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Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens

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The trypanocide Suramin was tested as a possible antagonist at the P_2-purinoceptor of the mouse vas deferens. At a concentration of 100 μM, Suramin antagonized the response to α,β-methylene ATP, while responses to carbachol and noradrenaline were unaffected. These results suggest that Suramin may provide a starting point for the development of specific antagonists for P_2-purinoceptors.

Introduction The trypanocidal drug Suramin is a potent inhibitor of a number of hydrolytic and oxidative enzymes (Wills & Wormall, 1950) and inhibits Na-K-ATPase at an intracellular site, possibly by interfering with the binding of adenosine triphosphate (ATP) (Fortes *et al.*, 1973).

Adenosine triphosphate (ATP) may act as a co-transmitter with noradrenaline at the sympathetic neuromuscular junction (Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984) and can cause either contraction or relaxation of smooth muscle by the activation of P_{2x}- and P_{2y}-purinoceptors respectively (Burnstock & Kennedy, 1985). Characterization of purinoceptors and our understanding of the role of ATP in sympathetic neuromuscular transmission has been hampered by the lack of suitable antagonists. Most studies on the excitatory P_{2x}-receptor have used desensitization of the receptors by ATP or α,β-methylene ATP to abolish purinergic responses, although this technique has been claimed to affect responses to noradrenaline in the immature rat basilar artery (Byrne & Large, 1986). Alternatively, photoaffinity labelling with arylazido aminopropionyl adenosine triphosphate ($ANAPP_3$) has been used to produce irreversible antagonism. However, this compound is also a P_2 agonist, and produces an initial activation of the receptor (Hogaboom *et al.*, 1980). Although reactive blue 2 is a selective, non competitive, antagonist of the P_{2y}-purinoceptor, it is without effect at the P_{2x}-purinoceptor (Burnstock & Warland, 1987).

In the search for a suitable P_{2x}-purinoceptor antagonist, we have examined the action of Suramin on contraction of the mouse vas deferens to α,β-methylene ATP and found it to be an effective and apparently selective antagonist.

Methods Male, C57BL6 mice (6–18 weeks old) were killed by cervical dislocation, the vasa deferentia were removed, and suspended, under a resting tension of 200 mg, in a small organ bath (volume 0.2 ml). The bath was perfused at 5 ml min^{-1} with Krebs solution containing (mM): NaCl 118.4, KCl 4.7, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, $CaCl_2$ 2.1, $MgCl_2$ 1.1 and glucose 11, heated to 35°C and saturated with 95% O_2: 5% CO_2. Isometric contractions were measured with a force transducer, and recorded on a u.v. oscillograph. Drugs were applied by changing the superfusing solution. Agonists were applied, for 10 s (α,β-methylene ATP) or 15 s (carbachol and noradrenaline) during which time a maximal response was obtained. Agonists were applied at 15 min intervals to avoid any problems of desensitization. When Suramin was used, it was present for 2 min before and during the agonist application. Drugs used were α,β-methylene ATP, (−)-noradrenaline bitartrate, carbachol (Sigma). Suramin was a gift from ICI and Bayer U.K.

Results α,β-methylene ATP produced a rapid contraction, which reached a maximum in 5 to 10 s and then started to decline. Superfusion of the vas deferens with Suramin alone produced no contraction. However, following 2 min superfusion with Suramin, there was a concentration-dependent reduction in the response to α,β-methylene ATP (Figure 1a); 15 min after washing out Suramin, the response to α,β-methylene ATP had recovered to 98 ± 7% (mean ± s.e.mean, $n = 4$) of the control response. In the presence of Suramin (100 μM) the log concentration-response curve for α,β-methylene ATP was shifted to the right, and appeared to have a steeper slope (Figure 1b).

To test the specificity of Suramin, we compared its effect on matched responses to noradrenaline, carbachol and α,β-methylene ATP (Figure 1c). While the response to α,β-methylene ATP was abolished, responses to noradrenaline and carbachol, were unaffected.

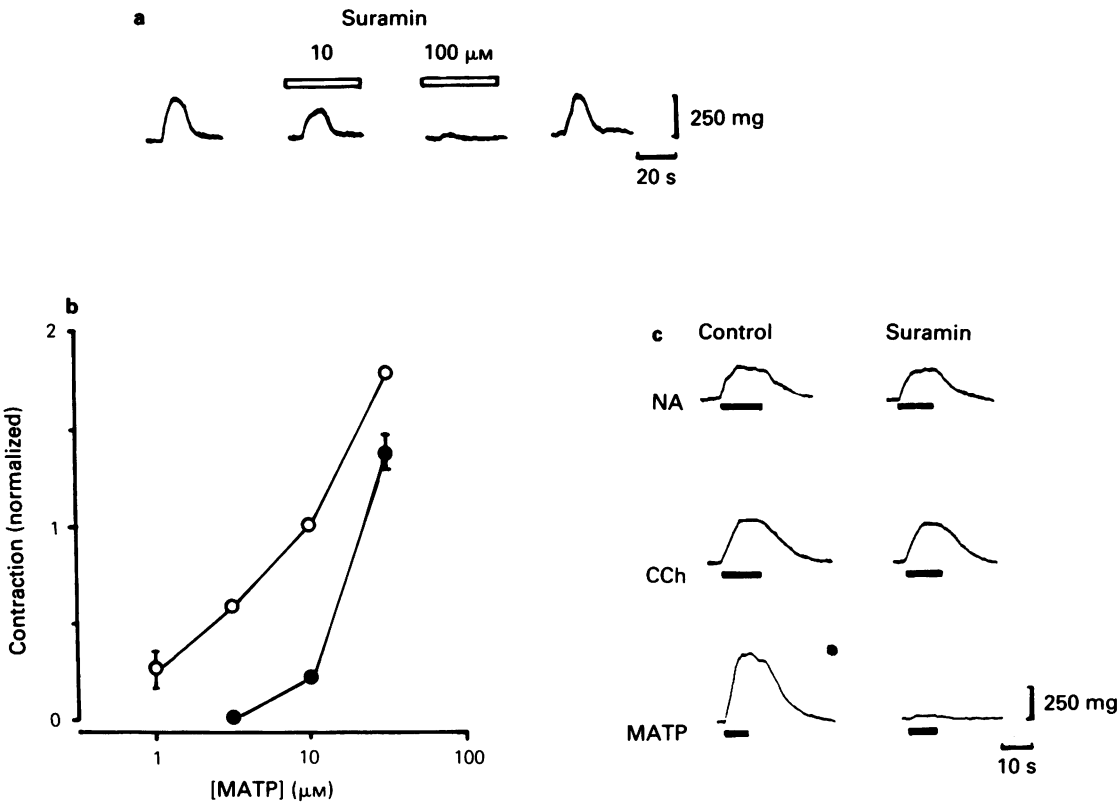

Figure 1 Effect of Suramin on the mechanical response of the mouse vas deferens. (a) Responses produced by α,β-methylene ATP at 15 min intervals in the absence and presence of 10 and 100 μM Suramin, which was present for 2 min before and during the agonist application. (b) Log concentration-response curves for the contraction produced by α,β-methylene ATP in the absence (○) and presence (●) of Suramin (100 μM); all responses were normalized with respect to the 10 μM response. Each point represents the mean from 3 experiments, vertical bars show s.e.mean where it exceeded the size of the symbol. (c) Responses to noradrenaline (NA, 10 μM) carbachol (CCh, 10 μM) and α,β-methylene ATP (MATP, 10 μM) in the absence and presence of Suramin (100 μM). The bars indicate the duration of the agonist application.

Discussion These results indicate that Suramin is a reversible antagonist at the P_2-purinoceptor in the mouse vas deferens. This effect of Suramin is apparently specific since responses to noradrenaline and carbachol were unaffected, but we do not know whether Suramin exhibits any selectivity for different types of purinoceptor. Furthermore, in view of the range of enzymes that are inhibited by Suramin (Wills & Wormall, 1950), we cannot rule out the possibility that it may interact with other receptors. However, since Suramin does not readily cross the cell membrane (Fortes *et al.*, 1973), the antagonism observed presumably resulted from an interaction at an extracellular site.

The antagonism produced by Suramin caused a shift in the α,β-methylene ATP log concentration-response curve, and may have increased its slope. Although the reason for this possible change in slope is unclear, the same effect has been observed with the irreversible antagonist $ANAPP_3$ (Hogaboom *et al.*, 1980).

In conclusion, Suramin appears to be a selective antagonist at the P_2-purinoceptor in the mouse vas deferens, and although not very potent, it may provide a starting point for the development of specific, reversible P_2-purinoceptor antagonists, which are without intrinsic activity.

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