RESEARCH REPORT

Suramin inhibits the early effects of PLA_2 neurotoxins at mouse neuromuscular junctions: A twitch tension study

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ABSTRACT

Several phospholipase A_2 (PLA_2) neurotoxins from snake venoms can affect acetylcholine release at the neuromuscular junction. In isolated nerve-muscle preparations three distinct phases have been described for this phenomenon: An initial transient decrease in twitch tension; a second facilitatory phase during which twitch height is greater than control twitch height; and the last phase which causes a reduction in twitch height that finally results in paralysis. Suramin has been reported to inhibit the toxic effects of β-bungarotoxin and another PLA_2 neurotoxin, crotoxin *in vitro* and *in vivo*. We have further examined the effects of suramin on the three phases of the effects of the presynaptic PLA_2 neurotoxins β-bungarotoxin, taipoxin and ammodytoxin on mouse phrenic nerve-hemidiaphragm preparations. When preparations were pre-treated with suramin (0.3mM), the early biphasic effects (depression followed by facilitation) were abolished, and the time taken for final blockade induced by β-bungarotoxin, taipoxin and ammodytoxin A was significantly prolonged. In contrast, suramin did not significantly affect the facilitation induced by the potassium channel blocking toxin dendrotoxin I when applied under the same conditions. In addition, application of 0.3mM suramin did not prevent the facilitatory actions of 3,4-diaminopyridine (3,4-DAP) and tetraethylammonium chloride (TEA). Overall, the mechanism whereby suramin reduces the effects of PLA_2 neurotoxins remains elusive. Since suramin reduces both enzyme-dependent and enzyme-independent effects of the toxins, suramin is not acting as a simple enzyme inhibitor. Furthermore, the observation that suramin does not affect actions of standard K^+ channel blockers suggests that suramin does not stabilise nerve terminals.

KEYWORDS: PLA_2 neurotoxins, β-bungarotoxin, Taipoxin, Ammodytoxin, Suramin, Mouse phrenic nerve hemidiaphragm preparations

INTRODUCTION

Suramin (an anti-trypanosomiasis drug and antagonist of P2 purinoceptors; Hoyle et al, 1990) has been reported to reverse the blocking action of non-depolarizing relaxants such as tubocurarine and pancuronium on twitch tension of rat diaphragm. This effect has not been observed on the paralysis caused by a depolarizing relaxant agent such as suxamethonium (Henning et al, 1992). Also suramin has an inhibitory effect on nerve terminal Ca^{2+} currents recorded from mouse triangularis sterni preparations (Henning et al, 1996; Lin et al, 2000). In addition, it has been reported that suramin can prevent the inhibitory effects of neurotoxins which block P-type Ca^{2+} channels, ω-conotoxin MVIIC and ω-agatoxin IVA, but has no effect on the non-selective Ca^{2+} channel blocker, Cd^{2+}, on nerve-evoked muscle contractions of mouse diaphragm preparations (Lin et al, 2000). Suramin has also been shown to interfere with the pharmacological effects of some snake venoms and toxins, for example; the PLA_2 activity of *Bothrops jararacussu* snake (Sifuentes et al, 2008), the myotoxic and paralyzing effect of bothropstoxin-I (Oliveira et al, 2003), and the pharmacology of some crotalid venoms (Arruda et al, 2002).

Suramin has also been shown to inhibit the physiological activity β-bungarotoxin, both *in vivo* and *in vitro* (Lin-Shiau and Lin, 1999). Suramin significantly delayed the time to paralysis induced by β-bungarotoxin in mice when administered intravenously 30min before toxin. Also, suramin at 0.3mM effectively delayed the neuromuscular blocking effect of β-bungarotoxin and crotoxin in mouse phrenic nerve-muscle preparations when applied 20 to 30min before, or after application of toxins. In contrast, suramin had no significant effect on the blocking action of a postsynaptic neurotoxin, α-bungarotoxin (Lin-Shiau and Lin, 1999). Recently, suramin was shown to antagonise the haematoxin action of *Echis carinatus* (Iran) snake venom, and significantly delaying time to death of envenomed mice (Fathi et al, 2010). Suramin also has a neuroprotective effect against β-bungarotoxin-induced cytotoxicity on cultured cerebellar granule neurons (Tseng and Lin-Shiau, 2003). In addition, suramin abolished the increase of frequency and amplitude of miniature end plate potentials (m.e.p.ps) induced by β-bungarotoxin (Lin-Shiau and Lin, 1999) and prolonged the time course of block of end plate potentials (e.p.ps) by β-bungarotoxin.

The purpose of the present study was to investigate the effects of suramin on a panel of prejunctionally active toxins, particularly looking at the early phases of their effects which are believed to be independent of their enzymatic activity.

MATERIALS AND METHODS

Reagents and materials

β-Bungarotoxin (T-5644, Lots 124H40081, 33H40141 and 68H4003) was supplied by Sigma Chemical Co Ltd (Poole, Dorset, England) and Latoxan, 20 Rue Leon Blum, 2600 Valence-France. Taipoxin was a gift from Dr David Eaker (Biochemistry Department, Uppsala University, Sweden) and was also purchased from Latoxan. Dendrotoxin I (DpI) was purchased from Ventoxin (Frederick, MD, USA). Two PLA_2 toxins from the long-nosed viper (*Vipera ammodytes ammodytes*), ammodytoxin A and C, were gifts from Dr Igor Krizaj (Department of Biochemistry and Molecular Biology, University of Ljubljana, Slovenia). Tetraethylammonium chloride (TEA) and 3,4-diaminopyridine (3,4-DAP) and materials required for making salt solutions were purchased from Sigma Chemical Co Ltd or Gibco BRL Life Technologies Ltd. Suramin (sodium salt) was obtained from Bayer (Leverkusen, Germany).

Twitch tension recording of mouse phrenic nerve-hemidiaphragm preparations

Mouse hemidiaphragms and their phrenic nerves were removed from male mice (Balb C strain, 20-25gm) killed by CO_2, in compliance with UK Home Office guidelines, immediately before experiments and placed in a dish containing physiological salt solution. The preparations were cleaned under the microscope of any connective tissue and the diaphragm was divided into two triangular or wedge-shaped parts. Each preparation was attached along its origin at the rib margin to a special tissue holder. The preparations were mounted in 10ml organ baths, under a resting tension of approximately 1gm in a physiological salt solution (Krebs solution) of the following composition: NaCl 118.4mM; KCl 4.7mM; $NaHCO_3$ 25mM; KH_2PO_4 1.2mM; $MgSO_4.7H_2O$ 1.4mM; $CaCl_2$ 2.5mM; glucose 11.1mM, pH 7.3-7.4 and bubbled with oxygen containing 5% carbon dioxide and maintained at 27°C or 37°C. Twitches were evoked by stimulating the phrenic nerves via platinum ring electrodes (0.2Hz with square wave pulses of 0.1ms and sufficient strength to elicit maximal contractions) and recorded isometrically using Grass Model 79 and Grass Model 7D polygraphs, and Grass Force-Displacement Transducers FT03. In order to reveal any facilitation of neuromuscular transmission in twitch tension experiments, preparations were partially paralysed (to 15-20% of control) by either the addition of 9-10mM $MgCl_2$ applied directly into the organ bath or by using physiological salt solution containing low Ca^{2+} (0.27-0.45mM). In some experiments, to ensure that direct stimulation of the nerve-muscle preparation did not contribute to the overall tension recorded, indirectly evoked twitches were blocked by adding successively greater concentrations of Mg^{2+} or low Ca^{2+} (less than 0.2mM) to the tissue bath and then re-adjusted with required amount of Mg^{2+} or Ca^{2+} to stabilise the twitch at 20% of control.

RESULTS

Effect of suramin on phrenic nerve-hemidiaphragm preparations

In order to determine the effect of suramin on twitch tension in the absence of PLA_2 toxins, we first tested its effect on mouse phrenic nerve-hemidiaphragm preparations. The preparations were indirectly stimulated and partly paralysed with low Ca^{2+} or high Mg^{2+} at 27°C or 37°C. Under these conditions, suramin (0.3mM) blocked the twitches. The blocking effect of suramin appeared without any delay and was temperature-dependent. This effect was accelerated significantly by increasing the temperature from 27°C to 37°C. The time to block was 35 ±6.6min at 27°C (n = 3) and 13 ±3min at 37°C (n = 4) (Figure 1).

Effect of suramin on the responses to PLA_2 neurotoxins

To monitor any changes in the amplitude of twitch tension caused by the PLA_2 neurotoxins, it was necessary to maintain the twitch height at a stable level of 20 to 30% of control twitch height in the presence of suramin. This was achieved by adding a few drops (<100μl) of normal Krebs solution

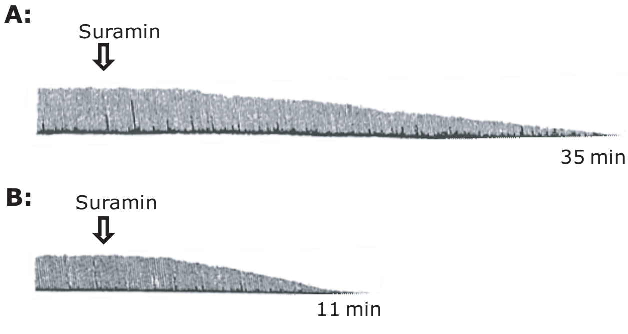

Figure 1. Effect of suramin (0.3mM) on twitch tension of mouse hemi-diaphragm at 27°C (**A**) in low Ca^{2+} and (**B**) at 37°C, in high Mg^{2+} (9-11mM) Krebs solution. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response.

to the organ bath (at 4-6min intervals) until twitch tension height stabilised at desired height. Under these conditions the interaction between suramin and toxins was examined.

Phospholipase A_2 neurotoxins cause triphasic changes on twitch tension of phrenic nerve-hemidiaphragm preparations. In view of the reported reversal action of suramin on neuromuscular blockade induced by these toxins, the effect of suramin on the triphasic action of three presynaptic PLA_2 neurotoxins, β-bungarotoxin (3µg/ml = 0.15µM), taipoxin (1µg/ml = 20nM) and ammodytoxin A (10µg/ml = 0.2µM) was investigated.

After partial paralysis of preparations by reducing the concentration of Ca^{2+} (0.27-0.45mM) or increasing the concentration of Mg^{2+} (9-11mM), the preparations were pre-treated with 0.3mM suramin for 15-20min before application of toxins. Suramin abolished the early biphasic effects, *i.e.*, depression and facilitation, and significantly prolonged the time taken to achieve twitch block. Figures 2-4 show the effects of suramin on the triphasic actions of β-bungarotoxin (n = 3), taipoxin (n = 4) and ammodytoxin A (n = 3), respectively.

Lack of effect of suramin on the facilitatory action of dendrotoxin I (DpI)

The effect of suramin on the facilitatory action of dendrotoxin I (DpI) (1µg/ml = 0.14µM) was investigated. Under the same conditions used for β-bungarotoxin, taipoxin, and ammodytoxin, preparations were incubated with 0.3mM suramin for 15-20min before application of DpI. In the control experiments, the facilitatory action of this toxin appeared without delay and without initial depression, the amplitude of twitch height increasing to more than twice of that of the control twitch height (263 ±13%) (n = 3) (Figure 5). Similar effects of DpI were observed in the experiments in which suramin was applied

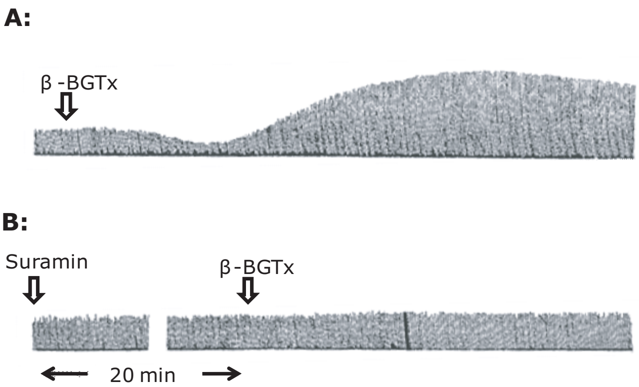

Figure 2. A. Effect of β-bungarotoxin (3µg/ml = 0.15µM) on twitch tension of mouse hemi-diaphragm partly paralysed by low Ca^{2+} Krebs solution. **B.** Effect of suramin (0.3mM) on triphasic action of β-bungarotoxin (3µg/ml = 0.15µM) when applied 20 min before application of toxin. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response.

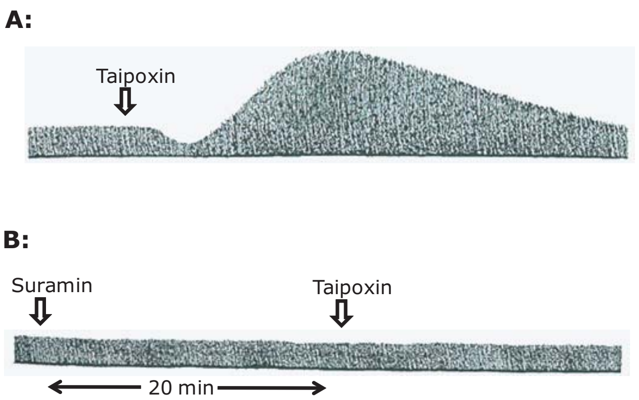

Figure 3. A. Effect of taipoxin (1µg/ml = 20nM) on twitch tension of mouse hemi-diaphragm partly paralysed by low Ca^{2+} Krebs solution. **B.** Effect of suramin (0.3mM) on the triphasic action of taipoxin (1µg/ml = 20nM) when applied 20 min before application of toxin. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response.

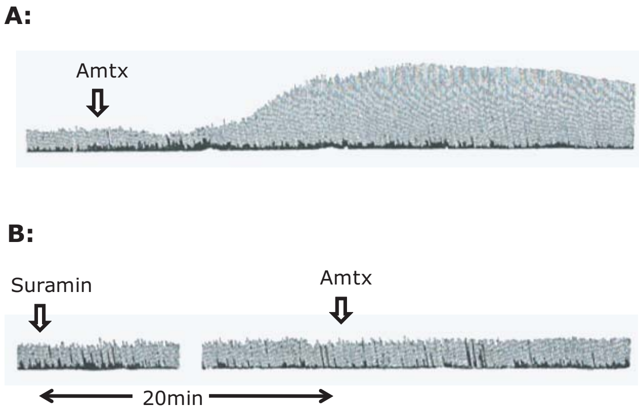

Figure 4. A. Effect of ammodytoxin A (10µg/ml = 2µM) on twitch tension of mouse hemi-diaphragm partly paralysis by high Mg^{2+} (9-11mM) Krebs solution. **B.** Effect of suramin (0.3mM) on the triphasic action of ammodytoxin when applied 20 min before application of toxin. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response.

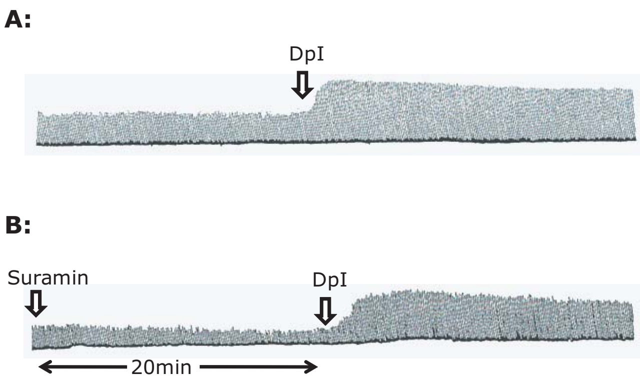

Figure 5. A. Effect of dendrotoxin I (DpI) (1µg/ml = 0.14 µM) on twitch tension of mouse hemi-diaphragm partly paralysed by high Mg^{2+} (9-11mM) Krebs solution. **B.** Lack of effect of suramin (0.3mM) on the facilitatory action of dendrotoxin I (DpI) (1µg/ml = 0.14µM) when applied 20min before application of toxin. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response.

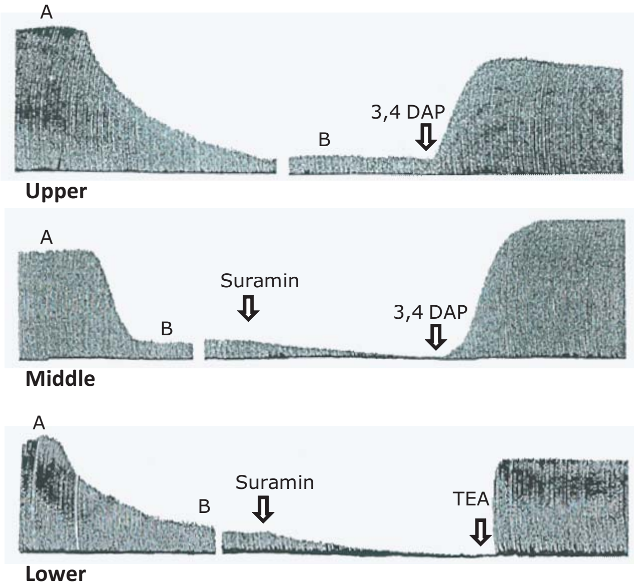

Figure 6. Upper. Effect of 3,4-diaminopyridine (3,4-DAP) (0.1mM) on twitch tension of mouse hemi-diaphragm partly paralysis by high Mg^{2+} (9-11mM) Krebs solution. **Middle and Lower**. Lack of effect of suramin (0.3mM) on the facilitatory action of 3,4-DAP (0.1mM) and TEA (2mM) when applied 20min before application of chemical agents. Preparations were stimulated indirectly at 0.2Hz with pulses of 0.1ms duration and voltage greater than required to produce the maximum response. Control twitch tension in normal Krebs solution (B) Part paralysis of twitch by high Mg^{2+} (9-11mM).

20min before DpI. In these experiments, suramin did not significantly affect the facilitation by dendrotoxin I; twitch height increased to 275 ±11% of control.

Interaction of suramin with 3, 4-diaminopyridine (3, 4-DAP) and tetraethylammonium (TEA)

Under similar experimental condition to the previous experiments with PLA_2 neurotoxins and dendrotoxin I, application of 0.3mM suramin in low Ca^{2+} at 27°C did not prevent the facilitatory actions of 3,4-DAP (0.1mM) and TEA (1-2mM) (Figure 6).

DISCUSSION

The present study used twitch tension experiments on mouse hemidiaphragm preparations and revealed that the early effects of PLA_2 neurotoxins, depression (phase I) and facilitiation (phase II), were abolished by suramin. Under the same conditions, suramin had no detectable effect on the facilitatory actions of dendrotoxin I (DpI), tetraethylammonium (TEA) and 3, 4-diaminopyridine (3,4-DAP).

It is generally accepted that the initial reduction of twitch tension (phase I) is due to the binding of the PLA_2 toxin to the nerve terminal (Chang, 1985). Suramin abolished this early effect of PLA_2 neurotoxins but only delayed the onset of the blocking phase. This suggests that suramin may compete with a PLA_2 toxin acceptor on the nerve terminal to delay the binding of the toxins to their binding site, as twitch block eventually takes place in the continued presence of suramin. The facilatatory phase of β-Bungarotoxin, crotoxin, taipoxin, notexin and ammodytoxin coincides with a block a fraction of the K^+ current recorded as perineural waveforms from mouse triangularis sterni preparations (Rowan and Harvey, 1988; Krizaj et al, 1995; Lin-Shiau and Lin, 1999). As suramin did not affect the facilitatory action of K^+ channel blockers, TEA, 3,4-DAP and dendrotoxin it is unlikely that the pharmacology of suramin is mediated through potassium channels. Suramin is a polysulfate anionic compound rich in negative charges that can interact with positive charges on peptides and proteins, thus it is possible that suramin could directly bind with the PLA_2 toxin. This interaction would be predicted to cause a conformational change which may delay binding to acceptors on the nerve terminals. However, it is difficult to explain the lack of effect of suramin on the neuromuscular blocking effect of a postsynaptic neurotoxin α-bungarotoxin or of the facilitatory toxin DpI, both of which also have positively charged residues. The mechanism of action of suramin at the neuromuscular junction is still not clear, although it was suggested that suramin inhibited Ca^{2+} entry to the nerve terminal by binding weakly to presynaptic voltage-dependent Ca^{2+} channels and reducing the release of acetylcholine (Henning et al, 1996; Lin et al, 2000). Such a mode of action can explain the twitch blocking effect of suramin on mouse nerve-hemidiaphragms partially paralysed by low Ca^{2+} or high Mg^{2+} but cannot account for the antagonistic effect of suramin on the neuromuscular effects of β-bungarotoxin, as Cd^{2+}, a Ca^{2+} channel blocker, does not alter the pharmacology of such toxins on neuromuscular transmission (Lin-Shiau and Lin, 1999). Overall, the mechanism whereby suramin reduces the effects of PLA_2 neurotoxins remains elusive. Since suramin reduces both enzyme-dependent and enzyme-independent effects of the toxins, suramin is not acting as a simple enzyme inhibitor. As suramin does not affect the actions of standard K^+ channel blockers, it is not stabilising nerve terminals. Perhaps there is a direct physical interaction between suramin and the toxins as has been suggested to account for the effect of suramin on the myotoxic activity of Lys49 homologues (Murakami et al, 2007).

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STATEMENT OF COMPETING INTERESTS

None declared.

REFERENCES

Arruda EZ, Silva NM, Moraes RA and Melo PA. 2002. Effect of suramin on myotoxicity of some crotalid snake venoms. Braz J Med Biol Res, 35, 723-726.

Chang CC. 1985. Neurotoxins with phospholipase A_2 activity in snake venoms. Proc Natl Sci Coun ROC, 9, 126-142.

Fathi B, Amani F, Jami-al-ahmadi A and Zare A. 2010. Antagonistc effect of suramin against the venom of the Iranian snake *Echis carinatus* in mice. Iranian J Vet Sci Technol, 2, 19-15.

Ginsborg BL and Warriner JN. 1960. The isolated chick biventer cervicis nerve-muscle preparation. British J Pharmacol, 15, 410-411.

Harvey AL, Barfaraz A, Thomson, E, Faiz A, Preston S and Harris JB. 1994. Screening of snake venoms for neurotoxic and myotoxic effects using simple in vitro preparations from rodents and chicks. Toxicon, 32, 257-265.

Henning RH, Nelemans A, Scaf A HJ, Eekeren JV, Agoston S and Hertog AD. 1992. Suramin reverses non-depolarizing neuromuscullar blockade in rat diaphragm. Eur J Pharmacol, 216, 73-79.

Henning RH, Nelemans A, Braga EG, Rowan EG and Harvey AL. 1996. The prejunctional inhibitory effect of suramin on neuromuscular transmission *in vitro*. Eur J Pharmacol, 301, 91-97.

Hoyle CH, Knight GE and Burnstock G. 1990. Suramin antagonizes responses to P2-purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. British J Pharmacol, 3, 617-621.

Krizaj I, Rowan EG and Gubenšek F. 1995. Ammodytoxin A acceptor in bovine brain synaptic membranes. Toxicon, 33, 437-449.

Lin MJ, Tan CT, Lee SY and Lin-shiau SY. 2000. Suramin protects the murine motor nerves from the toxic effects of presynaptic Ca^{2+} channel inhibitors. Neurosci Letts, 287, 97-100.

Lin-Shiau SY and Lin MJ. 1999. Suramin inhibits the toxic effects of presynaptic neurotoxins at the mouse nerve terminals. Eur J Pharmacol, 382, 75-80.

Murakami MT, Vicoti MM, Abrego JRB et al. 2007. Interfacial surface charge and free accessibility to the PLA_2–active site-like region are essential requirements for the activity of Lys49 PLA_2 homologues. Toxicon, 49, 378-387.

Oliveira DM, Cavalcante WL, Arruda EZ, Melo PA, Dal-Pai Silva M and Gallacci M. 2003. Antagonism of myotoxic and paralysis activities of bothropstoxin-I by suramin. Toxicon, 42, 373-379.

Rowan EG and Harvey AL. 1988. Potassium channel blocking actions of beta-bungarotoxin and related toxins on mouse and frog motor nerve terminals. British J Pharmacol, 94, 839-847.

Sifuentes DN, El-Kik CZ, Ricardo HD et al. 2008. Ability of suramin to antagonize the cardiotoxic activities of Bothrops jararacussu venom. Toxicon, 52, 28-36.

Su MJ and Chang CC. 1981. Effects of bivalent cations on the presynaptic actions and phospholipase A_2 activity of notexin. A comparison with other complex presynaptic neurotoxins. Proc Natl Sci Coun ROC, 1, 82-90.

Su MJ and Chang CC. 1984. Presynaptic effects of snake venom toxins which have phospholipase A_2 activity (ß-bungarotoxin, taipoxin, crotoxin). Toxicon, 22, 631-640.

Tseng WP and Lin-Shiau SY. 2003. Suramin inhibits ß-bungarotoxin-induced activation of N-methyl-D-aspartate receptors and cytotoxicity in primary neurons. Toxicol Appl Pharmacol, 189, 45-55.