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SPECIAL REPORT

Suramin – a powerful inhibitor of neural ecto-diadenosine polyphosphate hydrolase

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The neural ecto-diadenosine polyphosphate hydrolase (ecto-Ap_nAase) from plasma membranes of *Torpedo* synaptic terminals is inhibited by suramin. This study was carried out by discontinuous h.p.l.c. and continuous fluorometric methods. The concentration-dependence studies showed a non-competitive mechanism for suramin in the Dixon plot, with a K_i value of 1.79 ± 0.03 μM with respect to ε-(Ap_3A) as the substrate and 1.69 ± 0.05 μM and 1.86 ± 0.06 μM for ε-(Ap_4A) and ε-(Ap_5A) respectively. These results indicate that suramin could be a base compound inhibiting ecto-Ap_nAase and providing an alternative way of studying the pharmacology of diadenosine polyphosphate receptors.

Keywords: Diadenosine polyphosphates; *Torpedo* synaptic membranes; ecto-nucleotidases; purinoceptors; suramin

Introduction Diadenosine polyphosphates (Ap_nA, $n=3-6$) have been found in neuro-secretory granules, co-stored with catecholamines or acetylcholine, and released upon stimulation in a calcium-dependent manner (Pintor *et al.*, 1992). The presence of high affinity receptors for Ap_nA in neural tissues has been reported, and also the induction of calcium influx in synaptic terminals from rat brain (Pintor & Miras-Portugal, 1995). The destruction of these compounds by an ecto-diadenosine polyphosphate hydrolase (ecto-Ap_nAase) terminates their extracellular action (Ramos *et al.*, 1995).

Suramin is one of the scarce antagonists available for most of the P_2 purinoceptor families and is also reported to be an inhibitor of the ecto-ATPase (Leff *et al.*, 1990; Bailey & Hourani, 1995; Beukers *et al.*, 1995). The present study demonstrates the high efficiency of suramin as an inhibitor of the ecto-Ap_nAase from the cholinergic terminals of *Torpedo* electric organ, where these compounds are stored and the existence of high affinity receptors has also been proved (Pintor *et al.*, 1992).

Methods Synaptic plasma membranes from *Torpedo* electric organ were isolated as described by Pintor *et al.* (1992).

Ecto-Ap_nAase activity was measured with the fluorescent derivatives of Ap_nA, the etheno-diadenosine polyphosphates ε-(Ap_3A), ε-(Ap_4A) and ε-(Ap_5A), by two different techniques. The enzymatic hydrolysis of the polyphosphate chain induces substantial emission fluorescence increases of 9, 7 and 6 fold, respectively, for ε-(Ap_3A), ε-(Ap_4A) and ε-(Ap_5A), with wavelengths of excitation and emission of 305 and 410 nm respectively. For the h.p.l.c. technique samples of plasma membranes containing 100 μg protein ml^{-1} in 20 mM Tris-HCl, pH 7.5, plus 4 mM $MgCl_2$ and 2 mM $CaCl_2$ and the ε-(Ap_nA) as substrate were incubated at 37°C and processed as described by Ramos *et al.* (1995). The h.p.l.c. equipment was from Waters (Milford, MA, U.S.A.). Peaks were identified with commerical standards from Sigma (St. Louis, U.S.A.). ε-(Ap_nA) compounds were synthesized as described by Ramos *et al.* (1995).

The continuous fluorometric method is based on measuring the fluorescence increase after hydrolysis and was carried out in the incubation conditions as above. Reaction progress was followed by recording the increase of fluorescence emission in an LS 50 fluorometer (Perkin-Elmer Ltd, Beaconsfield, Buckinghamshire, U.K.). Both techniques are described fully by Ramos *et al.* (1995).

Results The cholinergic plasma membranes from synaptic terminals of *Torpedo* electric organ hydrolyse the ε-(Ap_nA), $n=3-5$, to a similar extent. The hydroylsis always produces ε-AMP and the corresponding etheno-mononucleotide, ε-$Ap_{(n-1)}$ (Ramos *et al.*, 1995). The h.p.l.c. chromatograms from the ε-(Ap_3A) hydrolysis are shown sequentially as a function of the incubation time (Figure 1a). ε-ADP and ε-AMP appear as hydrolytic products, as does ε-Ado, the last being the result of the sequential action of ecto-nucleotidases and ecto-5'-nucleotidase present in these neural membranes (Zimmermann, 1994). The K_M value obtained for ε-(Ap_3A) hydrolysis was 0.5 μM in *Torpedo* synaptic terminals.

The inhibitory action of suramin in this preparation is evident even at very low concentrations as shown in the h.p.l.c. chromatograms (Figure 1a) and in the continuous fluorometric method (Figure 1b), where the inhibitory action of increasing amounts of suramin is shown. Very similar results were obtained for ε-(Ap_4A) and ε-(Ap_5A).

The concentration-dependence of inhibition by suramin is shown in Figure 2, where the Dixon plot indicates the existence of a non-competitive mechanism with a K_i value of 1.79 ± 0.03 μM with respect to ε-(Ap_3A) as substrate. Values of 1.69 ± 0.05 μM and 1.86 ± 0.06 μM were obtained for ε-(Ap_4A) and ε-(Ap_5A) respectively, with identical non-competitive inhibitory behaviour.

Discussion This study demonstrates that suramin, a compound frequently used as an antagonist of P_{2Y} and P_{2X} purinoceptors (Leff *et al.*, 1990; Abbracchio & Burnstock, 1994; Bailey & Hourani, 1995) and also reported to be an inhibitor of ecto-ATPase (Bailey & Hourani, 1995; Beukers *et al.*, 1995), is a potent inhibitor of ecto-Ap_nAase. Moreover, although suramin exhibits a non-competitive mechanism with respect to both ecto-enzymes, ecto-Ap_nAase and ecto-ATPase, the K_i values are significantly different, and suramin is much more powerful as an inhibitor of ecto-Ap_nAase (K_i under 2 μM) than ecto-ATPase, which exhibits K_i and IC_{50} values ranging from 43 μM to more than 1 mM, depending on the tissues studied.

The diadenosine polyphosphates have been reported to be agonists on P_{2Y} and some subtypes of P_{2X} and P_{2U} purinoceptors (Abbracchio & Burnstock, 1994; Hoyle *et al.*, 1995). Suramin acts as an antagonist on most of the P_{2X} ionotropic

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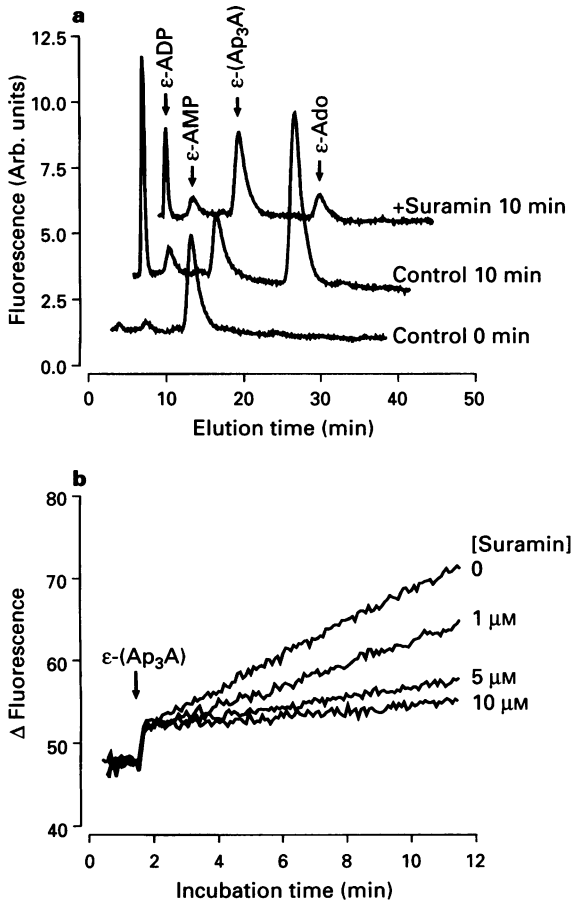

Figure 1 Effect of suramin on the degradation of ε-(Ap_3A) by synaptic terminals from *Torpedo* electric organ. Plasma membranes were incubated as described in Methods. (a) H.p.l.c. chromatograms from aliquots of the reaction medium (20 μl) containing 1 μM ε-(Ap_3A) at 0 min, 10 min, and 10 min in the presence of 5 μM suramin. The ε-(Ap_3A) hydroylsis was inhibited 80% with respect to control. (b) Fluorescence increase associated with the cleavage of 0.75 μM ε-(Ap_3A) at several concentrations of suramin, applied 1 min before the addition of the substrate. Both figures represent typical experiments easily reproducible.

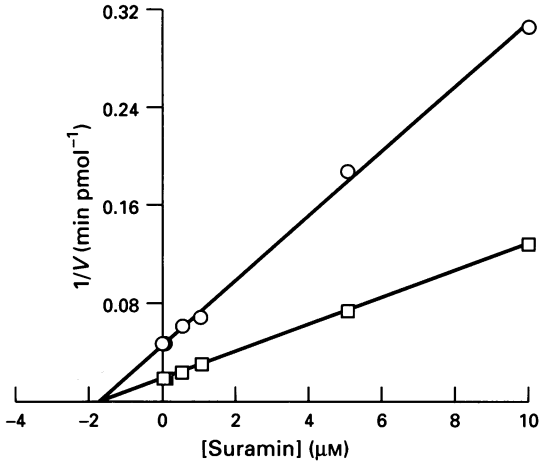

Figure 2 Dixon plot of inhibitory effects of suramin on ε-(Ap_3A) hydrolysis. Plasma membranes were incubated as described in Methods with ε-(Ap_3A) 0.25 μM (○) or 0.75 μM (□), and suramin at various concentrations. The correlation coefficients for the linear regressions are $r=0.9988$ and $r=0.9999$ respectively for ε-(Ap_3A) 0.25 μM and ε-(Ap_3A) 0.75 μM. The graph represents the mean of three experiments.

purinoceptors with IC_{50} values in the range 1–50 μM, and on some, but not all, of the metabotropic P_{2Y} and P_{2U} purinoceptors at much higher concentrations, 100 μM to 1 mM being frequently reported. Besides, it is noteworthy that in rat brain synaptic terminals the Ap_nA compounds – via specific receptors, different from those of ATP – elicit a calcium transient that is not blocked by suramin (Pintor & Miras-Portugal, 1995). These data suggest that the study of the effect of diadenosine polyphosphates in the case of (1) metabotropic P_2 purinoceptors; (2) their specific synaptic receptors; and (3) suramin-insensitive P_{2X} subtypes, can be undertaken in the presence of very small amounts of suramin. In this situation, destruction of Ap_nA will be avoided without interference at their receptor level. A better approach and understanding of purinergic cellular communication could therefore be achieved.

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References

ABBRACCHIO, M.P. & BURNSTOCK, G. (1994). Purinoceptors: are there families of P_{2X} and P_{2Y} purinoceptors? *Pharmacol. Ther.*, **64**, 445–475.

BAILEY, S.J. & HOURANI, S.M.O. (1995). Effects of suramin on contractions of the guinea-pig vas deferens induced by analogues of adenosine 5′-triphosphate. *Br. J. Pharmacol.*, **114**, 1125–1132.

BEUKERS, M.W., KERKHOF, C.J.M., VAN RHEE, A.M., ARDANUY, U., GURGEL, C., WIDJAJA, H., NICKEL, P., IJZERMAN, A.P. & SOUDIJN, W. (1995). Suramin analogs, divalent cations and ATPgammaS as inhibitors of ecto-ATPase. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **351**, 523–528.

HOYLE, C.H.V., POSTORINO, A. & BURNSTOCK, G. (1995). Pre- and postjunctional effects of diadenosine polyphosphates in the guinea-pig vas deferens. *J. Pharm. Pharmacol.*, **47**, 926–931.

LEFF, P., WOOD, B.E. & O'CONNOR, S.E. (1990). Suramin is a slowly-equilibrating but competitive antagonist at P_{2X}-receptors in the rabbit isolated ear artery. *Br. J. Phramacol.*, **101**, 645–649.

PINTOR, J., KOWALEWSKI, H.J., TORRES, M., MIRAS-PORTUGAL, M.T. & ZIMMERMANN, H. (1992). Synaptic vesicle storage of diadenosine polyphosphates in the *Torpedo* electric organ. *Neurosci. Res. Commun.*, **10**, 9–14.

PINTOR, J. & MIRAS-PORTUGAL, M.T. (1995). A novel receptor for diadenosine polyphosphates coupled to calcium increase in rat midbrain synaptosomes. *Br. J. Pharmacol.*, **115**, 895–902.

RAMOS, A., PINTOR, J., MIRAS-PORTUGAL, M.T. & ROTLLAN, P. (1995). Use of fluorogenic substrates for detection and investigation of ectoenzymatic hydrolysis of diadenosine polyphosphates: a fluorometric study on chromaffin cells. *Anal. Biochem.*, **228**, 74–82.

ZIMMERMANN, H. (1994). Signalling via ATP in the nervous system. *Trends Neurosci.*, **17**, 420–426.

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