Br. J. Pharmacol. (1990), **101**, 224–226

Reversible and selective antagonism by suramin of ATP-activated inward current in PC12 phaeochromocytoma cells

[1]Ken Nakazawa, Kannosuke Fujimori, Akira Takanaka & Kazuhide Inoue

Division of Pharmacology, National Institute of Hygienic Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

1 The effects of suramin on an adenosine 5'-triphosphate (ATP)- activated inward current were investigated in PC12 phaeochromocytoma cells with whole-cell voltage-clamp techniques.

2 Suramin (30 to 300 μM) inhibited the ATP-activated current in a dose-dependent manner. The inhibitory effects were reversible and competitive.

3 Suramin also suppressed the current activated by adenosine 5'-O-(3-thiotriphosphate) but did not affect the current activated by nicotine. Suramin did not affect the suppression of a K current induced by methacholine.

4 The results suggest that suramin antagonizes the ATP-receptor-operated membrane current reversibly and selectively.

Introduction

Evidence has been provided to suggest that adenosine 5'-triphosphate (ATP) is released with noradrenaline or acetylcholine from nerve terminals in smooth muscle and neuronal cells (Burnstock & Kennedy, 1985, Bean & Fiel, 1989). Recent studies revealed that excitatory responses mediated by P_2-purinoceptors are triggered by ATP-activated cationic inward currents in smooth muscle cells (Benham *et al.*, 1987; Benham & Tsien, 1987; Nakazawa & Matsuki, 1987; Friel, 1988; Bean & Fiel, 1989) and non-muscle cells (Krishtal *et al.*, 1988; Inoue *et al.*, 1989). It was reported that suramin, a trypanocide drug, antagonized an excitatory P_2-purinoceptor response, namely, smooth muscle contraction, in mouse vas deferens (Dunn & Blakeley, 1988). This finding suggested the possibility that suramin is a reversible blocker of P_2-purinoceptors. However, there have been no reports concerning effects of suramin on ATP-activated currents.

In the present study, we have examined the effects of suramin on the ATP-activated current in PC12 phaeochromocytoma cells. We also examined whether suramin affected other receptor-operated membrane currents.

Methods

PC12 cells were cultured as previously described by Inoue & Kenimer (1988). Briefly, PC12 cells (1×10^6; passage 50–60) were plated in collagen-coated polystyrene dishes and cultured for 2 to 3 days in Dulbecco's modified Eagle's medium containing 5% foetal bovine serum, 5% heat-inactivated horse serum, 2 mM L-glutamine and 50 μg ml^{-1} gentamicin sulphate at 37°C, with an atmosphere of 90% air: 10% CO_2.

Membrane currents were measured with whole-cell patch-clamp techniques (Hamill *et al.*, 1981) under conditions described elsewhere (Nakazawa *et al.*, 1989). The cells were superfused with an extracellular solution containing (mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 11.1 and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 10.0 (adjusted with NaOH to pH 7.4). Patch pipettes were filled with an intracellular solution containing (mM): CsCl 150, HEPES 10.0 and glycoletherdiamine- N,N,N',N'-tetracetic acid (EGTA) 5.0. Membrane current measurements were made at room temperature (about 22°C). In calculating the amplitude of currents activated by depolarizing voltage-steps, a linear component of leak current, estimated from a hyperpolarizing step from −60 mV to −100 mV, was subtracted.

Drugs used were ATP (adenosine 5'-triphosphate disodium salt, Sigma), suramin monosodium salt (Bayer), ATPγS (adenosine 5'-O-(3-thiotriphosphate) tetralithium salt, Boehringer Mannheim), nicotine (Sigma) and methacholine (acetyl-β-methylcholine chloride, Sigma). Drugs were dissolved in extracellular solution and applied to the cells by superfusion.

Results

ATP activated a rapid inward current, which reached a maximum within 1 to 2 s and inactivated with a time constant of about 5 s, in PC12 cells (Inoue *et al.*, 1989). Figure 1a shows the blocking effect of suramin on the ATP-activated current. Suramin (30 μM) partially blocked an inward current activated by 100 μM ATP. The effect was reversible and the current comparable to the control response was obtained after washout of suramin. Suramin blocked the ATP-activated current in a dose-dependent manner between 30 and 300 μM (Figure 1b). Figure 1 shows the dose–response curves for the ATP-activated current in the presence and absence of 30 μM suramin. The dose–response curve was shifted to the right in a parallel manner with suramin. Maximal responses obtained with ATP (up to 3 mM) were not affected by 30 μM suramin (control: 1016 ± 120 pA at −60 mV (means ± s.e.mean, $n = 5$); suramin: 1143 ± 175 pA at −60 mV ($n = 5$)).

ATPγS activated a current similar to that produced by ATP but the response was somewhat more sustained (Nakazawa *et al.*, 1990). Suramin also suppressed the current activated by 300 μM ATPγS (Figure 2a). The current was decreased upon superfusion with suramin (100 μM) and reversed by returning to the solution containing ATPγS alone. Suramin (100 μM) inhibited the ATPγS (300 μM)-activated current by about 80% in 5 cells tested.

We examined the selectivity of the antagonistic effect of suramin. First, we studied the effects on a nicotinic receptor-operated inward current (Bormann & Matthaei, 1983; Nakazawa *et al.*, 1989). Nicotine activates an inward current much smaller than that activated by ATP in PC12 cells (Inoue *et al.*, 1989). As shown in Figure 2b, suramin (300 μM) did not affect

[1] Author for correspondence.

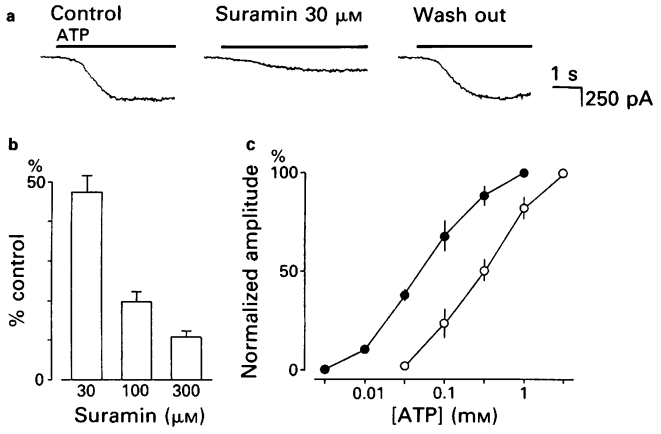

Figure 1 Effect of suramin on ATP-activated inward current in PC12 cells. Holding potential was −60 mV. (a) Current activated by 100 μM ATP at 5 min intervals in the absence (left and right) and presence (middle) of 30 μM suramin. Suramin was added 2 min before ATP application. (b) Dose-dependence of the inhibitory effect of suramin on the ATP-activated current. Peak amplitude of the current activated by 100 μM ATP in the presence of suramin was normalized to that in the absence of suramin. Each column represents the mean from 4 to 6 cells tested. Vertical bars show s.e.mean. (c) Log concentration curves for peak amplitudes of the current activated by ATP in the absence (●) and presence (○) of suramin (30 μM). All responses were normalized with respect to the maximal response in each cell. Each point represents the mean from 4 to 6 cells tested. Vertical bars show s.e.mean where it exceeded the size of the symbol.

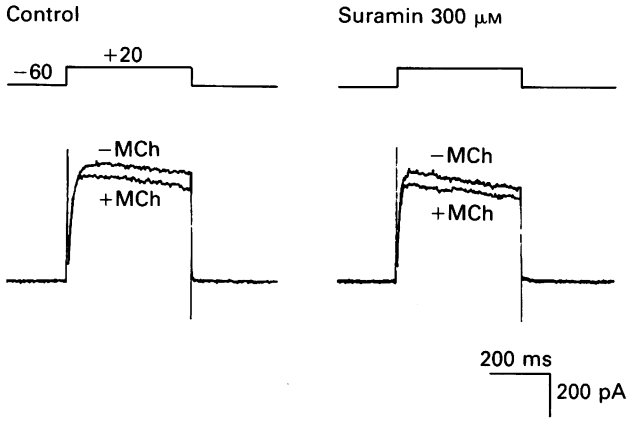

Figure 3 Lack of effect of suramin on the suppression by methacholine of a K current. The K current was activated by a 400 ms voltage-step to +20 mV from a holding potential of −60 mV every 5 s. The cell was first exposed to 300 μM methacholine and rinsed with drug-free solution for 5 min. The cell was next superfused with 300 μM suramin for 1 min and then exposed to 300 μM methacholine (suramin was continuously present). The current traces just before (−MCh) and 30 s after (+MCh) the exposure to methacholine in the absence (left) or presence of 300 μM suramin (right) were superimposed.

the current activated by 10 μM nicotine (93.0 ± 4.3% of control, $n = 5$). Next, we studied the effects of suramin on a muscarinic receptor-mediated response in PC12 cells, namely, suppression of a voltage-gated K current (Nakazawa *et al.*, 1989). In preliminary experiments, suramin (300 μM) itself slightly decreased the K current (92.4 ± 3.5% of control, $n = 5$). Suramin did not affect the suppression of the K current induced by 300 μM methacholine, a selective agonist for muscarinic receptors. The K current which remained after the application of 300 μM methacholine was 76.5 ± 3.7% of control in the presence of 300 μM suramin ($n = 6$; the current in the presence of suramin was taken as control) whereas the value was 83.6 ± 2.2% of control in the absence of suramin ($n = 6$). The values were not significantly different (Student's t test, $P > 0.05$).

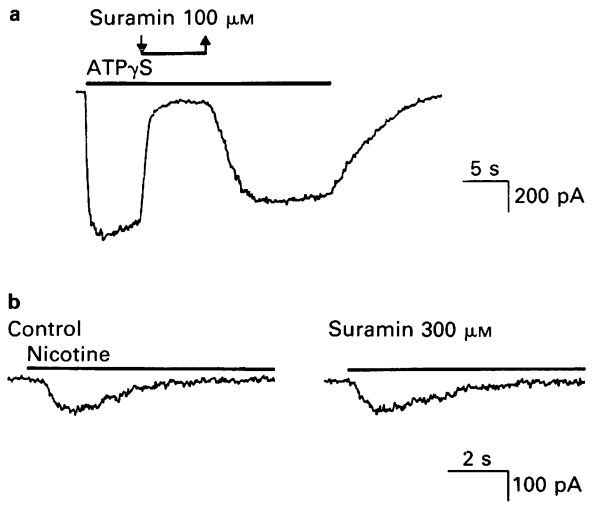

Figure 2 Effects of suramin on inward currents activated by adenosine 5'-O-(3-thiotriphosphate) (ATPγS) (a) and nicotine (b). Holding potential was −60 mV. (a) Current activated by 300 μM ATPγS. Suramin (100 μM) was applied during the ATPγS-activated current. The current was suppressed during the application of suramin and recovered after washout. (b) Current activated by 10 μM nicotine in the absence (left) and presence (right) of 300 μM suramin. Note that suramin did not affect the nicotine-activated current but depressed markedly the response to ATPγS.

Discussion

It was reported that suramin antagonizes both excitatory and inhibitory P_2-purinoceptor responses in smooth muscle tissues (Dunn & Blakeley, 1988; Den Hertog *et al.*, 1989). In the present study, suramin reversibly and competitively antagonized the ATP-activated inward current in PC12 cells. This finding for the first time showed that suramin is also an antagonist for purinoceptors in non-muscle cells. Suramin antagonized the current activated by ATPγS but did not affect the changes in membrane currents induced by nicotine and methacholine.

ATP exerts excitatory effects by activation of receptor-operated channels in smooth muscle cells (Benham *et al.*, 1987; Benham & Tsien, 1987; Nakazawa & Matsuki, 1987; Friel, 1988) and neuronal cells (Krishtal *et al.*, 1988; Bean & Friel, 1989). The receptor-operated channels in smooth muscle and neurones appear to have many properties in common (Bean & Friel, 1989). We recently characterized the ATP-activated current in PC12 cells (Nakazawa *et al.*, 1990): the ionic pathway for the current has very similar properties including kinetics, voltage-dependency, ion-selectivity, and agonist-selectivity to those reported for the ATP-activated channels in other excitable cells (Bean & Fiel, 1989). Therefore, it is probable that the blocking of the ATP-activated current is a common mechanism underlying antagonism by suramin of excitatory effects of ATP both in muscle and non-muscle cells. Competitive antagonism by suramin of the ATP-activated current in the present study suggests that suramin may compete with ATP for the receptor which seems directly coupled to the channels (Benham *et al.*, 1987; Bean & Friel, 1989).

In conclusion, we have shown that suramin reversibly and selectively antagonizes the ATP-activated current in PC12 cells. The blockade of receptor-operated channels may be a common method of action of suramin on excitatory responses mediated by P_2-purinoceptors.

We would like to thank Prof. B.P. Bean of Harvard Medical School for sending us their preprint paper. We also thank Mrs T. Obama for her skilled technical assistance.

References

BEAN, B.P. & FRIEL, D.D. (1989). ATP-activated channels in excitable cells. In *Ion Channels*, Vol. 2. ed. Narahashi, T. pp. 169–203. New York: Plenum Press.

BENHAM, C.D., BOLTON, T.B., BYRNE, N.G. & LARGE, W.A. (1987). Action of externally applied adenosine triphosphate on single smooth muscle cells dispersed from rabbit ear artery. *J. Physiol.*, **387**, 473–488.

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature*, **328**, 275–278.

BORMANN, D.A. & MATTHAEI, H. (1983). Three types of acetylcholine induced single channel currents in clonal rat phaeochromocytoma cells. *Neurosci. Lett.*, **40**, 193–197.

BURNSTOCK, G. & KENNEDY, C. (1985). A dual function for adenosine 5'-triphosphate in the regulation of vascular tone; excitatory cotransmitter with noradrenaline from perivascular nerves and locally released inhibitory intravascular agent. *Circ. Res.*, **58**, 319–330.

DEN HERTOG, A., NELEMANS, A. & DEN AKKER, J.V. (1989). The inhibitory action of suramin on the P_2-purinoceptor response in smooth muscle cells of guinea-pig taenia caeci. *Eur. J. Pharmacol.*, **166**, 531–534.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93**, 243–245.

FRIEL, D.D. (1988). An ATP-sensitive conductance in single smooth muscle cells from the rat vas deferens. *J. Physiol.*, **401**, 361–380.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free patches. *Pflügers Arch.*, **391**, 85–100.

INOUE, K. & KENIMER, J.G. (1988). Muscarinic stimulation of calcium influx and norepinephrine release in PC12 cells. *J. Biol. Chem.*, **263**, 8157–8161.

INOUE, K., NAKAZAWA, K., FUJIMORI, K. & TAKANAKA, A. (1989). Extracellular adenosine 5'-triphosphate-evoked norepinephrine secretion not relating to voltage-gated Ca channels in phaeochromocytoma PC12 cells. *Neurosci. Lett.*, **106**, 294–299.

KRISHTAL, O.A., MARCHENKO, S.M., OBUKHOV, A.G. & VOLKOVA, T.M. (1988). Receptors for ATP in rat sensory neurons: the structure-function relationship for ligands. *Br. J. Pharmacol.*, **95**, 1057–1062.

NAKAZAWA, K., FUJIMORI, K., TAKANAKA, A. & INOUE, K. (1989). Existence of muscarinic suppression of a K current in PC-12 phaeochromocytoma cells. *Am. J. Physiol.*, **257**, C1030–C1033.

NAKAZAWA, K., FUJIMORI, K., TAKANAKA, A. & INOUE, K. (1990). An ATP-activated conductance in PC12 phaeochromocytoma cells and its modification by extracellular calcium. *J. Physiol.* (in press).

NAKAZAWA, K. & MATSUKI, N. (1987). Adenosine triphosphate-activated inward current in isolated smooth muscle cells from rat vas deferens. *Pflügers Arch.*, **409**, 644–646.

(Received March 13, 1990
Revised May 1, 1990
Accepted May 14, 1990)