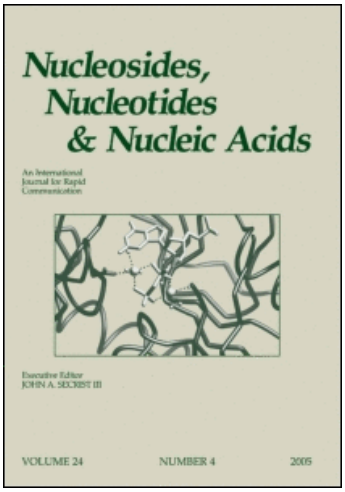

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Suramin is a Competitive But Slowly-Equilibrating Antagonist at P_{2x}-Receptors in the Rabbit Ear Artery

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SURAMIN IS A COMPETITIVE BUT SLOWLY-EQUILIBRATING ANTAGONIST AT P_{2X}-RECEPTORS IN THE RABBIT EAR ARTERY

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Abstract: Antagonist effects of suramin at the P_{2X}-receptor in the rabbit ear artery were associated with agonist curve depression and a steep Schild plot. Kinetic analysis revealed a problem of slow equilibration and established conditions under which suramin fulfilled all criteria for simple competition.

The quantitative classification of P_{2X}-purinoceptors has been limited by the lack of selective competitive antagonists. However, an agent which has been claimed to possess antagonistic properties at these receptors is the trypanocide, suramin[1]. This substance showed inhibition of P_{2X}-receptor-mediated contractions in the mouse vas deferens[1] and in the guinea-pig bladder[2], although it was previously reported to be devoid of effect in the latter tissue[3]. However, no study has appeared which establishes, according to quantitative pharmacological criteria[4,5], that suramin's antagonistic action is genuinely competitive. Indeed, where antagonist action has been demonstrated[1,2], deviations from competitive behaviour are evident.

The present study investigated the antagonistic properties of suramin in the rabbit ear artery, a preparation which, according to agonist potency order information, contains P_{2X}-receptors[6].

Isolated rings of endothelially-denuded ear artery from NZW-rabbits were prepared for organ bath studies. In an initial experiment, α,ß-methylene-ATP (αßmeATP) concentration-effect (E/[A]) curves were constructed cumulatively in a paired curve design in the absence and presence of increasing concentrations of suramin incubated for 45 min.

Although the resulting E/[A] curves were displaced in parallel to the right the slope of the associated Schild plot was significantly greater than unity (1.50 ± 0.08, SE, 18 df).

One possible explanation for a steep Schild plot is insufficient antagonist equilibration time[7]. This was investigated by examining the effects of a low ($3x10^{-5}$M) and a high (10^{-3}M) concentration of suramin using a short (15 min) and a long (3 hr) incubation period. $3x10^{-5}$M suramin produced greater displacement of αßmeATP curves when incubated for 3 hr than when incubated for 15 min, whereas 10^{-3}M suramin produced the same degree of shift at the two times. These results confirmed that slow equilibration by suramin was a problem which could be overcome by extending its equilibration period. However, in these experiments 10^{-3}M suramin also produced a depression in the αßmeATP E/[A] curve when incubated for 3 hr, compromising quantitative analysis of antagonism.

Therefore, it was necessary to seek conditions under which suramin could achieve an effective equilibrium with receptors but under which the depressive effect would be minimised. To do this we investigated the kinetics of equilibration by suramin in greater detail.

In each tissue an αßmeATP curve was constructed in the absence of suramin and then following 30, 60, 120, 180, 240 min incubation with suramin ($3x10^{-5}$M). The resulting dose-ratios (r) were calculated at each time point. Paton[8] showed that the fractional occupancy of receptors by an antagonist (B) is given by the expression (r-1)/r and that the relationship between fractional occupancy (p_B) and time (t) is $p_B = p_{Beq}\ (1 - \exp\{-(k_1[B]+k_{-1})t\})$ in which p_{Beq} is the equilibrium value of occupancy. Thus, estimates of the onset (k_1) and offset (k_{-1}) rate constants can be obtained from a plot of (r-1)/r against t. The results of this analysis applied to suramin are shown in Figure 1.

The estimated values of k_1 and k_{-1} were 306 ± 45 min^{-1} M^{-1} and 4.40 ± 1.52 x 10^{-3} min^{-1} respectively. We could then calculate, from the exponential function, the time for different concentrations of suramin to achieve a given occupancy level. We assumed that 95% occupancy (that is, $p_{Beq} = 0.95p_{Beq}$) represents effectively complete equilibrium and calculated the times for $3x10^{-5}$, 10^{-4}, $3x10^{-4}$ and 10^{-3} M suramin to achieve this value. Respectively, these were 220, 86, 31 and 10 min.

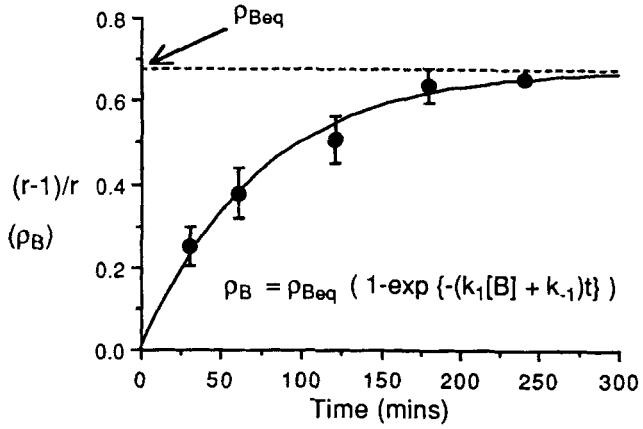

FIG.1
Kinetics of equilibration by suramin (30 μM). Fractional occupancy (p_B) values were calculated from dose-ratios ((r-1)/r), plotted against time and analysed as described in the text to estimate onset and offset rate constants.

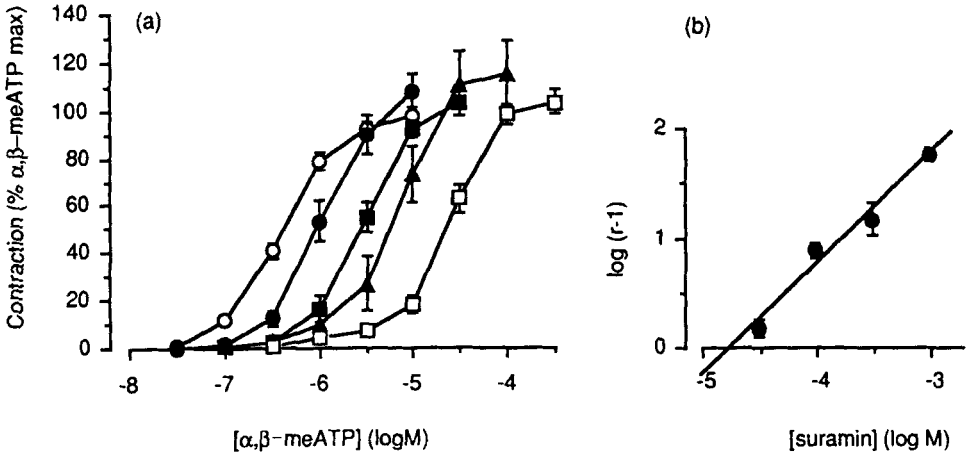

FIG 2.
Optimised analysis of suramin antagonism. Panel (a) α βmeATP curves were obtained in the absence (o) and presence of suramin: 30 μM (o); 100 μM (■); 300 μM (▲); 1 mM (□) using incubation times calculated from the kinetic analysis. Curves were displaced in parallel. Panel (b) Schild plot. The plot has a slope of unity and the estimated pK_B was 4.79.

Using these incubation times, a second analysis of antagonism was performed, the results of which are shown in Figure 2. Under these optimised conditions, suramin demonstrated antagonist dynamics consistent with simple competition, that is, parallel displacement of αßmeATP E/[A] curves and a Schild plot slope of unity (1.00 ± 0.09, 23 df). The pK_B estimate was 4.79 (± 0.05, 24 df).

In an experiment of identical design but using L-ß, γmeATP as the agonist, the effects of suramin were again consistent with simple competition. In this case a pK_B value of 5.17 (± 0.04,17df) was obtained which although significantly different would not be considered as meaningfully different from the previous estimate.

Suramin (10^{-3}M, 15 min) had no effect on KCl E/[A] curves and produced only slight rightward displacements of phenylephrine (0.22 ± 0.12, 10 df) and histamine (0.36 ± 0.06, 8 df) curves. These experiments indicated that suramin is some 50x selective for P_{2X}-receptors over other vasoconstrictor mechanisms.

In conclusion suramin is a genuinely competitive but slowly-equilibrating antagonist at P_{2X}-receptors in the rabbit ear artery.

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