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Reversible skeletal neuromuscular paralysis induced by different lysophospholipids

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Abstract **Lysophosphatidylcholine rapidly paralyses the neuromuscular junction (NMJ), similarly to snake phospholipase A2 neurotoxins, implicating a lipid hemifusion-pore transition in neuroexocytosis. The mode and kinetics of NMJ paralysis of different lysophospholipids (lysoPLs) in high or low [Mg^{2+}] was investigated. The following order of potency was found: lysophosphatidylcholine > lysophosphatidylethanolamine > lysophosphatidic acid > lysophosphatidylserine > lysophosphatidylglycerol. The latter two lysoPLs closely mimic the profile of paralysis caused by the toxins in high [Mg^{2+}]. This paralysis is fully reversed by albumin washing. These findings provide novel insights on the mode of action of snake neurotoxins and qualify lysoPLs as novel agents to study neuroexocytosis.**

Keywords: Neuromuscular junction; Paralysis; Snake neurotoxins; Phospholipase A2; Lysophospholipids

1. Introduction

Many venomous snake species produce presynaptic neurotoxins endowed with phospholipase A2 activity (SPANs) which play a major role in envenomation. These neurotoxins cause a persistent blockade of neurotransmitter release from nerve terminals [1–5].

The induction of neuromuscular transmission failure by SPANs is traditionally measured in nerve–muscle preparations isolated from the mouse hemidiaphragm or the chicken biventer cervicis. Such measurements can be performed in buffers containing either low (1 mM) or high (5–7 mM) Mg^{2+} concentrations ([Mg^{2+}]). The former one is close to the physiological concentration, but the latter [Mg^{2+}] provides the advantage of faster toxin effect and higher reproducibility. Nearly all the reports on the neuromuscular blockade with SPANs, present in the literature, were conducted under the latter condition. In the presence of 5–7 mM Mg^{2+}, the curve of the paralysis induced by SPANs is triphasic, as shown in Fig. 1A. An initial phase of weak inhibition of acetylcholine release is followed by a second prolonged phase of facilitated release which merges into a third phase of progressive decline of neurotransmission [6–8]. Electron microscopy performed at the third stage shows swollen and enlarged axon terminals with depletion of synaptic vesicles (SVs) [1,9–13]. We recently showed that SPANs block the neurotransmission by promoting fusion of SVs with the presynaptic membrane and by inhibiting their retrieval, at the same time [14,15]. Moreover, we showed that all the functional and morphological alterations caused by SPANs can be induced by incubation with their phospholipids hydrolysis products, i.e. lysophosphatidylcholine (lysoPC) and fatty acids (FA) [16]. These findings were explained on the basis of the known effect of the inverted cone-shaped lysoPC and cone-shaped FA on membrane fusion, as they promote the transition from lipid hemifusion intermediates to an open pore [16–18].

Among lysophospholipids (lysoPLs), only lysoPC was investigated previously [16]. We have extended these studies to the major classes of lysoPL, with different head-groups, either alone or mixed with fatty acid, and we present here their effects on the mode and kinetics of induction of paralysis of the mouse hemidiaphragm neuromuscular junction. Oleic acid (OA) was used in each case because it is a major FA of the neuronal membrane and for the sake of comparison with previous data. We also report the remarkable finding that the nerve terminal inhibition induced by these lipid mixtures and by SPANs is reversed by washings with the lipid binding protein albumin showing that these inhibitors induce paralysis without causing permanent damage to the tissue under our experimental conditions.

2. Materials and methods

2.1. Materials

Taipoxin and textilotoxin were purchased from Venom Supplies (Tanunda, South Australia); β-BTx was from Sigma (Saint Louis, MS, USA). Purity was checked by sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis (SDS–PAGE) and PLA2 activity measured with a secretory PLA2 assay kit (Cayman Chemical Company, Ann Arbor, MI) [19]. Bovine serum albumin (BSA) was from Roche (Indianapolis, IN, USA). Lysophosphatidylcholine (1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine and 1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine) and OA (sodium salt) were purchased from Sigma. Lysophosphatidylethanolamine (1-myristoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine), lysophosphatidylserine (1-oleoyl-2-hydroxy-sn-glycero-3-phosphoserine), lysophosphatidylglycerol (1-myristoyl-2-hydroxy-sn-glycero-3-[phospho-rac-(1-glycerol)]) (sodium

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Abbreviations: FA, fatty acids; OA, oleic acid; SPAN, snake phospholipase A2 presynaptic neurotoxin; lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; lysoPS, lysophosphatidylserine; lysoPG, lysophosphatidylglycerol; lysoPA, lysophosphatidic acid; lysoPL, lysophospholipid; SVs, synaptic vesicles; [Mg^{2+}], magnesium concentration; PLA2, phospholipase A2; neuromuscular junction, NMJ

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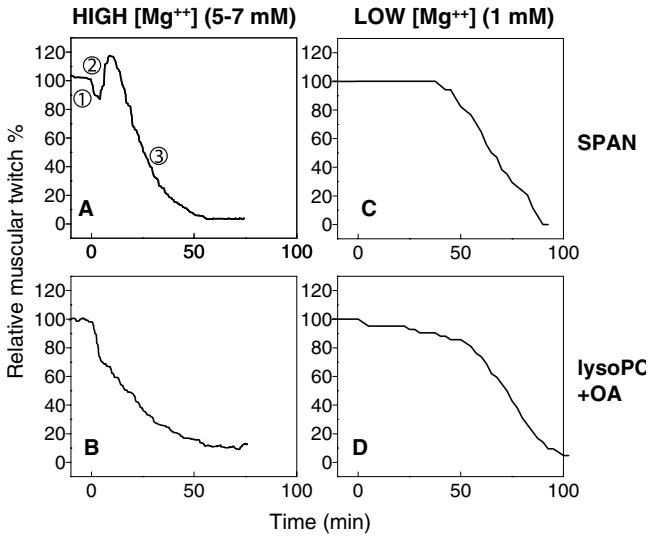

Fig. 1. Paralysis of the neuromuscular preparation in low or high [Mg^{2+}] buffers. Time courses of the inhibition of the mouse hemidiaphragm NMJ caused by taipoxin (1 μg/ml) in high (A) and low [Mg^{2+}] (C) buffers. Closely similar traces were obtained with β-bungarotoxin and textilotoxin (not shown). These neurotoxins induce a triphasic profile, consisting of an initial rapid depression phase (1), a facilitation phase (2), and a final run-down phase (3). The inhibition profile obtained in low [Mg^{2+}] is characterized by a long lag phase before the run-down phase. Panels B and D report the paralysis profile obtained upon addition in the bathing medium of a 1:1 mixture of myristoyl-lysoPC and OA (final concentration 150 μM each). Also in this case the low [Mg^{2+}] trace (D) shows a long lag phase. Toxins or lipids are added at time 0. For comparison of different experiments, the stabilized initial twitching strength was taken as 100%; each trace is representative of at least three experiments.

salt), and lysophosphatidic acid (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate) (sodium salt) were obtained from Avanti Polar lipids (Alabaster, AL, USA).

2.2. Lipids preparation

Suitable aliquotes of lysoPL were dissolved in small volumes of $CHCl_3$:CH_3OH (3:1, v/v), dried to a thin film under a gentle nitrogen flow and vacuum pumped for at least 2 h to remove residual traces of organic solvents. The dried lipid film was suspended in buffer L (Hepes-Na 10 mM, pH 7.4, NaCl 150 mM) at final concentration of 2.5 mM, extensively vortexed and then ultrasonically dispersed in a bath sonicator at 37°– 40 °C, until optical clarity was obtained. OA was suspended in buffer L (final concentration 10 mM), vortexed and ultrasonically dispersed with the same procedure. When required, LysoPL and OA suspensions were mixed in a 1:1 molar ratio and ultrasonically dispersed for additional 10 min. All suspensions were kept at 37°– 40 °C until use.

2.3. Mouse phrenic nerve-hemidiaphragm preparation

All experimental procedures were carried out in accordance to the European Communities Council Directive no. 86/609/EEC. Mouse phrenic nerve hemidiaphragms were isolated from CD-1 mice weighing about 20–30 g as previously described [20] and mounted in 20 ml oxygenated (95% O_2, 5% CO_2) physiological buffer (139 mM NaCl, 12 mM $NaHCO_3$, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4 and 11 mM glucose, pH 7.4). Two innervated hemidiaphragm preparations were isolated from each animal.

The phrenic nerve was stimulated via two ring platinum electrodes with supramaximal stimuli of 10 V amplitude and 0.1 ms pulse duration, with a frequency of 0.1 Hz (Stimulator 6002, Harvard Apparatus, Massachusetts, USA). Muscle contraction was monitored with an isometric transducer (Harvard Apparatus); data were recorded via an NI DAQCard-6062E and analyzed by a LabView-based computer program (National Instruments, Austin, Texas, USA); the amplitude of twitch was calculated as a difference from basal muscular tension and the mean of peak value measured after stimulation.

Muscles were stretched to the optimal length for twitch tension and the muscle twitch allowed to stabilize for at least 20 min at 37 °C. In control experiments the amplitude of muscle contraction under this type of stimulation was constant for at least 8 h. In high [Mg^{2+}] experiments, aliquots of a 0.5 M solution of $MgCl_2$ were added to the bath to achieve a twitch reduction from 45 to 55% of initial stabilized twitch, which correspond to a final [Mg^{2+}] of 5–7 mM, in different preparations. Muscles were equilibrated for further 15–20 min; the mean twitch value measured during the last 5–10 min before the addition of toxin or lipids was taken as 100% in order to normalize the experiments.

Taipoxin, textilotoxin (1 μg/ml) or β-bungarotoxin (3 μg/ml) were added to the nerve–muscle preparations in the minimal volume of buffer; lipids or lipid mixtures were added to 20 ml bath medium at the final concentration of 150 μM (for each lipids) and twitch failure was monitored. In all cases, after complete paralysis of the neuromuscular preparation, direct stimulation of the muscle was performed to exclude any myotoxic effect of the compounds tested.

2.4. Albumin washing

After muscle paralysis in high [Mg^{2+}], nerve stimulation was suspended and the medium was rapidly discarded and replaced with physiological buffer (pre-oxygenated and pre-warmed) containing 0.2% w/v of bovine albumin (washing buffer). After 5 min, the washing buffer was removed, the nerve–muscle preparation extensively washed with pre-warmed physiological buffer, and then nerve stimulation was re-established.

3. Results

3.1. Effects of [Mg^{2+}] on the neuromuscular junction paralysis induced by snake neurotoxins or lysophospholipids/fatty acid mixtures

The triphasic curve of paralysis of the neuromuscular junction (NMJ) exhibited by SPANs is observed only when the bathing medium contains a high [Mg^{2+}] (Fig. 1A), whilst in physiological [Mg^{2+}] a simple run-down type of curve is obtained (Fig. 1C). Very similar paralysis curves were observed with the three SPAN neurotoxins used here: β-bungarotoxin, taipoxin and textilotoxin. The molecular events underlying phases 1 and 2 (only present in high [Mg^{2+}]), are not known. A clarifying contribute may derive from the use of simple NMJ inhibitory agents such as the lysoPLs, which are the product of the phospholipase A2 enzymatic activity of these neurotoxins [16]. In low [Mg^{2+}], a lysoPC + OA mixture caused a decrease of neurotransmission that proceeded until complete blockade; the onset of paralysis was preceded by a significant lag phase (Fig. 1D), similar to that observed with SPANs under the same experimental conditions (Fig. 1C). Unlike lysoPC, all the other lysoPL mixtures, at the concentration tested here, failed to paralyze the NMJ in low [Mg^{2+}] (not shown). Total block of neurotransmission was achieved faster for both lysoPC + OA and SPANs in high [Mg^{2+}], though this lipid mixture failed to reproduce the triphasic profile typical of SPANs (Fig. 1B). Nevertheless one cannot exclude that the initial inhibitory phase was present but superimposed to the run-down phase (Fig. 1B).

We tested a group of lysoPL that include zwitterionic lipids like lysophosphatidylethanolamine (lysoPE), and negatively charged lysoPLs, like lysophosphatidylserine (lysoPS), lysophosphatidic acid (lysoPA), and lysophosphatidylglycerol (lysoPG). In high [Mg^{2+}], all these lipids were able to completely paralyze the NMJ, although with different potency.

The time periods required to give 50% muscle paralysis are given in Table 1 for lysoPLs and for their equimolar mixture with OA. The lysoPL alone is sufficient to inhibit the synapse in high [Mg^{2+}], with a paralysis time shorter than, or equal to that induced by their mixture with OA, suggesting that lysolipids are the major effectors of the NMJ blockade. Only lysoPE has a potency comparable to LysoPC, whereas all the other lysoPL tested are much less effective. At variance, in low [Mg^{2+}] lysoPC alone gave a partial inhibitory effect, and full inhibition was only achieved when it was mixed with OA [16]. Clearly, [Mg^{2+}] is a major determinant of both the inhibitory potency and the shape of the curve of lysoPL induced paralysis. All these lysoPLs do not paralyse the muscle directly as no change in muscle twitch was found with direct muscle stimulation (not shown).

3.2. Different lysophospholipids induce different paralysis profiles of the neuromuscular junction

Membrane phospholipids differ not only for their head groups but also with respect to their FAs. We used the oleoyl or myristoyl derivatives, depending on commercial availability. The myristoyl derivatives were found to be more potent than their oleoyl counterparts, possibly because of their more pronounced inverted cone shape, due to the shorter hydrocarbon chain, and to their more efficient partition into the plasma membrane.

Fig. 2 shows the NMJ paralysis profiles of different lysoPLs in high [Mg^{2+}]. The shape of curves obtained is very similar for lysoPL alone and for lysoPL/OA mixtures (not shown). LysoPC and lysoPE paralyze the NMJ with a simple run-down curve. A very interesting finding is that lysoPS and lysoPG induce a triphasic curve of paralysis, reminiscent of that elicited by SPANs; lysoPA causes a biphasic curve with a rapid initial inhibition which then progresses at a lower rate. This mimicking of the first phase of the SPAN-induced neuromuscular paralysis by some lysoPL is puzzling and interesting at the same time because it challenges previous conclusions that the initial SPAN-induced inhibitory phase was not dependent on PLA2 activity [5,7,8].

Table 1
Comparison of the neuromuscular paralysis time induced by different lysoPLs and lysoPls mixtures with OA

High Mg (5–7 mM)				
	lysoPL		lysoPL + OA	
	T50%	RP[a]	T50%	RP[a]
LPC myristoyl	10′ ± 3 $n = 3$	100	14′ ± 4 $n = 7$	100
LPC oleoyl	19′ ± 11 $n = 5$	53(100)	35′ ± 8 $n = 5$	45(100)
LPE myristoyl	18′ ± 13 $n = 3$	55	37′ ± 9 $n = 3$	38
LPA oleoyl	79′ ± 48 $n = 4$	8(24)	188′ ± 13 $n = 3$	7(19)
LPS oleoyl	172′ ± 49 $n = 4$	6(11)	177′ ± 52 $n = 4$	8(20)
LPG myristoyl	230′ ± 99 $n = 4$	4	224′ ± 50 $n = 4$	6

The inhibitory activity of lysophospholipids is expressed as the time required to reduce the muscular twitch to 50% of its initial value (T50%). Comparison between the relative potency (RP) of the different lysoPL was made by taking the T50% of myristoyl-lysoPC as 100%. The same comparison made for the oleoyl derivatives are given in parenthesis.
[a]RP = relative potency (T50% LPC/T50% other lipid ×100).

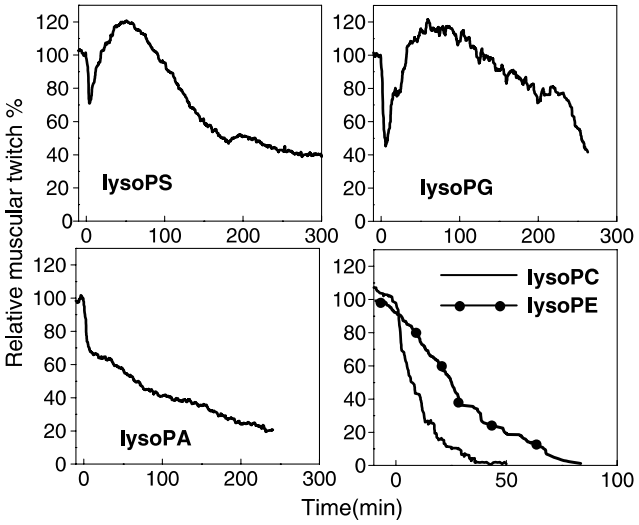

Fig. 2. Paralysis of the neuromuscular preparation caused by different lysoPLs in high [Mg^{2+}] bathing medium. Typical paralysis profiles obtained after addition of the specified lysophospholipid; abbreviations used are as given in the text. The negatively charged lysoPLs cause a rapid depression of the muscle twitch, comparable to phase (1) described in Fig. 1 for the snake neurotoxins. LysoPS and lysoPG also induced a facilitation phase similar to the phase (2) of snake neurotoxins shown in Fig. 1; this latter facilitation effect is absent in the case of lysoPA. Zwitterionic lysoPLs (lysoPC and LysoPE) cause a more rapid paralysis of NMJ with a run-down curve. Lipids are added at time 0.

3.3. Reversibility of paralysis of the neuromuscular junction

The partition of lysoPL and FA into the presynaptic membrane from the medium is responsible for the blockade of the nerve terminals. Conversely, one would expect that their removal from the membrane would restore synaptic function, if a permanent damage is not established within the present experimental time. Removal of FA and their derivatives can be achieved by addition of albumin, a serum protein which contains several FA binding sites [21,22]. Fig. 3 shows a typical twitch profile of the lysoPC+OA treated NMJ in high [Mg^{2+}]; the simple washing with a lipid-free and albumin containing medium completely restores the nerve-stimulated muscle twitch. Recovery of NMJ function was completed within few minutes, suggesting that the lysoPL and FA equilibration between the membrane and the medium and their binding to albumin are very rapid. Similar recoveries were obtained with the other lysoPL tested here (not shown). Rapid albumin binding of FA and FA derivatives with consequent effect of the membrane fusion of liposomes was very recently reported [23]. If the NMJ paralysis induced by SPANs is indeed mediated by lysoPL and FA, for the same physico-chemical reasons, albumin washing should be effective also in the case of the SPAN-induced inhibition. Fig. 3 shows that this is indeed the case. However, recovery is only partial and then paralysis progresses again. The same pattern was recorded with all toxins tested in high [Mg^{2+}]. This temporary relief of inhibition is expected on the basis of the known fact that SPAN binding becomes rapidly irreversible, as SPANs cannot be washed away and become insensitive to the action of specific anti-toxin antibodies [24,25]. Thus, the toxin can continue to hydrolyse phospholipids on the presynaptic membrane, with the production of the lysoPL and FA which then inhibit the synapse.

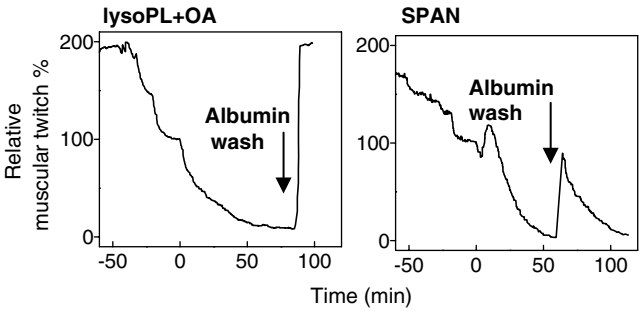

Fig. 3. Albumin washing reverses the neuromuscular paralysis induced by SPANs or by lysoPLs. A muscle was bathed in physiological buffer and, after stabilization of its twitching strength, Mg^{2+} was added as described in materials and methods . When twitch was stabilized to its typical high [Mg^{2+}] value (100%), the lipid mixture was added. At complete paralysis the lysoPL-containing medium was replaced with a medium containing 0.2% albumin (arrow) in physiological buffer. The albumin washing restored the twitch amplitude to the initial values (left panel). A similar behaviour was obtained after intoxication with all SPANs tested, but in this case the recovery was partial and temporary (right panel).

3.4. Discussion

Previous studies have provided evidence that the facilitation phase of SPAN action on the NMJ corresponds to stimulation of exocytosis, whereas the third phase is characterized by inhibition of endocytosis and SV recycling. The nature of the first transient phase of inhibition remains unexplained. Here, we show that this initial depression of the NMJ function is linked to the presence of high [Mg^{2+}], and therefore is not likely to be present in vivo, during envenomation. This ion appears to act on the presynaptic membrane rather than on the SPAN itself, as there are lysoPLs (lysoPS, lysoPG) which mimic the toxin effect, with a triphasic paralysis profile, and high [Mg^{2+}] does not affect the phospholipase A2 activity of SPAN (our unpublished observations). We propose here that Mg^{2+} at high concentrations interacts with the "active zones" of the presynaptic membrane, which are the preferred sites of SV fusion with the plasma membrane, making them more sensitive to membrane fusion with SVs. This suggestion is based on the following observations: (a) lysoPL alone are able to inhibit the NMJ in high [Mg^{2+}], while lysoPC association with FA is required for inhibition in low [Mg^{2+}] [16]; (b) no lag phase is present in the action of SPAN and of lysoPL in high [Mg^{2+}]; (c) generally, paralysis develops more rapidly in high [Mg^{2+}]. All these experimental features speak in favour of a facilitated SV fusion with the presynaptic membrane. The implication of "active zones" in the inhibitory effect of SPANs and lysoPL also offers an explanation for the different lipid behaviour found here with respect to the first inhibitory phase. In fact, LysoPG, LysoPS and lysoPA, possibly owing to their net negative charge, may insert into the "active zones" and change their structure in such a way as to make them less prone to fuse with SVs. On the contrary, the initial lysoPC or lysoPE partition into these zones does not affect their propensity to fuse. When more lysoPC or lysoPE are incorporated into the "active zones" their effects on membrane curvature develops and then affect exocytosis and endocytosis. The initial inhibitory effect of lysoPG, lysoPS and lysoPA is overcome as more of them are incorporated, thus becoming sufficient to promote the pore formation which leads to acetylcholine release. The situation is different with SPANs as they release the products of phospholipids hydrolysis at their membrane binding sites. The data would be reconciled if one considers our recent proposal that SPANs bind within the "active zones" of the presynaptic membrane [4].

Another aim of the present work was to test the possibility that lysoPL others than lysoPC could be more powerful in paralysing the NMJ, but none of them was found to be more effective than lysoPC. This indicates that the more common lysoPC is probably the best lysoPL to be used in studies of the mechanism of membrane fusion. This finding may also have an evolutionary significance as phosphatydylcholine is by far the major class of phospholipids present on the outer leaflet of the presynaptic membrane which is the first one to become accessible to the snake neurotoxins.

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References

[1] Dixon, R.W. and Harris, J.B. (1999) Nerve terminal damage by beta-bungarotoxin: its clinical significance. Am. J. Pathol. 154, 447–455.
[2] Kini, R.M. (1997) Venom phospholipase A2 enzymes. Structure, function and mechanism. Wiley and Sons, Eds., Chichester.
[3] Montecucco, C. and Rossetto, O. (2000) How do presynaptic PLA2 neurotoxins block nerve terminals? Trends Biochem. Sci. 25, 266–270.
[4] Rossetto, O., Morbiato, L., Caccin, P., Rigoni, M. and Montecucco, C. (2006) Presynaptic enzymatic neurotoxins. J. Neurochem. 97, 1534–1545.
[5] Rowan, E.G. (2001) What does β-bungarotoxin do at the neuromuscular junction? Toxicon 39, 107–118.
[6] Harris, J.B. (1997) Toxic phospholipases in snake venom: an introductory review. Symp. Zool. Soc. Lond. 70, 235–250.
[7] Howard, B.D. and Gundersen Jr., C.B. (1980) Effects and mechanisms of polypeptide neurotoxins that act presynaptically. Annu. Rev. Pharmacol. Toxicol. 20, 307–336.
[8] Kelly, R.B., Oberg, S.G., Strong, P.N. and Wagner, G.M. (1976) β-Bungarotoxin, a phospholipase that stimulates transmitter release. Cold Spring Harb. Symp. Quant. Biol. 40, 117–125.
[9] Chen, I.L. and Lee, C.Y. (1970) Ultrastructural changes in the motor nerve terminals caused by beta-bungarotoxin. Virchows Arch. B Cell Pathol. 6, 318–325.
[10] Cull-Candy, S.G., Fohlman, J., Gustavsson, D., Lullmann-Rauch, R. and Thesleff, S. (1976) The effects of taipoxin and notexin on the function and fine structure of the murine neuromuscular junction. Neuroscience 1, 175–180.
[11] Gopalakrishnakone, P. and Hawgood, B.J. (1984) Morphological changes induced by crotoxin in murine nerve and neuromuscular junction. Toxicon 22, 791–804.
[12] Harris, J.B., Grubb, B.D., Maltin, C.A. and Dixon, R. (2000) The neurotoxicity of the venom phospholipases A(2), notexin and taipoxin. Exp. Neurol. 161, 517–526.
[13] Lee, C.Y., Tsai, M.C., Chen, Y.M., Ritonja, A. and Gubensek, F. (1984) Mode of neuromuscular blocking action of toxic phospholipases A2 from *Vipera ammodytes* venom. Arch. Int. Pharmacodyn. Ther. 268, 313–324.
[14] Bonanomi, D., Pennuto, M., Rigoni, M., Rossetto, O., Montecucco, C. and Valtorta, F. (2005) Taipoxin induces synaptic vesicle exocytosis and disrupts the interaction of synaptophysin I with VAMP2. Mol. Pharmacol. 67, 1901–1908.
[15] Rigoni, M., Schiavo, G., Weston, A.E., Caccin, P., Allegrini, F., Pennuto, M., Valtorta, F., Montecucco, C. and Rossetto, O. (2004) Snake presynaptic neurotoxins with phospholipase A2 activity induce punctate swellings of neurites and exocytosis of synaptic vesicles. J. Cell Sci. 117, 3561–3570.
[16] Rigoni, M., Caccin, P., Gschmeissner, S., Koster, G., Postle, A.D., Rossetto, O., Schiavo, G. and Montecucco, C. (2005)

Equivalent effects of snake PLA2 neurotoxins and lysophospholipid–fatty acid mixtures. Science 310, 1678–1680.
[17] Chernomordik, L.V. and Kozlov, M.M. (2003) Protein-lipid interplay in fusion and fission of biological membranes. Annu. Rev. Biochem. 72, 175–207.
[18] Zimmerberg, J. and Chernomordik, L.V. (2005) Neuroscience. Synaptic membranes bend to the will of a neurotoxin. Science 310, 1626–1627.
[19] Reynolds, L.J., Hughes, L.L. and Dennis, E.A. (1992) Analysis of human synovial fluid phospholipase A2 on short chain phosphatidylcholine-mixed micelles: development of a spectrophotometric assay suitable for a microtiterplate reader. Anal. Biochem. 204, 190–197.
[20] Bulbring, E. (1946) Observations on the isolated phrenic nerve diaphragm preparation of the rat. Brit. J. Pharmacol. 1, 38–61.
[21] Simard, J.R., Zunszain, P.A., Hamilton, J.A. and Curry, S. (2006) Location of high and low affinity fatty acid binding sites on human serum albumin Revealed by NMR drug-competition analysis. J. Mol. Biol. 361, 336–351.
[22] Curry, S., Mandelkow, H., Brick, P. and Franks, N. (1998) Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. Nat. Struct. Biol. 5, 827–835.
[23] Melia, T.J., You, D., Tareste, D.C. and Rothman, J.E. (2006) Lipidic antagonists to SNARE-mediated fusion. J. Biol. Chem.
[24] Kamenskaya, M.A. and Thesleff, S. (1974) The neuromuscular blocking action of an isolated toxin from the elapid (*Oxyuranus scutellactus*). Acta Physiol. Scand. 90, 716–724.
[25] Simpson, L.L., Lautenslager, G.T., Kaiser, I.I. and Middlebrook, J.L. (1993) Identification of the site at which phospholipase A2 neurotoxins localize to produce their neuromuscular blocking effects. Toxicon 31, 13–26.