ELSEVIER

European Journal of Pharmacology 301 (1996) 91–97

ejp

The prejunctional inhibitory effect of suramin on neuromuscular transmission in vitro

Robert H. Henning [a,*], Edward G. Rowan [b], M. Fatima M. Braga [b], Adriaan Nelemans [a], Alan L. Harvey [b]

[a] *Department of Clinical Pharmacology, Groningen Institute for Drug Studies, University of Groningen, Bloemsingel 1, 9713 BZ Groningen, Netherlands*
[b] *Department of Physiology and Pharmacology and Institute for Drug Research, University of Strathclyde, Glasgow, G1 1XW, UK*

Received 28 September 1995; revised 19 December 1995; accepted 19 January 1996

Abstract

The P_2 purinoceptor antagonist suramin reverses skeletal muscle paralysis evoked by non-depolarizing neuromuscular blocking agents in vitro and in vivo. To further study the action of suramin on neuromuscular transmission, (miniature) endplate potentials ((m.)e.p.ps), motor nerve terminal currents and the release of radiolabeled acetylcholine was measured in isolated nerve–muscle preparations. In preparations paralysed by low Ca^{2+}/high Mg^{2+} conditions, suramin (10 μM–1 mM) induced a concentration-dependent decrease in quantal content of the e.p.ps without affecting m.e.p.ps. Suramin reversed neuromuscular block by *d*-tubocurarine in these preparations. In erabutoxin paralysed preparations, suramin (40 μM–1 mM) inhibited the motornerve terminal currents related to Ca^{2+} influx concentration-dependently, but did not affect Na^+ currents. Suramin-induced inhibition of Ca^{2+} currents was not antagonized by ATPγS. Suramin (300 μM) reduced [^{14}C]acetylcholine outflow in non-paralysed rat phrenic nerve–hemidiaphragm preparations by 32%. As suramin did not chelate Ca^{2+}, these results indicate that suramin inhibits neuromuscular transmission by blocking prejunctional Ca^{2+} channels, thereby decreasing acetylcholine release upon nerve stimulation.

Keywords: Neuromuscular transmission; Suramin; P_2 purinoceptor; Non-depolarizing neuromuscular relaxant; Ca^{2+} channel

1. Introduction

Suramin is a polysulphonated naphtylurea that has been used for many years in the treatment of trypanosomiasis. Suramin is known for its antagonistic properties on all subtypes of P_2 purinoceptors (Dunn and Blakely, 1988; Den Hertog et al., 1989; Hoyle et al., 1990; Leff et al., 1990; Nakazawa et al., 1991), including those on skeletal muscle myotubes (Henning et al., 1992a, 1993a).

Both in vitro and in vivo it was demonstrated that suramin also reverses the action of non-depolarizing neuromuscular blockers in rats, whereas it does not affect neuromuscular blockade by depolarizing blockers (Henning et al., 1992b, 1993b). Suramin's mode of action in reversal of neuromuscular blockade is not yet fully understood. At the neuromuscular junction, ATP is co-released with acetylcholine from the motor nerve ending (Silinsky and Hubbard, 1973), and different receptors sensitive to ATP and its breakdown products (P_2 purinoceptors and P_1 purinoceptors) are present at both the prejunctional (Ribeiro and Walker, 1975; Silinsky et al., 1989; Correia-de-Sá et al., 1991; Hamilton and Smith, 1991) and postjunctional site (Kolb and Wakelam, 1983; Häggblad and Heilbron, 1988; Henning et al., 1992a, 1993a, 1993c; Webb et al., 1993). Therefore, it was initially thought that the suramin-induced reversal of non-depolarizing blockade was due to an action on the purinergic system, i.e. either by an antagonistic action on P_2 purinoceptors or by blocking hydrolysis of ATP into active P_1 purinoceptors agonists. However, experiments in isolated rat nerve–hemidiaphragm preparations suggest that such actions on purinergic systems are presumably not involved in the reversal action of suramin on non-depolarizing neuromuscular blockade (Henning et al., 1992b).

In this study, we examined the prejunctional action of suramin in isolated nerve–muscle preparations in an at-

* Corresponding author. Tel.: (31) (50) 632810; fax: (31) (50) 632812; e-mail: r.h.henning@med.rug.nl

Elsevier Science B.V.
PII S0014-2999(96)00065-9

tempt to further elucidate its intriguing action on non-depolarizing neuromuscular block.

2. Materials and methods

2.1. Intracellular recording

Intracellular recordings of endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps) were made from mouse triangularis sterni nerve–muscle preparations as previously described in detail (Braga et al., 1991). Experiments were carried out at room temperature (20–22°C) using 3 M KCl-filled microlectrodes with a resistance of 5–15 MΩ. To obtain m.e.p.ps characteristics, 200 m.e.p.ps were analysed. Quantal content was determined in preparations paralyzed by continuous superfusion with a low Ca^{2+}/high Mg^{2+} extracellular solution bubbled with 95% O_2/5% CO_2 of the following composition (mM): NaCl 118.5, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 0.5, $MgCl_2$ 1–4, $NaHCO_3$ 25, glucose 11.1 (pH 7.3). Membrane potential was monitored continuously. Quantal content of e.p.ps was determined by the direct method (mean e.p.p. amplitude ($n = 50$)/mean m.e.p.p. amplitude ($n = 200$)) after the data were normalised to a membrane potential of −80 mV. The reversal effect of suramin on non-depolarizing neuromuscular block was determined in *d*-tubocurarine (0.2 μM) paralyzed preparations in external solution of the following composition (mM): NaCl 118.5, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11.1 (pH 7.3). Suramin was added to the superfusing solution cumulatively every 15 min unless indicated otherwise.

2.2. Perineural recording

Perineural recordings of waveforms associated with nerve terminal action potentials were made using mouse triangularis sterni nerve–muscle preparations as previously described (Braga et al., 1991). Muscles were paralysed by 0.5 μM erabutoxin a to prevent twitching (Braga et al., 1991; Pillet et al., 1993). This toxin was chosen to avoid excessive concentrations of non-depolarizing neuromuscular blockers as suramin competitively antagonizes their paralyzing action (Henning et al., 1992a; Henning et al., 1993b) and because suramin effects on Ca^{2+}-related waveforms cannot be obtained in preparations paralyzed by low Ca^{2+}/high Mg^{2+} extracellular solution. Experiments were carried out at room temperature (20–22°C) using 3 M KCl-filled microlectrodes with a resistance of 5–15 MΩ in preparations continuously superfused with external solution of the following composition (mM): NaCl 118.5, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11.1 (pH 7.3). Drugs were added to the superfusing solution.

2.3. Radiolabeled acetylcholine outflow

The experiments were performed on innervated muscle strips of ~1.5 cm width of the left phrenic nerve–hemidiaphragm from 150 g male Wistar rats. Evoked transmitter release was assessed by measuring radioactive outflow from preparations labeled with [^{14}C]choline as described by Wessler and Kilbinger (1986). The preparations were superfused at a rate of 3 ml · min^{-1} in a 2 ml tissue bath at 37°C with a physiological salt solution bubbled with 95% O_2 and 5% CO_2, containing (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 11.2 and choline 1 μM. After a 40 min equilibration period, the perfusion was stopped and nerve endings were loaded with 1 μM [^{14}C]choline (specific activity 53 mCi · $mmol^{-1}$) by electrical stimulation of the nerve with supramaximal rectangular pulses (6 V, 0.3 ms duration) at 1 Hz for 40 min. Nerve stimulation was then stopped and the preparation was washed for 50 min by superfusion at a rate of 20 ml · min^{-1} with physiological salt solution, supplemented with 10 μM hemicholinium-3 to prevent re-uptake of choline. After this period, the superfusion rate was decreased to 1 ml · min^{-1} and the effluent was collected every 3 min. Transmitter release was evoked by phrenic nerve stimulation at 5 Hz for 3 min during three stimulation periods: 12–15 min (S_1); 39–42 min (S_2) and 66–69 min (S_3) to obtain detectable outflow of radiolabeled acetylcholine over basal values. Radioactive content of the samples was measured by liquid scintillation spectrometry and expressed as dps · g^{-1} wet weight. Suramin was added to the superfusing solution 15 min before the start of S_3.

2.4. Materials

Suramin was obtained from Bayer (Leverkusen, Germany) and [^{14}C]choline from Du Pont (Den Bosch, Netherlands). Erabutoxin a was a gift from Professor N. Tamiya (Tohoku University, Sendai, Japan). Other compounds were from Sigma (Poole, UK).

2.5. Statistics

Results are presented as mean ± S.E. of at least four experiments unless otherwise stated. Statistical analysis was by paired or unpaired Student's *t*-test, $P < 0.05$ being regarded as significant.

3. Results

3.1. Intracellular recordings

Suramin (10 μM–1 mM) had no effect on the resting membrane potential of mouse triangularis sterni prepara-

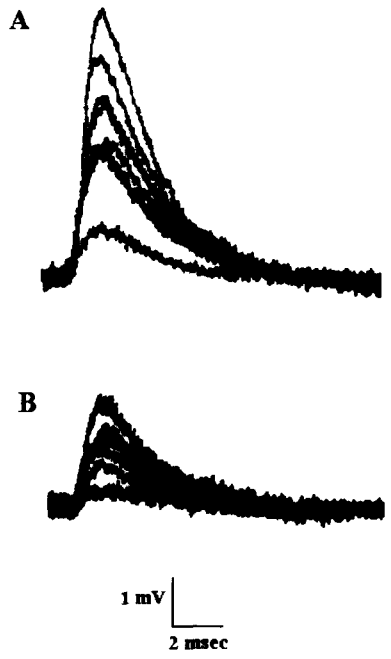

Fig. 1. The effect of suramin on the endplate potential (e.p.p.) of a mouse triangular sterni preparation. Superimposed e.p.ps in a preparation paralysed by low Ca^{2+}/high Mg^{2+} solution ($CaCl_2$ 0.5 mM, $MgCl_2$ 3 mM). A: control; B: the same preparation after the addition of suramin (1 mM, 15 min). Each panel shows 7 superimposed e.p.ps recorded at room temperature (membrane potential = −80 mV). Registrations are representative of four experiments in which 50 e.p.ps and 200 m.e.p.ps were analysed to determine quantal content. Initial and postexperiment quantal content did not differ (4.6 ± 2.2 and 4.3 ± 2.5, respectively; $n = 4$).

tions paralysed by low Ca^{2+}/high Mg^{2+} conditions, which measured −79.4 ± 3.4 and −80.6 ± 2.7 mV in the absence and the presence of suramin (1 mM), respectively ($n = 5$). Addition of suramin (10 μM–1 mM) did not change the time course, amplitude or frequency of miniature endplate potentials (m.e.p.ps) either (m.e.p.p frequency: 5.1 ± 2.1 s^{-1} and 5.2 ± 1.8 s^{-1}; m.e.p.p amplitude: 0.73 ± 0.1 and 0.78 ± 0.2 mV, in the absence or presence of suramin (1 mM), respectively; $n = 5$). Suramin, however, induced a concentration-dependent decrease in the amplitude of the endplate potential (e.p.p.) without affecting the e.p.p. time course (Fig. 1). This implies that suramin reduced the average quantal content of e.p.ps (Fig. 3). The effect of suramin on quantal content was reversible by washing the preparation with suramin-free buffer for 15 min (initial and postexperiment quantal content: 4.6 ± 2.2 and 4.3 ± 2.5, respectively; $n = 4$).

This apparently prejunctional inhibitory effect of suramin, as indicated by the decrease in quantal content, is difficult to reconcile with its reported reversal of neuromuscular block induced by non-depolarizing neuromuscular blocking agents in rat. To exclude species differences, the reversal action of suramin on non-depolarizing neuromuscular block was examined in *d*-tubocurarine paralysed mouse triangular sterni preparations. In tubocurarine-paralysed preparations (0.2 μM), suramin (100 μM) increased the amplitude of e.p.ps, subsequently resulting in muscle-twitching within 5 min ($n = 3$).

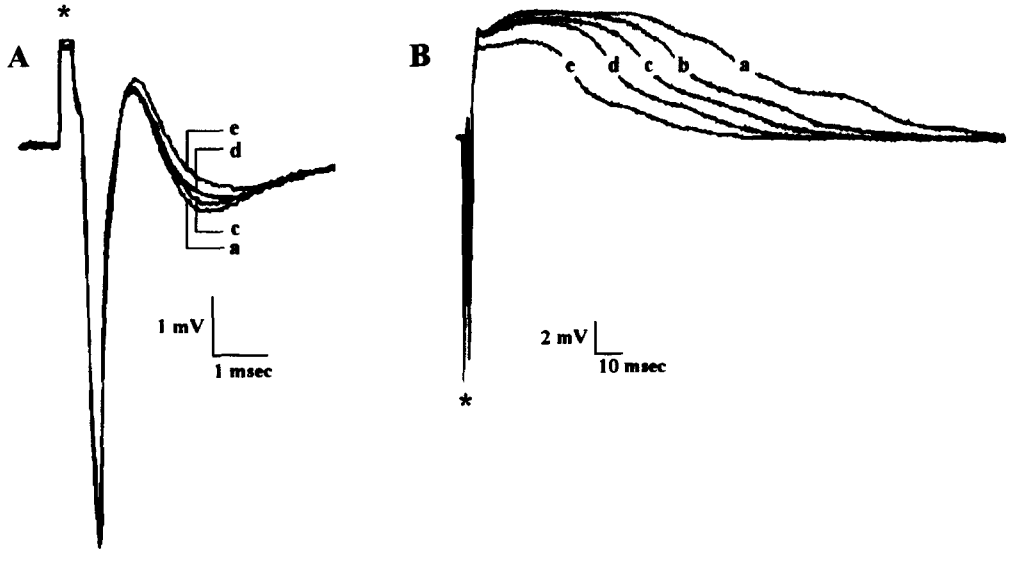

Fig. 2. The effect of cumulative concentrations of suramin at the motor nerve terminal of mouse triangular sterni nerve-muscle preparation. A: inhibition of the calcium-activated K^+ current by cumulative concentrations of suramin. Voltage-dependent K^+ channels were inactivated by 3,4-diaminopyridine (400 μM). The first negative deflection is due to influx of Na^+ at the last nodes of Ranvier of the parent axon. B: inhibition of the Ca^{2+} current caused by cumulative concentrations of suramin. The K^+ channels were blocked by 3,4-diaminopyridine (400 μM) and tetraethylammonium (4 mM). The initial downstroke is due to Na^+ influx, followed by the upstroke of the unmasked Ca^{2+} influx. Suramin was added every 15 min to obtain the following cumulative concentrations: a, control; b, 10 μM; c, 30 μM; d, 100 μM; e, 300 μM. * Stimulus artefact. Note the difference in the time scale of recordings A and B.

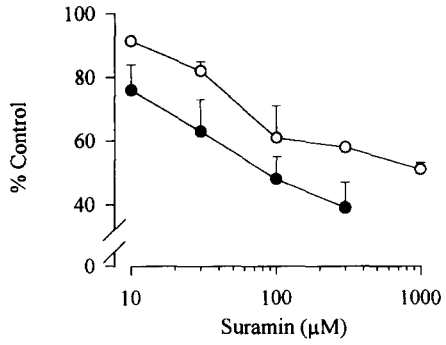

Fig. 3. Concentration-dependent inhibitory effect of cumulative concentrations of suramin on average quantal content of the e.p.ps and Ca^{2+} influx in mouse triangular sterni muscle-nerve preparation. Quantal content in preparations paralyzed by low Ca^{2+}/high Mg^{2+} solution (○; $n = 4$). Initial and postexperiment quantal content did not differ (4.6 ± 2.2 and 4.3 ± 2.5, respectively). Area of the Ca^{2+} plateau of perineural waveforms in the presence of 3,4-diaminopyridine (400 μM) and tetraethylammonium (4 mM) (●; $n = 5$). Values are expressed as the percentage of initial values ± S.E. All values in the presence of suramin (30–1000 μM) are significantly different from initial values ($P < 0.05$, paired Student's t-test).

3.2. Perineural recordings

The prejunctional inhibitory action of suramin on neuromuscular transmission was studied further using the perineural recording technique in preparations paralysed with erabutoxin a, an irreversible nicotinic acetylcholine receptor antagonist (Pillet et al., 1993). Under these conditions, the perineural waveform shows two major components related to an inward Na^+ current at the last few nodes of Ranvier and an outward K^+ current at the nerve terminal (Mallart, 1985; Braga et al., 1991). Suramin (10 μM–1 mM) had no effect on either of these components. In the presence of 3,4-diaminopyridine (400 μM) to inactivate voltage-dependent K^+-channels, the perineural waveform consists of three phases. The initial fast downward deflection is due to Na^+ influx, the second positive phase of the waveform is related to an inward Ca^{2+} current, while the third phase, a slower negative deflection, is due to an outward Ca^{2+}-activated K^+ current at the nerve endings (Fig. 2A; Mallart, 1985; Anderson et al., 1988). Suramin (10–300 μM) reduced the amplitude of the Ca^{2+}-related waveforms in a concentration-dependent fashion (Fig. 2A). The Na^+-dependent portion of the waveform as represented by the initial fast downward deflection, was not affected by suramin (Fig. 2A).

In the presence of 3,4-diaminopyridine, the Ca^{2+}-activated K^+ channels were subsequently blocked by tetraethylammonium (4 mM). Under these conditions, a prolonged Ca^{2+}-dependent plateau developed (Fig. 2B; Mallart, 1985; Penner and Dreyer, 1986). Suramin (10 μM–300 μM) produced a reversible and concentration-dependent decrease of the Ca^{2+} plateau (Fig. 2B, Fig. 3).

To investigate whether the suramin-induced decrease of the Ca^{2+} plateau is related to the P_2 purinoceptor antagonistic property of this compound, the effect of ATPγS was studied on perineural recordings. Cumulative concen-

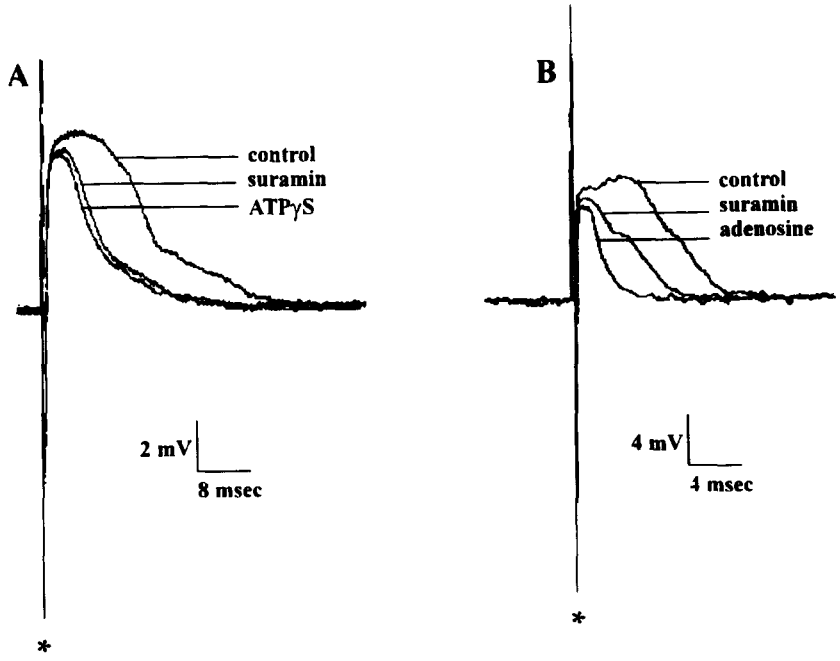

Fig. 4. The effect of purinoceptor agonists on suramin depressed Ca^{2+} currents in mouse triangular sterni nerve-muscle preparations. A: the P_2 purinoceptor agonist, ATPγS (300 μM), did not affect the depressed Ca^{2+} plateau of a suramin treated preparation (300 μM; 15 min). B: the P_1 purinoceptor agonist adenosine (300 μM) further depressed the Ca^{2+} plateau of another treated preparation suramin (300 μM; 15 min). The K^+ channels were blocked by 3,4-diaminopyridine (400 μM) and tetraethylammonium (4 mM). The initial downstroke is due to Na^+ influx, followed by the upstroke of the unmasked Ca^{2+} influx. * Stimulus artefact. Recordings are representative of three or more experiments.

trations of ATPγS (10 μM–300 μM) did not affect the Ca^{2+} plateau phase of the perineural recording (not shown), nor did ATPγS affect the decline in the plateau phase of suramin (300 μM) treated preparations (Fig. 4A). To investigate a possible action of suramin on prejunctional P_1 purinoceptors, the interaction between the P_1 purinoceptor agonist adenosine and suramin was studied. Adenosine (1 μM–1 mM) progressively decreased the Ca^{2+} plateau of perineural recordings in untreated preparations (not shown) as well as those of preparations pretreated with suramin (300 μM; Fig. 4B).

As these experiments indicated an action of suramin on Ca^{2+} influx in the nerve ending, it was checked whether suramin chelates Ca^{2+}, by addition of suramin to a buffer containing (mM) NaCl 145, KCl 5, $MgCl_2$ 1.2, glucose 11, HEPES 5, $CaCl_2$ 2.5. Addition of suramin (10–600 μM) did not change the voltage difference recorded by the calcium sensitive electrode (percentage of the initially recorded voltage at suramin 600 μM: 102.2 ± 3.8%, n = 5).

3.3. Radiolabeled acetylcholine outflow

To examine the prejunctional inhibitory action of suramin in non-paralysed nerve–muscle preparations, the outflow of [^{14}C]acetylcholine from nerve endings was measured in indirectly stimulated rat phrenic nerve–hemidiaphragm preparations. Suramin did not change basal acetylcholine release (rate constants of exponential decline: 74.1 ± 9.3 s^{-1} and 73.4 ± 10.3 s^{-1} in the absence and the presence of suramin, respectively (n = 4)). During the first stimulation period (S_1), the ^{14}C outflow amounted 30.6 ± 9.1 dps · g^{-1} wet weight (n = 8). The evoked [^{14}C]acetylcholine outflow during the following stimulation periods (S_2, S_3) was expressed as percentage of that during the first period of stimulation (S_1). Suramin (300 μM), added after the second stimulation period, decreased the evoked [^{14}C]acetylcholine outflow during S_3 by ~ 30% compared with control preparations (Table 1).

Table 1
Inhibitory effect of suramin on evoked [^{14}C]acetylcholine outflow from phrenic nerve–hemidiaphragm preparations

	S_2/S_1	S_3/S_2
Control	0.89 ± 0.08	0.62 ± 0.02
Suramin	0.90 ± 0.09	0.42 ± 0.07 [a]

The preparation was stimulated for 3 min at 5 Hz during three subsequent stimulation periods (S_1, S_2, S_3). Suramin (300 μM) was introduced immediately after S_2, 15 min before the start of S_3. Data are expressed as mean ratios of release (± S.E.) of dps · g^{-1} wet tissue during the respective stimulation periods (n = 4). [a] P = 0.03 as compared with S_3/S_2 ratio from control experiments (Student's t-test). The release of [^{14}C]acetylcholine over basal levels during S_1 amounted 46.6 ± 4.6 dps · g^{-1} wet tissue (n = 4).

4. Discussion

This is the first study to describe a prejunctional action of the P_2 purinoceptor antagonist suramin on adult neuromuscular transmission. The prejunctional inhibitory effect of suramin was demonstrated in isolated nerve–muscle preparations by the concentration-dependent reduction of the average quantal content of e.p.ps and the diminished release of [^{14}C]acetylcholine. Furthermore, suramin depressed the Ca^{2+}-carried plateau phase of the perineural recording without affecting currents dependent on voltage-activated Na^+ and K^+ channels, indicating that suramin primarily inhibits the Ca^{2+} entry in the motor nerve terminal. Since the amount of acetylcholine released upon nerve stimulation is dependent on the quantity of Ca^{2+} entering the motor nerve terminal, the action of suramin on e.p.ps and acetylcholine outflow can readily be explained by inhibition of the Ca^{2+} entry process. As suramin did not chelate Ca^{2+}, it seems likely that its mode of action is related to a blockade of the prejunctional voltage-dependent Ca^{2+} channel, which is presumably of the P-type (Uchitel et al., 1992). Such a mode of action may also underlie the blockade by suramin of presynaptic Ca^{2+} levels following initial nerve–muscle contact in cell co-cultures (Dai and Peng, 1993). Further, at present it is not known whether suramin exerts a similar inhibitory action at other synapses than the neuromuscular junction.

Although suramin is known as a competitive antagonist for different P_2 purinoceptors receptors (Dunn and Blakely, 1988; Den Hertog et al., 1989; Hoyle et al., 1990; Leff et al., 1990; Nakazawa et al., 1991; Henning et al., 1992a, 1993a) and exerts its action at concentrations expected to antagonize actual ATP concentrations at the neuromuscular junction (Wagner et al., 1978; Volknandt and Zimmerman, 1986; Smith, 1991), it seems unlikely that P_2 purinoceptors are involved in the prejunctional inhibitory action of suramin. ATPγS, a stable P_2 purinoceptor agonist, did not affect the Ca^{2+}-regulated components of the perineural recording and did not restore the suramin-induced depression of the Ca^{2+}-related waveforms. Also, it has been reported that ATP and analogues other than ATPγS do not affect perineural waveforms recorded at motor nerve endings (Hamilton and Smith, 1991).

Further, it seems unlikely that the suramin evoked prejunctional inhibition is related to an action through prejunctional P_1 purinoceptors (Ribeiro and Walker, 1975; Silinsky et al., 1989; Hamilton and Smith, 1991; Correia-de-Sá et al., 1991). First, an antagonistic effect of suramin on P_1 purinoceptors has not been described (Henning et al., 1993c). Second, it may be that the action of suramin is due to inhibition of ectonucleotidases (Smolen and Weissmann, 1978; Hourani and Chown, 1989), causing a decrease in the formation of adenosine. In such case, the inhibition by suramin should have been overcome by addition of adenosine, which clearly was not the case.

Suramin has been reported to reverse paralysis induced by non-competitive neuromuscular blocking agents both in the isolated rat diaphragm (Henning et al., 1992b) and in the anaesthetized rat (Henning et al., 1993b). This reversal effect of suramin was confirmed here in mouse nerve–muscle preparations. Several observations oppose the possibility that the reversal action of suramin can be explained by suramin possessing an opposite effect on acetylcholine release depending on initial quantal content, i.e. reduction of quantal content in preparations with low initial quantal content (low Ca^{2+}/high Mg^{2+}) and augmentation in preparations with assumed high initial quantal content (*d*-tubocurarine paralyzed). The prejunctional inhibiting effect was confirmed on perineural recordings and in radiolabeled acetylcholine outflow experiments, both preparations with assumed high initial quantal content. Also, suramin was observed not to change the concentration–twitch height relationship for the prejunctionally evoked paralysis by lowering Ca^{2+} or increasing Mg^{2+} in the extracellular solution in indirectly stimulated rat hemidiaphragm (Henning et al., 1992b). Thus, the mechanism underlying the reversal action of suramin of non-depolarizing neuromuscular blockers is still unclear. The prejunctional inhibitory action of suramin is just opposite of what is needed to reverse a non-depolarizing blockade. Therefore, a prejunctional action of suramin cannot explain its reversal properties. As suramin does not affect acetylcholinesterase activity (Henning et al., 1992b), the last possible action of suramin to explain its reversal properties on non-depolarizing neuromuscular blockers is a postjunctional action. However, as suramin did not change the amplitude and time course of m.e.p.ps, skeletal muscle membrane potential or directly evoked twitch (Henning et al., 1992b), it is unlikely that suramin by itself possesses a postjunctional action on neurotransmission. Therefore, it is concluded that the reversal action of suramin is not due to a stimulatory action of the compound on neurotransmission. Consequently, the reversal action of suramin has to be related to interference with the action of non-depolarizing blockers on the nicotinic receptor as suggested before (Henning et al., 1992b). The β- and δ-subunits of the nicotinic acetylcholine receptor of *Torpedo* have been shown to possess extracellular binding sites for ATP (Carlson and Raftery, 1993). A possible explanation for the reversal action of suramin might be the overlap of the binding domains of suramin (to the ATP site) and non-competitive neuromuscular blockers (to the acetylcholine site).

In conclusion, a prejunctional inhibitory action of suramin on neuromuscular transmission was found, not related to its purinoceptor antagonistic properties, but most likely caused by the inhibition of prejunctional Ca^{2+} entry, resulting in a diminished release of acetylcholine from the motor nerve terminal. The absence of a stimulatory action of suramin on pre- and postjunctional sites of neurotransmission indicate that the reversal of non-depolarizing neuromuscular blockade by suramin is due to a direct interference with the action of this class of neuromuscular blocking agents.

Acknowledgements

The authors wish to thank Dr. G. Smith for assistance in experiments with the calcium sensitive electrode and Dr. P. Correia-de-Sá for his valuable recommendations with respect to the acetylcholine outflow experiments. This study was partially sponsored by Grant 903–42–004 from the Dutch Organization for Scientific Research (NWO).

References

Anderson, A.J., A.L. Harvey, E.G. Rowan and P.N. Strong, 1988, Effects of charybdotoxin, a blocker of Ca^{2+}-activated K^+ channels, on motor nerve terminals, Br. J. Pharmacol. 95, 1299.

Braga, M.F.M., E.G. Rowan and A.L. Harvey, 1991, Effects of tacrine, velnacrine (HP029), suronacrine (HP128), and 3,4-diaminopyridine on skeletal neuromuscular transmission in vitro, Br. J. Pharmacol. 102, 909.

Carlson B.J. and M.A. Raftery, 1993, Specific binding of ATP to extracellular sites on *Torpedo* acetylcholine receptor, Biochemistry 32, 7329.

Correia-de-Sá, P., A.M. Sebastião and J.A. Ribeiro, 1991, Inhibitory and excitatory effects of adenosine receptor agonists on evoked transmitter release from phrenic nerve endings of the rat, Br. J. Pharmacol. 103, 1614.

Dai Z. and H.B. Peng, 1993, Elevation in presynaptic Ca^{2+} level accompanying initial nerve–muscle contact in tissue culture, Neuron 10, 827.

Den Hertog, A., J. Van den Akker and S.A. Nelemans, 1989, Suramin and the inhibitory junction potential in taenia caeci of the guinea-pig, Eur. J. Pharmacol. 173, 207.

Dunn P.M. and A.G. Blakely, 1988, Suramin: a reversible P_2 purinoceptor antagonist in mouse vas deferens, Br. J. Pharmacol. 93, 243.

Häggblad, J. and E. Heilbron, 1988, P_2 purinoceptor-stimulated phosphoinositide turnover in chick myotubes, FEBS Lett. 235, 133.

Hamilton, B.R. and D.O. Smith, 1991, Autoreceptor-mediated purinergic and cholinergic inhibition of motor nerve terminal calcium currents in the rat, J. Physiol. (London) 432, 327.

Henning, R.H., S.A. Nelemans, J. Van den Akker and A. Den Hertog, 1992a, The nucleotide receptors on mouse C2C12 myotubes, Br. J. Pharmacol. 106, 853.

Henning, R.H., S.A. Nelemans, A.H.J. Scaf, J. Van Eekeren, S. Agoston and A. Den Hertog, 1992b, Suramin reverses non-depolarizing neuromuscular blockade in rat diaphragm, Eur. J. Pharmacol. 216, 73.

Henning, R.H., M. Duin, A. den Hertog and S.A. Nelemans, 1993a, Activation of the phospholipase C pathway by ATP is mediated exclusively through nucleotide type P_2 purinoceptors in C2C12 myotubes, Br. J. Pharmacol. 110, 747.

Henning, R.H., M.C. Houwertjes, S.A. Nelemans and S. Agoston, 1993b, Reversal by suramin of neuromuscular block produced by pancuronium in the anaesthetized rat, Br. J. Pharmacol. 108, 717.

Henning, R.H., M. Duin, A. den Hertog and S.A. Nelemans, 1993c, Characterization of P_2 purinoceptor mediated cyclic AMP formation in mouse C2C12 myotubes, Br. J. Pharmacol. 110, 133.

Hourani, S.M.O. and J.A. Chown, 1989, The effects of some possible inhibitors of ectonucleotidases on the breakdown and pharmacological effects of ATP in the guinea pig urinary bladder, Gen. Pharmacol. 20, 177.

Hoyle, C.H.V., G.E. Knight and G. Burnstock, 1990, Suramin antagonizes responses to P_2 purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli, Br. J. Pharmacol. 99, 617.

Kolb, H.A. and M.J.O. Wakelam, 1983, Transmitter-like action of ATP on patched membranes of cultured myoblasts and myotubes, Nature 303, 621.

Leff, P., B.E. Wood and S.E. O'Connor, 1990, Suramin is a slowly-equilibrating but competitive antagonist at P_2 receptors in rabbit isolated ear artery, Br. J. Pharmacol. 101, 645.

Mallart, A., 1985, Electric current flow inside perineurial sheats of mouse motor nerves, J. Physiol. (London) 368, 565.

Nakazawa K., K. Fujimori, A.. Takanaka and K. Inoue, 1991, Reversible and selective antagonism by suramin of ATP-activated inward current in PC12 phaeochromocytoma cells, Br. J. Pharmacol. 102, 581.

Penner, R. and F. Dreyer, 1986, Two different presynaptic calcium currents in mouse motor nerve terminals, Pflügers Arch. 406, 190.

Pillet, L., O. Tremeau, F. Ducancel, P. Drevet, S. Zinn-Justin, S. Pinkasfeld, J.C. Boulain and A. Menez, 1993, Genetic engineering of snake toxins. Role of invariant residues in the structural and functional properties of a curaremimetic toxin, as probed by site-directed mutagenesis, J. Biol. Chem. 268, 909.

Ribeiro, J.A. and J. Walker, 1975, The effect of adenosine triphosphate and adenosine diphosphate on transmission at the rat and frog neuromuscular junctions, Br. J. Pharmacol. 54, 213.

Silinsky, E.M. and J.I. Hubbard, 1973, Release of ATP from rat motor nerve terminals, Nature 243, 404.

Silinsky, E.M., C. Solsona and J.K. Hirsh, 1989, Pertussis toxin prevents the inhibitory effect of adenosine and unmasks adenosine-induced excitation at mammalian motor nerve endings, Br. J. Pharmacol. 97, 16.

Smith, D.O., 1991, Sources of adenosine released during neuromuscular transmission in the rat. J. Physiol. (London) 432, 343.

Smolen, J.E. and G. Weissmann, 1978, Mg^{2+}-ATPase as a membrane ecto-enzym of human granulocytes: Inhibitors, activators and response to phagocytosis, Biochim. Biophys. Acta 512, 525.

Uchitel, O.D., D.A. Protti, V. Sanchez, B.D. Cherksey, M. Sugimori and R. Llinas, 1992, P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses, Proc. Natl. Acad. Sci. USA 89, 3330.

Volknandt, W. and H. Zimmerman, 1986, Acetylcholine, ATP, and proteoglycan are common to synaptic vesicals isolated from the electric organs of eel and electric catfish as well as from rat diahragm, J. Neurochem. 47, 1449.

Wagner, J.A., S.S. Carlson and R.B. Kelly, 1978, Chemical and physical characterization of cholinergic synaptic vesicels, Biochemistry 17, 1199.

Webb, T.E., J. Simon, B.J., Krishek, A.N. Bateson, T.G. Smart, B.F. King, G. Burnstock and E.A. Barnard, 1993, Cloning and functional expression of a brain G-protein-coupled ATP receptor, FEBS Lett. 324, 219.

Wessler, I. and H. Kilbinger, 1986, Release of [^{3}H]acetylcholine from a modified rat phrenic nerve–hemidiaphragm preparation, Naunyn-Schmied. Arch. Pharmacol. 334, 357.