after 6 min of exposure, to $S_5$, after 48 min of exposure (Figure 1; β,γ-methylene-ATP not tested in the mouse). 2-Chloroadenosine caused similar inhibition, irrespective of whether it was added 6 min (Figure 1) or 3 min before $S_3$ (not shown), and the same was true for α,β-methylene-ATP ($n = 3$–4 for the 3-min experiments). The effects of α,β- and β,γ-methylene-ATP were not changed when the superfusion medium contained adenosine deaminase (rat; Figure 1c). Tetrodotoxin practically abolished the overflow response (Figure 1). An exposure time of 6 min before the respective stimulation period was chosen for all subsequent experiments.

When added before $S_3$, $S_4$ and $S_5$ at increasing concentrations, 2-chloroadenosine progressively reduced the evoked overflow of tritium (mouse: Figure 2b; rat: Figure 2e). The $IC_{50}$ (maximal inhibition) values obtained by fitting a sigmoid to the averaged concentration-response curves were 0.24 μM (47%) in mouse vas deferens slices (closed symbols in Figure 3) and 0.20 μM (70%) in rat vas deferens slices (closed symbols in Figure 4). In both species, DPCPX 10 nM caused a large shift of the concentration-response curve to the right without a depression of the maximal inhibition (Figures 3a and 4a). The apparent $pK_B$ value (see Methods) of DPCPX against 2-chloroadenosine was 10.2 in the mouse and 9.7 in the rat. In contrast to DPCPX, suramin 300 μM caused little if any change of the concentration-response curve of 2-chloroadenosine in the mouse (Figure 3b) and no change whatsoever in the rat (Figure 4b). α,β-Methylene-ATP 30 μM antagonized the effect of 2-chloroadenosine in the mouse, apparently with a depression of the maximal inhibition, but not, or not significantly, in the rat (Figures 3c and 4c).

β,γ-Methylene-ATP also progressively reduced the evoked overflow of tritium when added before $S_3$, $S_4$ and $S_5$ at increasing concentrations (mouse: Figure 2c; rat: Figure 2f). The $IC_{50}$ (maximal inhibition) values were 3.8 μM (48%) in mouse vas deferens slices (closed symbols in Figure 5) and 4.8 μM (84%) in rat vas deferens slices (closed symbols in

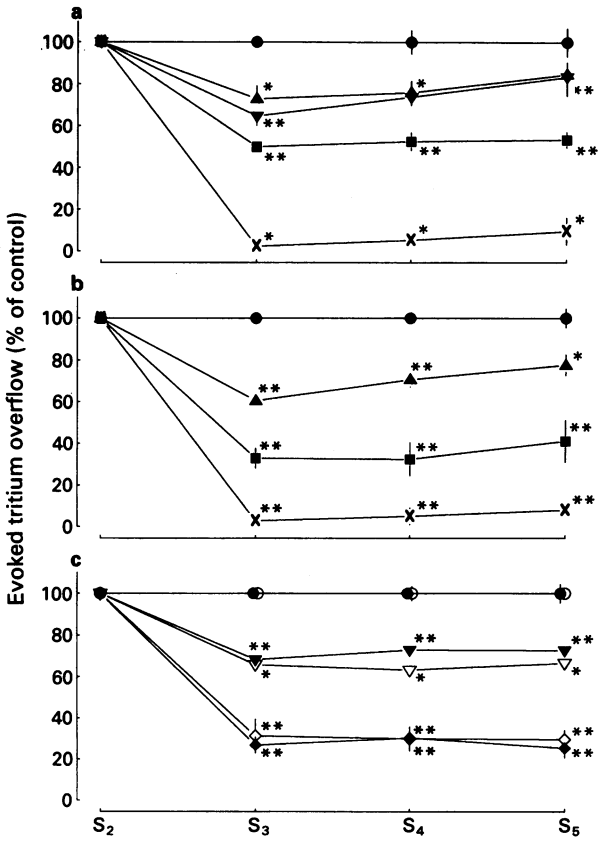


**Figure 1** Effects of purinoceptor agonists and tetrodotoxin on electrically evoked overflow of tritium from slices of mouse (a) and rat vas deferens (b, c). Slices were stimulated five times by 60 pulses, 1 Hz ($S_1$–$S_5$). Solvent (●, ○), 2-chloroadenosine 30 μM (■), 2-methylthio-ATP 30 μM (▲), α,β-methylene-ATP 30 μM (▼, ▽), β,γ-methylene-ATP 30 μM (◆, ◇) or tetrodotoxin 0.5 μM (X) was added 6 min before $S_3$ for the remainder of the experiment. Open symbols (c) represent experiments in which the medium contained adenosine deaminase 0.3 u ml$^{-1}$ from 36 min before $S_1$ onwards. Ordinates, evoked tritium overflow: $S_n/S_2$ ratios obtained in individual tissue slices were calculated as a percentage of the corresponding mean $S_n/S_2$ ratio in the appropriate control (solvent) group. Means ± s.e.mean of 4–11 experiments. Significant differences from corresponding control (solvent): $*P<0.05$ and $**P<0.01$.

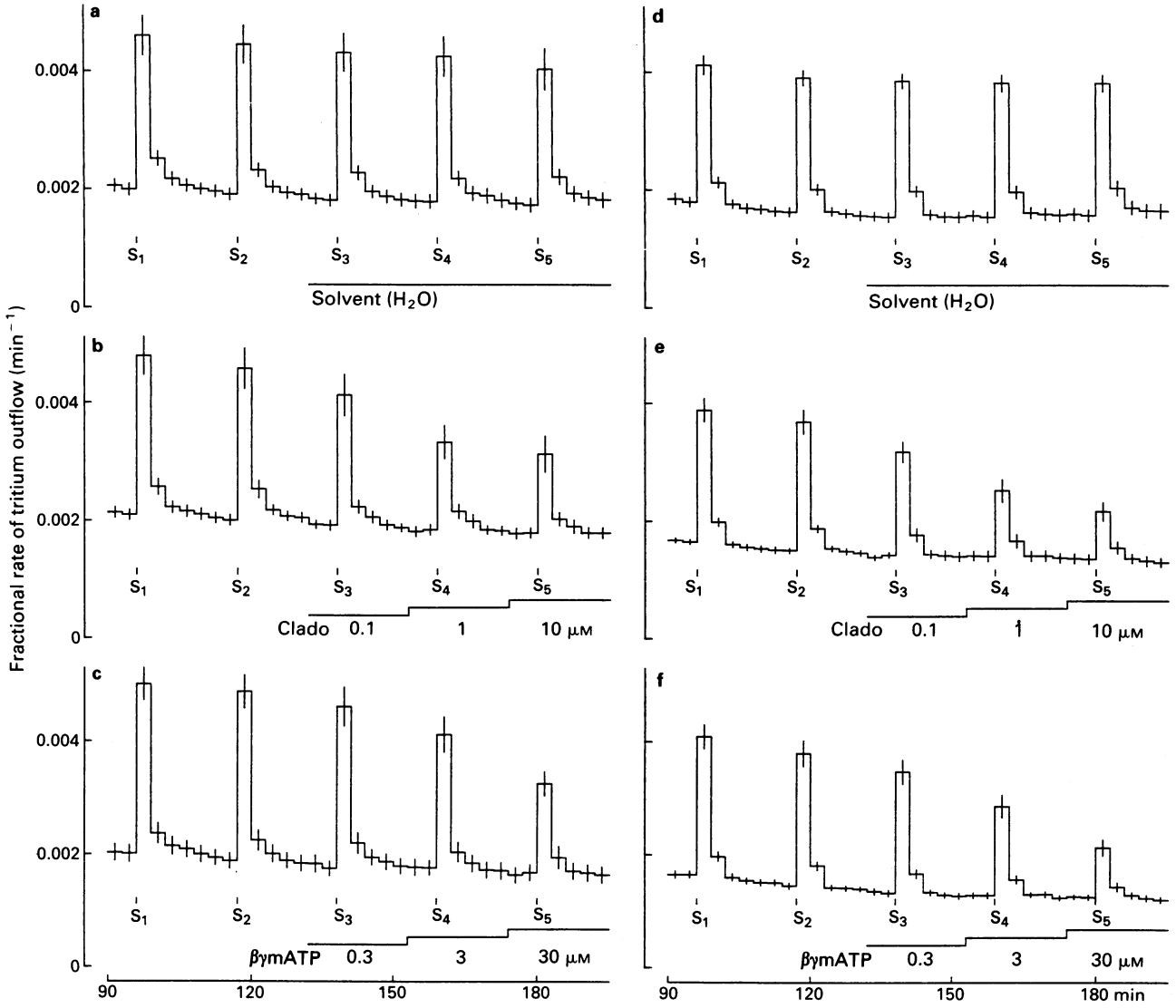


**Figure 2** Time course of tritium outflow from slices of mouse (a–c) and rat (d–f) vas deferens and effects of 2-chloroadenosine (b, e, Clado) and β,γ-methylene-ATP (c, f, βγmATP). Slices were stimulated five times with 60 pulses, 1 Hz ($S_{1-5}$). Solvent, 2-chloroadenosine and β,γ-methylene-ATP were added as indicated. Means ± s.e.mean of 10–15 experiments.

Figure 6). As in the case of 2-chloroadenosine, DPCPX (10 nM) shifted the concentration-response curve of β,γ-methylene-ATP to the right in both species (open diamonds in Figures 5a and 6a). The apparent $pK_B$ value (see Methods) of DPCPX against β,γ-methylene-ATP was 9.6 in both species, similar to the apparent $pK_B$ values against 2-chloroadenosine. Suramin (300 μM) displayed greater antagonist activity against β,γ-methylene-ATP than against 2-chloroadenosine at least in the mouse: there was a clear shift of the concentration-response curve of β,γ-methylene-ATP, with an apparent $pK_B$ of 4.5 (Figure 5b); in the rat, the antagonism was questionable (Figure 6b). α,β-Methylene-ATP (30 μM) antagonized the effect of β,γ-methylene-ATP in the mouse, but not, or not significantly, in the rat (Figures 5c and 6c) as had been the case for 2-chloroadenosine. The effect of DPCPX (10 nM) combined with suramin (300 μM) against β,γ-methylene-ATP was also examined. In the mouse, the combination caused a large shift beyond that produced by DPCPX alone (open circles in Figure 5a), approximately as large as the shift produced by suramin (300 μM) alone (Figure 5b). In the rat, there was at best a very small shift beyond that produced by DPCPX alone (open circles in Figure 6a).

Finally, concentration-response curves were determined for α,β-methylene-ATP. The $IC_{50}$ (maximal inhibition) values were 2.9 μM (27%) in the mouse (closed symbols in Figure 7a) and 3.0 μM (33%) in the rat (closed symbols in Figure 7b). DPCPX (10 nM) abolished the effect in both species (Figure 7). In some experiments, the 60 pulses of each stimulation period were applied at 8 instead of 1 Hz. The overflow of tritium at $S_2$ was then 1.023% of the tritium content of the tissue in mouse vas deferens ($n = 16$; as compared with 1.009% at 1 Hz; Table 1) and 1.197% in rat vas deferens ($n = 12$; as compared with 0.774% at 1 Hz; Table 1). α,β-Methylene-ATP 3, 30 and 300 μM, when administered before $S_3$, $S_4$ and $S_5$, failed to change the overflow of tritium evoked by 60 pulses, 8 Hz in both mouse and rat ($n = 6–10$).

2-Chloroadenosine (Figure 2b and e), β,γ-methylene-ATP (Figure 2c and f) and α,β-methylene-ATP did not change the basal efflux of tritium ($b_n/b_2$) except for a decrease caused by α,β-methylene-ATP (300 μM) in the rat (not shown).

## Discussion

The results indicate that the prejunctional purinoceptor mechanisms in mouse and rat vas deferens, although not identical, are similar.

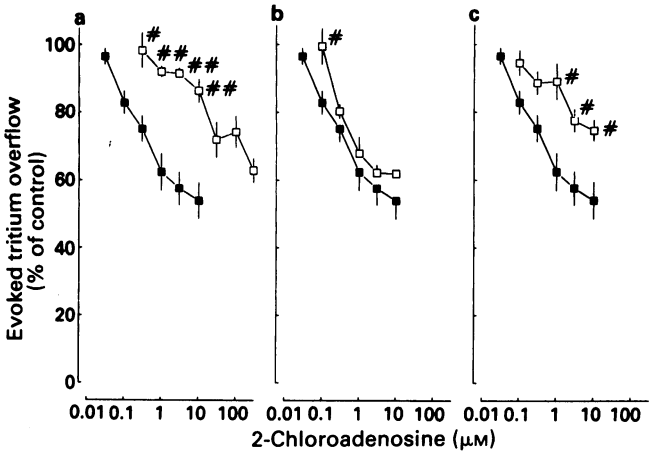


**Figure 3** Interaction of 2-chloroadenosine with 8-cyclopentyl-1,3-dipropylxanthine (a, DPCPX), suramin (b) and α,β-methylene-ATP (c) on electrically evoked tritium overflow from slices of mouse vas deferens. Slices were stimulated five times with 60 pulses, 1 Hz. 2-Chloroadenosine was added at increasing concentrations from 6 min before to 15 min after the onset of $S_3$, $S_4$ and $S_5$. Closed symbols represent experiments in which 2-chloroadenosine was given alone. Open symbols represent experiments in which the medium contained DPCPX 10 nM (a), suramin 300 μM (b) or α,β-methylene-ATP 30 μM (c) from 36 min before $S_1$ onwards. Abscissae, concentration of 2-chloroadenosine. Ordinates, evoked tritium overflow: $S_n/S_2$ ratios obtained in individual tissue slices were calculated as a percentage of the corresponding mean $S_n/S_2$ ratio in the appropriate control group (solvent instead of 2-chloroadenosine). Means ± s.e.mean of 5–11 experiments. Significant differences from experiments without DPCPX, suramin and α,β-methylene-ATP: $\#P<0.05$ and $\#\#P<0.01$.

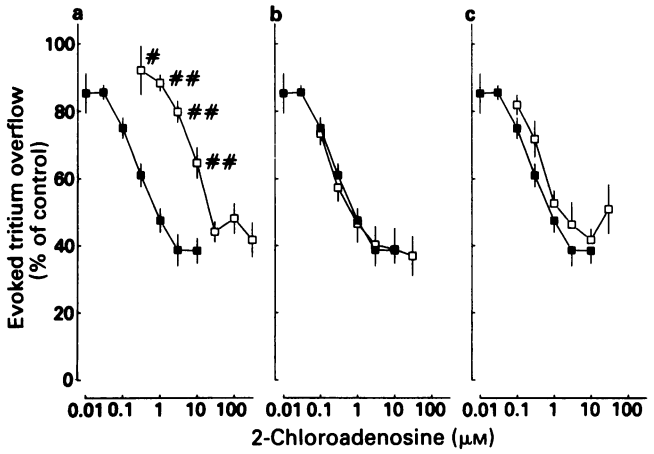


**Figure 4** Interaction of 2-chloroadenosine with 8-cyclopentyl-1,3-dipropylxanthine (a, DPCPX), suramin (b) and α,β-methylene-ATP (c) on electrically evoked tritium overflow from slices of rat vas deferens. Means ± s.e.mean of 4–13 experiments. Other details as explained in legend to Figure 3.

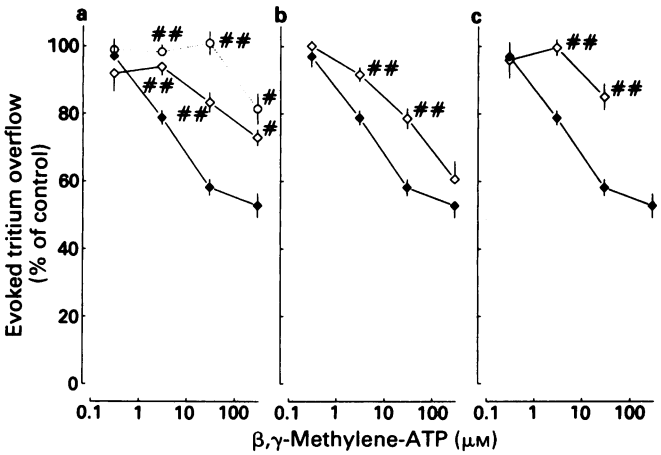


**Figure 5** Interaction of β,γ-methylene-ATP with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or DPCPX combined with suramin (a), suramin (b) and α,β-methylene-ATP (c) on electrically evoked tritium overflow from slices of mouse vas deferens. Slices were stimulated five times with 60 pulses, 1 Hz. β,γ-Methylene-ATP was added at increasing concentrations from 6 min before to 15 min after the onset of $S_3$, $S_4$ and $S_5$. Closed symbols represent experiments in which β,γ-methylene-ATP was given alone. Open diamonds represent experiments in which the medium contained DPCPX 10 nM (a), suramin 300 μM (b) or α,β-methylene-ATP 30 μM (c) from 36 min before $S_1$ onwards. Open circles represent experiments in which the medium contained both DPCPX 10 nM and suramin 300 μM from 36 min before $S_1$ onwards (a). Abscissae, concentration of β,γ-methylene-ATP. Ordinates, evoked tritium overflow: $S_n/S_2$ ratios obtained in individual tissue slices were calculated as a percentage of the corresponding mean $S_n/S_2$ ratio in the appropriate control group (solvent instead of β,γ-methylene-ATP). Means ± s.e.mean of 5–17 experiments. Significant differences from experiments without DPCPX, suramin and α,β-methylene-ATP: $^{\#}P<0.05$ and $^{\#\#}P<0.01$.

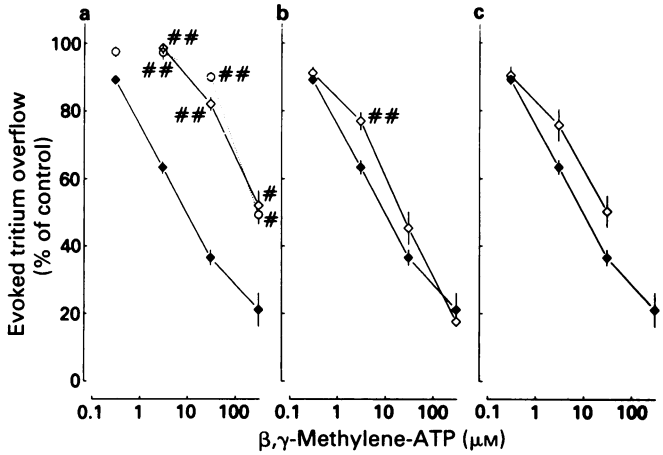


**Figure 6** Interaction of β,γ-methylene-ATP with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) or DPCPX combined with suramin (a), suramin (b) and α,β-methylene-ATP (c) on electrically evoked tritium overflow from slices of rat vas deferens. Means ± s.e.mean of 5–17 experiments. Other details as explained in legend to Figure 5.

*Mouse vas deferens*

The operation of prejunctional $P_1$-purinoceptors in mouse vas deferens (von Kügelgen *et al.*, 1989) is confirmed in the present study by the agonist effect of 2-chloroadenosine (see Blakeley *et al.*, 1988) and the antagonism exerted against this effect by DPCPX (Figure 3a). DPCPX is highly selective for the $A_1$ subtype of $P_1$-purinoceptor. Its apparent $pK_B$ value against 2-chloroadenosine (10.2) is similar to values found at $A_1$-receptors (Bruns *et al.*, 1987; Lohse *et al.*, 1987; Sebastião *et al.*, 1990) and, hence, identifies the prejunctional receptor as $A_1$. DPCPX also, and with a very similar apparent $pK_B$ value (9.6), antagonized the effect of β,γ-methylene-ATP, indicating that β,γ-methylene-ATP also acted to a great extent through the $A_1$-receptor (Figure 5a). The $A_1$ character of the receptor will be discussed further in the section on the rat vas deferens.

The results obtained with β,γ-methylene-ATP also confirm

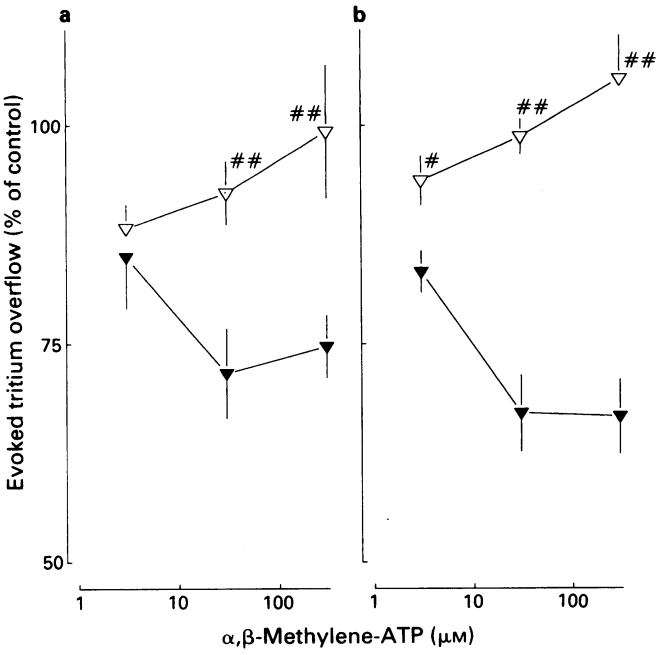


**Figure 7** Interaction of α,β-methylene-ATP with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on electrically evoked tritium overflow from slices of mouse (a) and rat vas deferens (b). Slices were stimulated five times with 60 pulses, 1 Hz. α,β-Methylene-ATP was added at increasing concentrations from 6 min before to 15 min after the onset of $S_3$, $S_4$ and $S_5$. Closed symbols represent experiments in which α,β-methylene-ATP was given alone. Open symbols represent experiments in which the medium contained DPCPX 10 nM from 36 min before $S_1$ onwards. Abscissae, concentration of α,β-methylene-ATP. Ordinates, evoked tritium overflow: $S_n/S_2$ ratios obtained in individual tissue slices were calculated as a percentage of the corresponding mean $S_n/S_2$ ratio in the appropriate control group (solvent instead of α,β-methylene-ATP). Means ± s.e.mean of 5–6 experiments. Significant differences from experiments without DPCPX: $^{\#}P<0.05$ and $^{\#\#}P<0.01$.

the operation of prejunctional $P_2$-purinoceptors in mouse vas deferens. Under conditions that left the concentration-response curve of 2-chloroadenosine unchanged or almost unchanged (Figure 3b), the $P_2$ antagonist, suramin, clearly shifted the curve of β,γ-methylene-ATP to the right (Figure 5b), with an apparent $pK_B$ value (4.5) close to that found at other $P_2$-purinoceptors (Hoyle *et al.*, 1990; Leff *et al.*, 1990; von Kügelgen *et al.*, 1990; Inoue *et al.*, 1991; Hourani *et al.*, 1992). A shift of similar magnitude was seen when the concentration-response curve of β,γ-methylene-ATP in the presence of suramin plus DPCPX was compared with the curve determined in the presence of DPCPX alone (Figure 5a); if DPCPX and suramin had blocked the same receptor, the combination should have caused a much smaller shift beyond that caused by DPCPX alone. It should be noted that the dual antagonism by both a xanthine and suramin against β,γ-methylene-ATP need not extend to all nucleotides. In our previous study, both 8-(*p*-sulphophenyl) theophylline and suramin antagonized the release-inhibiting effect of UTP. However, only suramin but not 8-(*p*-sulphophenyl) theophylline attenuated significantly the effect of ATPγS (von Kügelgen *et al.*, 1989); possibly the contribution of $P_1$-receptors, relative to the contribution of $P_2$-purinoceptors, is weaker in the case of ATPγS than of β,γ-methylene-ATP and UTP.

Two additional observations support the existence of prejunctional $P_2$-purinoceptors in mouse vas deferens. The ATP analogue 2-methylthio-ATP reduced the release of noradrenaline (Figure 1a). 2-Methylthio-ATP does not activate $A_1$-purinoceptors (Bailey & Hourani, 1990; von Kügelgen *et al.*, 1992a). Therefore, its effect in all likelihood was mediated by $P_2$-purinoceptors. Since 2-methylthio-ATP is selective for the $P_{2Y}$ subtype, the prejunctional $P_2$-receptors may be $P_{2Y}$-like as proposed (von Kügelgen *et al.*, 1989). The second observation is the increase, by suramin, of the release of noradrenaline elicited by the second stimulation period $S_2$ (Table 1). Such changes must be considered with caution because this kind of study is planned to compare release *ratios* ($S_n/S_2$ here) rather than single stimulation periods (Wichmann *et al.*, 1989). However, suramin also increases release *ratios* when properly administered and we have shown that it does so by blockade of a release-inhibiting, negative feedback, effect of endogenous ATP at prejunctional $P_2$-purinoceptors (von Kügelgen *et al.*, 1993).

α,β-Methylene-ATP reduced the release of noradrenaline elicited by stimulation at 1 but not at 8 Hz, possibly due to the general inverse relationship between stimulation frequency and the extent of prejunctional modulation (e.g. Kirpekar *et al.*, 1975). Antagonism by DPCPX suggests that the effect was $A_1$-purinoceptor-mediated (Figure 7a) and the lower maximum in comparison with 2-chloroadenosine and β,γ-methylene-ATP suggests that α,β-methylene-ATP acted at the $A_1$-receptor with low efficacy. If so, partial antagonism at the $A_1$-receptor may have been the reason for the attenuation, by α,β-methylene-ATP, of the inhibition produced by 2-chloroadenosine and β,γ-methylene-ATP (Figures 3c, 5c).

### *Rat vas deferens*

When the results obtained in the mouse are compared with those in the rat vas deferens, the similarity in the effects of 2-chloroadenosine (Figure 3a *versus* 4a) and β,γ-methylene-ATP (Figure 5a *versus* 6a) as well as in their interaction with DPCPX (same Figures) is striking. The inhibition of noradrenaline release in rat vas deferens by both 2-chloroadenosine and β,γ-methylene-ATP, and the attenuation of the inhibition by a xanthine derivative, confirm the findings of Westfall's group as well as their conclusion that the nucleoside and the nucleotide act at a common site (Forsyth *et al.*, 1991; see also Shinozuka *et al.*, 1988; 1990). Westfall and his colleagues suggested that the common site in the rat was a novel, $P_3$-purinoceptor. Here we disagree: the more complete evidence now available rather indicates a $P_1$ or, more specifically, $A_1$ character. First, the common site is blocked by 8-(*p*-sulphophenyl)theophylline, a known $P_1$-purinoceptor antagonist, although affinity constants of the antagonist were not determined (Shinozuka *et al.*, 1988; 1990; Forsyth *et al.*, 1991). Second, the apparent $pK_B$ values of DPCPX against 2-chloroadenosine and β,γ-methylene-ATP in the rat, 9.7 and 9.6, on the one hand support the identity of the two agonists' sites of action, and on the other hand are very close to $pK_B$ values of DPCPX at $A_1$-receptors, in support of an $A_1$ character of the common site, as already discussed for the mouse vas deferens. Third, a reason for the $P_3$ postulate was that nucleotides usually are considered to be poor agonists at $P_1$-purinoceptors (e.g. Forsyth *et al.*, 1991). However, there is now evidence showing that this may not be true. For example, nucleotides including β,γ-methylene-ATP potently inhibit cholinergic neuroeffector transmission in the guinea-pig ileum (Wiklund *et al.*, 1985; Wiklund & Gustafsson, 1986), contract the rat colon muscularis mucosae (Bailey & Hourani, 1990; Bailey *et al.*, 1992), inhibit the efferent function of capsaicin-sensitive neurones in guinea-pig atria (Rubino *et al.*, 1992), and inhibit the release of noradrenaline from rabbit brain noradrenergic axons (von Kügelgen *et al.*, 1992a) – all effects mediated by $P_1$- ($A_1$-) purinoceptors.

The prejunctional $P_2$-purinoceptor was more difficult to detect in the rat than in the mouse. Although suramin did not change the concentration-response curve of 2-chloroadenosine in the rat (Figure 4b), it also caused little, if any, shift to the right of the concentration-response curve of β,γ-methylene-ATP (Figure 6b) and produced little additional shift when it was combined with DPCPX (Figure 6a). Possibly the $P_2$ agonist activity of β,γ-methylene-ATP, relative to

its $A_1$ agonist activity, is weaker in the rat than in the mouse. As in the mouse, however, 2-methylthio-ATP reduced the release of noradrenaline in the rat vas deferens (Figure 1b), again indicating both the occurrence of prejunctional $P_2$-purinoceptors and their $P_{2Y}$-like character. Finally, as in the mouse, suramin increased the release of noradrenaline at $S_2$ (Table 1), and it also increased release *ratios* when properly administered, apparently by antagonism against endogenous ATP at $P_2$-purinoceptors (Kurz, von Kügelgen & Starke, unpublished observations).

The effect of α,β-methylene-ATP in the rat is a final example of similarity to, but not identity with, the mouse: as in the mouse, α,β-methylene-ATP reduced the release of noradrenaline at 1 but not 8 Hz, an effect blocked by DPCPX (Figure 7b) and with a lower maximum than found for 2-chloroadenosine and β,γ-methylene-ATP; in contrast to the mouse, however, α,β-methylene-ATP at best tended to attenuate the inhibition produced by 2-chloroadenosine and β,γ-methylene-ATP (Figures 4c, 6c). In the study of Forsyth *et al.* (1991), α,β-methylene-ATP did not change the release of noradrenaline in rat vas deferens but did antagonize the effect of β,γ-methylene-ATP; the difference may be due to the frequency of stimulation which was 2 Hz in Forsyth *et al.* (1991). Generally speaking, effects of α,β-methylene-ATP on the release of noradrenaline have been examined with discrepant results, from slight decrease via no change to increase (Ishikawa, 1985; Stjärne & Åstrand, 1985; Miyahara & Suzuki, 1987; von Kügelgen *et al.*, 1989; 1992a; Shinozuka *et al.*, 1990; Forsyth *et al.*, 1991; Sperlagh & Vizi, 1991). We suspected that the release-inhibiting $A_1$ effect of α,β-methylene-ATP might be indirect, due to release of adenosine in the vas deferens slices. However, the inhibition remained unchanged in the presence of adenosine deaminase (Figure 1) which, under similar conditions, has been shown to destroy exogenous as well as endogenous adenosine (Forsyth *et al.*, 1991; von Kügelgen *et al.*, 1992a,b). The $A_1$ effect of α,β-methylene-ATP, hence, seems to be direct. It should be noted, however, that α,β-methylene-ATP has little, if any, agonist or antagonist effect at other $A_1$-purinoceptors (Bailey & Hourani, 1990; Bailey *et al.*, 1992; von Kügelgen *et al.*, 1992a).

## *Conclusion*

Previous studies led to different conclusions concerning prejunctional purinoceptor mechanisms in the vas deferens of the mouse and the rat (see Introduction). The present direct comparison shows, however, that the mechanisms in the two species are similar. Simple inspection of the Figures makes the similarity obvious (compare Figure 3, mouse, with Figure 4, rat; compare Figure 5, mouse, with Figure 6, rat). Specifically, the results indicate that nucleosides and nucleotides have an important common prejunctional site of action in the vas deferens of either species, as originally suggested for the rat (Forsyth *et al.*, 1991; see also Lukacsko & Blumberg, 1982; Shinozuka *et al.*, 1988; 1990). The common site is a $P_1$-purinoceptor; there seems to be no need to postulate a novel $P_3$-purinoceptor. However, the results also demonstrate a second, $P_2$, prejunctional purinoceptor in both species, as originally suggested for the mouse (von Kügelgen *et al.*, 1989), although the nucleotide mainly used in the present study, β,γ-methylene-ATP, acts to a smaller extent through the $P_2$-receptor in the rat than in the mouse.

This study was supported by the Jung-Stiftung, Hamburg, and the Deutsche Forschungsgemeinschaft (SFB 325). We thank Bayer, Wuppertal, for suramin.

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*(Received July 7, 1993*
*Revised August 18, 1993*
*Accepted August 27, 1993)*