P2-Purinoceptor antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens

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Abstract. The sites of action at which ATP elicits contraction of the rat vas deferens were studied by means of the P2-purinoceptor antagonists pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), suramin and reactive blue 2.

Increasing concentrations of PPADS (up to 1 mM), suramin (up to 1 mM) and reactive blue 2 (up to 320 µM) reduced and eventually abolished contractions elicited by the P2X-purinoceptor-selective agonist α,β-methylene ATP 3 µM with IC50 values of 2.1, 10.1 and 27.0 µM, respectively. In contrast, PPADS and suramin caused only a partial inhibition of contractions elicited by ATP 1 mM, maximal reduction by about 40%, IC50 values 1.3 and 5.0 µM, respectively; reactive blue 2 did not change ATP-induced contractions. In tissues exposed to PPADS 320 µM throughout, increasing concentrations of reactive blue 2 or suramin decreased contractions elicited by ATP 1 mM, IC50 values 2.6 and 14.5 µM, respectively. In tissues exposed to suramin 320 µM throughout, increasing concentrations of PPADS decreased contractions elicited by ATP 1 mM, IC50 37.9 µM, whereas reactive blue 2 slightly enhanced these contractions. In tissues exposed to reactive blue 2 100 µM throughout, increasing concentrations of PPADS decreased contractions elicited by ATP 1 mM, IC50 26.6 µM, whereas suramin caused no change. Pre-exposure to α,β-methylene ATP 1 µM to desensitize P2X-purinoceptors reduced the response to ATP 1 mM by 91% in otherwise untreated tissues, but did not reduce the response to ATP 1 mM in tissues exposed throughout to PPADS 320 µM, suramin 320 µM or reactive blue 2 100 µM. Neither PPADS nor suramin nor reactive blue 2 altered contractions elicited by KCl 35 mM. The P1-purinoceptor antagonist 8-(p-sulfophenyl) theophylline 100 µM did not change contractions elicited by α,β-methylene ATP 3 µM or ATP 1 mM.

It is concluded that ATP 1 mM elicits contraction of the rat vas deferens through three sites: P2X-purinoceptors which are blocked by PPADS, suramin and reactive blue 2; P2Y-purinoceptors blocked by reactive blue 2 and suramin but resistant to PPADS; and non-P2X-non-P2Y-purinoceptors blocked by PPADS but resistant to inhibition by suramin and reactive blue 2.

Key words: Rat vas deferens — P2X-Purinoceptors — P2Y-Purinoceptors — α,β-Methylene ATP — ATP — Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) — Suramin — Reactive blue 2

Introduction

It is widely assumed that, in most smooth muscle tissues including the rodent vas deferens, nucleotides produce contraction by activation of P2X-purinoceptors (Table 2 of Burnstock 1991). This view, like the P2 subclassification generally, is mainly based on agonist potency series.

However, even before P2-purinoceptors were first divided into P2X and P2Y (Burnstock and Kennedy 1985) it was suggested that ATP and some related nucleotides act through more than a single site in guinea-pig vas deferens (Fedan et al. 1982a). Contractions elicited by ATP were biphasic: low concentrations caused a rapid transient response only, high concentrations caused in addition a more tonic but also transient contraction. The rapid component was selectively attenuated by the alkylating ATP derivative arylazido aminopropionyl ATP (Fedan et al. 1982b), whereas the late component was later shown to be selectively reduced by treatment with ATP-2',3'-dialdehyde (Fedan and Lamport 1990a). Contractions of the rat vas deferens elicited by ATP, although monophasic in untreated preparations, were similarly resolved into two components after desensitization to either P1P4-diadenosine-tetraphosphate, which suppressed an early component, or α,β-methylene ATP, which suppressed a late component (Stone and Paton 1989). In the mouse vas deferens, the P2 antagonist suramin (Dunn and Blakeley 1988) antagonized the effect of α,β-methylene ATP over the entire range of α,β-methylene ATP...
concentrations tested but antagonized only the effect of low but not of high concentrations of ATP, again indicating that ATP caused contraction through more than a single site (von Kügelgen et al. 1990).

Additional P2-purinoceptor antagonists besides suramin are now available. Pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) reduces P2X-purinoceptor-mediated contractions in the rabbit and guinea-pig vas deferens (Lambrecht et al. 1992; McLaren et al. 1993). Reactive blue 2 has been employed as an antagonist of P2X-purinoceptor-mediated responses in a variety of tissues. The reactive blue 2 now available is 97% pure and may be devoid of nonspecific effects previously found with less (60%) pure preparations (p. 43 of Kennedy 1990). We used these three antagonists to discriminate and characterize receptors activated by ATP and the prototypic P2X-purinoceptor agonist α,β-methylene ATP (see Burnstock 1991) in rat vas deferens.

Methods

Male Wistar rats (240–360 g) were killed by decapitation and the vasa deferentia removed and cleaned of adherent tissue. Prostatic thirds were suspended vertically in a 5.7 ml organ bath. The lower end was fixed and the upper end attached to an isometric force transducer (K30, Hugo Sachs Elektronik, Hugstetten, Germany) under an initial tension of 9.8 mN. The incubation medium contained (mM): NaCl 118, KCl 4.8, CaCl2 2.5, KH2PO4 0.9, NaHCO3 25, MgSO4 1.2, glucose 11, ascorbic acid 0.3 and disodium EDTA 0.03. It was saturated with 95% O2/5% CO2 and kept at 37°C. The medium was replaced every 15 min. Tissues relaxed to about 3 mN during a 60 min equilibration period. This final resting tension remained constant for the rest of the experiments. The tension was recorded on a Graphite thermal pen recorder (Ettlingen, Germany).

Contractions were elicited by α,β-methylene ATP 3 μM, ATP 1 mM or high K+, of which only one was tested on a single preparation. High K+ was added as 35 mM KCl, final K+ concentration therefore 40.7 mM, without osmotic compensation. Unless stated otherwise, nucleotides or high K+ were added 6 to 10 times at 60 min intervals and left in the bath for 10 to 20 s. Cumulative concentration-effect curves for PPADS, suramin or reactive blue 2 were determined by concentration increases immediately after the first and all following responses to α,β-methylene ATP. α,β-Methylene ATP was added every 60 min in the presence of solvent (by 97±10% at 9th addition, n = 11, and 66±16% at 10th addition, n = 4; Fig. 2, left-hand tracings). Unlike contractions elicited by α,β-methylene ATP, responses to ATP were only reduced but not abolished by increasing concentrations of PPADS (maximal reduction by 43±1% and suramin (maximal reduction by 39±1%) and were not changed at all by reactive blue 2 (Figs. 2, 3). The IC50 values of PPADS and suramin were close to experiments was dissolved in medium. Solutions of drugs were added to the organ bath in aliquots not exceeding 32 μl (70 μl for high K+).

The arithmetic mean ± SEM (for IC50 values the SE as defined by Waud 1976) is given throughout. Differences between means were tested for significance by the Mann-Whitney test. Differences with error probabilities <0.05 were taken to be statistically significant.

Results

α,β-Methylene ATP 3 μM elicited rapid, transient contractions amounting to 8.7±0.4 mN (n = 24; first addition; all experiments pooled; Fig. 7A below). The concentration of 3 μM is approximately an EC50 under the present conditions (R. Büttmann, unpublished data; see also Mallard et al. 1992). The contractions increased when α,β-methylene ATP was added every 60 min in the presence of solvent (by 52±9% at 7th addition; n = 5). PPADS, suramin and reactive blue 2 concentration-dependently reduced and finally abolished contractions elicited by α,β-methylene ATP, with potency decreasing in that order (Fig. 1). IC50 values are summarized in Table 1.

ATP 1 mM elicited rapid, transient contractions of 5.7±0.3 mN (n = 37; first addition; all experiments pooled; Fig. 2, top tracings, and Fig. 7B below). The place of ATP 1 mM in the concentration-response curve of ATP is not known since the curve does not level off at concentrations up to 3.2 mM (R. Büttmann, unpublished data; see also Taylor et al. 1983). The contractions increased when ATP was added every 60 min in the presence of solvent (by 97±10% at 9th addition, n = 11, and 66±16% at 10th addition, n = 4; Fig. 2, left-hand tracings). Unlike contractions elicited by α,β-methylene ATP, responses to ATP were only reduced but not abolished by increasing concentrations of PPADS (maximal reduction by 43±1%) and suramin (maximal reduction by 39±1%) and were not changed at all by reactive blue 2 (Figs. 2, 3).

![Fig. 1. Effect of PPADS, suramin and reactive blue 2 (RB 2) on contractions elicited by α,β-methylene ATP 3 μM. α,β-Methylene ATP was added to the bath at 60 min intervals. PPADS (○; n = 4), suramin (●; n = 4), or RB 2 (□; n = 5) was added at increasing concentrations after the first and all following responses to α,β-methylene ATP. Abscissae, antagonist concentration. Ordinates show contraction (mean ± SEM) as a percentage of pre-antagonist contraction (see text for magnitude), corrected for any change observed in controls (no antagonist).](image-url)