Suramin protects the murine motor nerves from the toxic effects of presynaptic Ca^{2+} channel inhibitors

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Abstract

The purpose of this study is to investigate whether suramin is capable of preventing the neurotoxic effects of Ca^{2+} channel inhibitors at the presynaptic sites. Mouse diaphragm and triangularis sterni preparations were used for this study in order to measure the muscle tension and nerve terminal Ca^{2+} current, respectively. Both ω -conotoxin MVIIC and ω -agatoxin IVA markedly inhibit the nerve-evoked muscle contractions as well as the nerve terminal Ca^{2+} current respectively. Pretreatment with suramin (0.3 mM) significantly reduced the inhibitory effect of nerve-evoked muscle contractions and Ca^{2+} current induced by either ω -conotoxin MVIIC or ω -agatoxin IVA but not that induced by the non-selective Ca^{2+} channel blocker, Cd^{2+} . Neither suramin nor Ca^{2+} -channel toxins significantly affect Na^{+} - and K^{+} currents of the nerve terminals. These findings indicate that suramin selectively interferes the action of presynaptic Ca^{2+} channel neurotoxins and thus reduces their depressant effects on the muscle contractions. The implication of these findings is that suramin and its derivatives may potentially become useful agents in management of intoxication of Ca^{2+} channel neurotoxins. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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Suramin is an antitrypanosomal agent for clinical use in human beings. We have previously demonstrated that suramin can inhibit the toxic effect of a presynaptic neurotoxin, β -bungarotoxin at mouse motor nerve terminal [8]. This protective effect of suramin was apparently mediated by a selective presynaptic site of action without purinoceptor involved. Since suramin can produce a weak inhibitory effect on the presynaptic Ca^{2+} current of motor nerve terminal [3], it is thus considered that suramin may exert an interference on the toxic effects of neurotoxins on the presynaptic Ca^{2+} channel.

The presynaptic Ca^{2+} channel toxins, ω -agatoxin IVA and ω -conotoxin MVIIC were isolated from the venom of spider *Agelenopsis aperta* [11] and sea snail *Conus magus* [4], respectively. They produce a potent inhibitory effect on the neurotransmitter release from the mouse motor nerve terminal [5,6]. It has been known that ω -agatoxin IVA is a P-type selective Ca^{2+} channel blocker, which can inhibit the neurotransmitter release from mouse motor nerve terminal

[12]. It is still awaited for searching the neuroprotective agents useful for management of the intoxication from these presynaptic Ca^{2+} channel neurotoxins in cases of spider bites and sea snail stings. Thus, it is important to explore whether suramin possesses the neuroprotective effect against presynaptic Ca^{2+} channel neurotoxins.

Mice of ICR strain (18–25 g) were sacrificed by rapid cervical dislocation. Phrenic nerve-hemidiaphragm preparations were isolated and suspended in 5–10 ml of oxygenated modified Krebs' solution with the compositions (in mM): NaCl 131, KCl 4.8, MgSO_4 1.2, CaCl_2 1–2.5, NaHCO_3 12.5 and glucose 11 (working temperature, 35–37°C, pH 7.2–7.4). Muscle contractions were elicited by electrical stimulation of the phrenic nerve with supramaximal rectangular pulses of 0.05 ms duration at 0.1 Hz and recorded with an isometric transducer on a Grass Model 7E polygraph. The muscle resting tension was adjusted to 1 g and maintained for 20–30 min to reach a steady-state before starting the experimental protocol. The left triangularis sterni intercostal nerve-muscle preparation was isolated according to the method described previously [6,9,10]. The isolated preparations were pinned out on the sylgard-coated glass chamber

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(2 ml) and visualized at 320 $\times$ magnification by a Zeiss microscope (Axioskop) equipped with Nomarski interference contrast optics. Preparations were continuously perfused at a rate of 3–5 ml min⁻¹ with oxygenated (95% O₂ plus 5% CO₂) modified Krebs' solution containing 5 mM Ca²⁺ performed at temperatures ranging from 24–28°C. The intercostal nerves were stimulated by a suction electrode using supramaximal voltage and square-wave pulses of 0.02 ms duration at 0.2 Hz (A-M systems model 2100 stimulator). Presynaptic waveforms were obtained from extracellular recording close to nerve terminal endings of intercostal nerves with glass microelectrodes filled with 2 M NaCl (resistance 5–15 M Ω) placed inside the perineural sheath [9]. Postsynaptic activity was blocked by adding 20–30 μ M

D-tubocurarine to the bathing medium. The signals of presynaptic waveforms were displayed on an oscilloscope (Tektronix 2221A) and stored in a videotape recorder (Neuro-Corder Recording Unit) and drawn by HC plotter (Tektronix). This study was conducted in accordance with the guideline for the care and use of Laboratory Animals by the Animal Research Committee in National Taiwan University, College of Medicine. All mice were housed in the cage and maintained on 12 h light and dark cycle and allow free access to food and water.

Suramin inhibits the depressant effects of presynaptic neurotoxins on nerve-evoked muscle contractions of diaphragm. Application of either ω -agatoxin IVA (80 nM) or ω -conotoxin MVIIC (120 nM) completely inhibited the

Fig. 1. Protection by suramin from the inhibitory effect of nerve-evoked muscle contractions induced by Ca²⁺ channel blockers in the mouse phrenic nerve-diaphragm. The preparation was bathed in the Krebs' solution containing 1 mM Ca²⁺. ω -Conotoxin MVIIC 120 nM (A) and ω -agatoxin IVA 80 nM (B) produced a reversible inhibition of nerve-evoked muscle contractions respectively. Pretreatment with suramin (0.3 mM) prevented the inhibitory effect of nerve-evoked muscle contractions induced by ω -conotoxin MVIIC (A) and ω -agatoxin IVA (B) respectively. However, suramin cannot prevent the inhibitory effect of nerve-evoked muscle contractions induced by Cd²⁺ (30 μ M) (C). Calibration: 10 min, 2 g.

Fig. 2. Suramin prevents the inhibitory effect of Ca²⁺-current induced by ω -agatoxin IVA and ω -conotoxin MVIIC on the nerve terminal of mouse triangularis sterni preparations. Pretreatment with suramin (0.1 mM) can prevent the further inhibitory effect on Ca²⁺ current induced by either 80 nM ω -agatoxin IVA (A) or 120 nM ω -conotoxin MVIIC (B), respectively. Note that suramin alone partially inhibits the Ca²⁺ current. Calibration: 5 ms, 1 mV.

Fig. 3. Suramin affects neither the inhibitory effect of Cd^{2+} on Ca^{2+} current nor Na^+ and K^+ currents of mouse triangularis-sterni nerve terminals. Suramin produced a partial inhibitory effect on Ca^{2+} current of triangularis sterni preparation (A). Cd^{2+} can produce a nearly complete inhibitory effect on Ca^{2+} current (B). Suramin cannot prevent the further inhibitory effect of Cd^{2+} ($30 \mu\text{M}$) on Ca^{2+} current (C). Suramin and Ca^{2+} channel blockers have no effects on Na^+ and K^+ current of triangularis sterni preparations. The Na^+ and K^+ currents were not significantly inhibited by treatment with 0.3 mM suramin and the subsequent application of 80 nM ω -agatoxin IVA or 120 nM ω -conotoxin MVIIC (D). (A–C) Calibration: 5 ms , 1 mV . (D) Calibration: 1 ms , 2 mV .

nerve-evoked muscle contractions of mouse diaphragm in 1 mM Ca^{2+} Krebs' solution. Pretreatment with suramin (0.3 mM) prevented the depressant effect elicited by either ω -agatoxin IVA or ω -conotoxin MVIIC (Fig. 1A,B; $n = 3$). Suramin also has protective effect when given 10 min after the application of the calcium channel neurotoxin (ω -agatoxin IVA: $95 \pm 4\%$ of control tension). However, suramin cannot prevent the blocking effect induced by $30 \mu\text{M}$ Cd^{2+} (Fig. 1C; $n = 3$).

Suramin inhibits the suppressive effects of presynaptic neurotoxins on Ca^{2+} current of triangularis-sterni. The Ca^{2+} currents were revealed by combined treatment with two K^+ channel blockers, 3,4-diaminopyridine (0.2 mM) and tetraethylammonium chloride (10 mM). The calcium currents were significantly inhibited by ω -agatoxin IVA 80 nM or ω -conotoxin MVIIC 120 nM (Fig. 2A,B; $n = 3$). Suramin (0.1 mM) alone partially inhibits Ca^{2+} current (Fig. 3A; $n = 4$). Pretreatment with suramin significantly prevents the inhibitory effect induced by presynaptic neurotoxin, ω -agatoxin IVA or ω -conotoxin MVIIC (Fig. 2A,B; $n = 3$) but not that induced by the non-selective Ca^{2+} channel blocker, Cd^{2+} (Fig. 3B,C; $n = 3$). Both suramin and presynaptic Ca^{2+} -channel neurotoxins are without effects on Na^+ and K^+ currents of triangularis-sterni (Fig. 3D; $n = 3$).

In this study, we have demonstrated that suramin can protect the motor nerve from the inhibitory effects of Ca^{2+} channel neurotoxins on the Ca^{2+} current. From these results, we propose that suramin can inhibit the toxic effects of presynaptic large molecular neurotoxins but not the small molecular heavy metal Cd^{2+} . Since suramin is a polysulfate anionic compound with rich negative charges, thus whether the rich negative charges of suramin to produce an interactive effect with the positive charges of presynaptic neurotoxins still need for further investigation. However, suramin cannot inhibit the neuromuscular blocking effect of a post-synaptic neurotoxin, α -bungarotoxin [7], suggesting that this presynaptic effect seems not absolutely dependent on the rich negative charges of suramin. Both ω -agatoxin IVA and ω -conotoxin MVIIC, the antagonists of P-type Ca^{2+} channels, blocked transmitter release from nerve endings [6,12]. Therefore, a P-type Ca^{2+} channel obviously exists in mouse motor nerve endings. In this study, we have found that suramin can produce a weak inhibitory effect on Ca^{2+} current (a partial antagonist). It is thus apparently that suramin can bind to the presynaptic P-type Ca^{2+} channel without the full effect of inhibition. The possible mechanism of suramin on the depressant effect of presynaptic Ca^{2+} toxins is that suramin occupies the presynaptic P-type Ca^{2+} channels and then prevents the further binding by presynaptic Ca^{2+} neurotoxins. This contention is in agreement with the previous finding that suramin can reverse the neuromuscular blocking actions of the non-depolarizing muscle relaxant such as D-tubocurarine [1,2] and tripeptide carbobenzoxy-GLY-GLY-GLY-ARG-naphthylamide [7], but not the depolarizing muscle relaxant succinylcholine. We still

don't know why suramin has antagonistic effects both on the postsynaptic nicotinic acetylcholine (ACh) receptor, e.g. d-TC and presynaptic Ca^{2+} channel neurotoxins, e.g. ω -agatoxin IVA. It could be a different mechanism between postsynaptic and presynaptic inhibitory effects of suramin. Nevertheless, these inhibitory effects of suramin are not related with its effect on ATP receptors (P2) as reported by Henning et al. [2]. In conclusion, suramin significantly inhibits neuromuscular blocking effect of the presynaptic Ca^{2+} neurotoxins, ω -agatoxin IVA and ω -conotoxin MVIIC. This effect of suramin is mediated by binding to the presynaptic Ca^{2+} channel, preventing the further blocking effect induced by other presynaptic Ca^{2+} channel toxins.

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