Changes of quantal transmitter release caused by gadolinium ions at the frog neuromuscular junction

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1 The actions of the trivalent cation, gadolinium (Gd^{3+}), were studied on frog isolated neuromuscular preparations by conventional electrophysiological techniques.

2 Gd^{3+} (450 μM) applied to normal or formamide-treated cutaneous pectoris nerve-muscle preparations induced, after a short delay, a complete block of neuromuscular transmission. The reversibility of the effect was dependent on the time of exposure.

3 Gd^{3+} (5–450 μM) had no consistent effect on the resting membrane potential of the muscle fibres.

4 Gd^{3+} (5–40 μM) applied to preparations equilibrated in solutions containing high Mg^{2+} and low Ca^{2+} reduced the mean quantal content of endplate potentials (e.p.ps) in a dose-dependent manner. Under those conditions, 3,4-diaminopyridine (10 μM) consistently reversed the depression of evoked quantal release.

5 The calcium current entering motor nerve terminals, revealed after blocking presynaptic potassium currents with tetraethylammonium (10 mM) in the presence of elevated extracellular Ca^{2+} (8 mM), was markedly reduced by Gd^{3+} (0.2–0.5 mM).

6 Gd^{3+} (40–200 μM) increased the frequency of spontaneous miniature endplate potentials (m.e.p.ps) in junctions bathed either in normal Ringer solution or in a nominally Ca^{2+} -free medium supplemented with 0.7 μM tetrodotoxin. This effect may be due to Gd^{3+} entry into the nerve endings since it is not reversed upon removal of extracellular Gd^{3+} with chelators (1 mM EGTA or EDTA). Gd^{3+} also enhanced the frequency of m.e.p.ps appearing after each nerve stimulus in junctions bathed in a medium containing high Mg^{2+} and low Ca^{2+} .

7 Gd^{3+} , in concentrations higher than 100 μM , decreased reversibly the amplitude of m.e.p.ps suggesting a postsynaptic action.

8 It is concluded that the block of nerve-impulse evoked quantal release caused by Gd^{3+} is related to its ability to block the calcium current entering the nerve endings, supporting the view that Gd^{3+} blocks N-type Ca^{2+} channels; while the enhancement of spontaneous quantal release is probably the result of Gd^{3+} entry into motor nerve endings. Besides its dual prejunctional effects on quantal release it is suggested that Gd^{3+} exerts a postsynaptic action on the endplate acetylcholine receptor-channel complex.

Keywords: Gadolinium; neuromuscular junction; acetylcholine release; motor nerve terminals; calcium channels

Introduction

It is generally accepted that nerve-impulse evoked transmitter release from nerve terminals is triggered by the phasic entry of calcium through voltage-sensitive membrane channels (for a recent review see Augustine *et al.*, 1987). Various types of calcium channels have been identified in neurones based on their single channel conductances, activation and inactivation properties and sensitivity to pharmacological agents (for a review see Miller, 1987). These channels have been tentatively termed T-, N-, and L-type calcium channels. Organic calcium channel antagonists, such as verapamil and dihydropyridines which block only L-channels, do not influence evoked acetylcholine release at frog neuromuscular junctions (Gotgilf & Magazanik, 1977; Nachshen & Blaustein, 1979; Arnon *et al.*, 1988). ω -Conotoxin which is a potent irreversible blocker of both L- and N-channels, consistently blocks nerve-impulse evoked transmitter release at frog neuromuscular junctions (Kerr & Yoshikami, 1984; Enomoto *et al.*, 1986; Sano *et al.*, 1987). Gadolinium (Gd^{3+}) has recently been shown in rodent neuroblastoma $\times$ glioma hybrid cells to block only one component of whole-cell current, possibly that through the N-type Ca^{2+} channel (Docherty, 1988; Brown *et al.*, 1989). As the

type of Ca^{2+} channel involved in nerve-impulse-evoked transmitter release from motor nerve terminals is not yet well defined, it was of interest to study the effects of Gd^{3+} on both quantal transmitter release and the presynaptic calcium current from frog motor nerve terminals.

Methods

Experiments were performed at 20–22°C on the cutaneous pectoris nerve-muscle preparation isolated from 20–25 g male frogs (*Rana esculenta*). The standard Ringer solution had the following composition in mM: NaCl 110.0, KCl 2.1, $CaCl_2$ 1.8, and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 5.0, buffered at pH 7.2. In some experiments the Ca^{2+} and Mg^{2+} concentrations were varied as specified in the results. All preparations were kept for 1 h in solutions with altered Ca^{2+} and Mg^{2+} concentrations to allow for equilibration. When changes were made in the ionic composition of the bathing solution, osmolarity was maintained by changing NaCl concentration.

When necessary, the mechanical activity of cutaneous pectoris muscles was uncoupled from the membrane surface depolarization by use of 2 M formamide (see del Castillo & Escalona de Motta, 1978). Preparations were not used until complete recovery of the resting membrane potential of the muscle fibres.

Membrane potentials, miniature endplate potentials (m.e.p.ps), endplate potentials (e.p.ps) and indirectly elicited action potentials were recorded with intracellular glass capillary microelectrodes filled with 3 M KCl (8–12 M Ω resistance)

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by conventional intracellular recording techniques. The nerve was stimulated, unless otherwise stated, at a rate of 0.5 Hz with supramaximal current pulses of 50 μ s duration through a glass suction electrode.

The quantal content (m) of e.p.ps was estimated either by the direct method (m = mean amplitude of e.p.ps/mean amplitude of me.p.ps) or by the method of failures (m = $\ln(N/N_0)$; where N = number of trials and N_0 number of failures of release).

Presynaptic currents were recorded either from nerve terminals by use of heat-polished microelectrodes (2–5 M Ω resistance) filled with standard Ringer solution or from the perineural space of fine superficial motor nerve bundles with 2 M NaCl-filled microelectrodes (10–15 M Ω resistance) as previously described (Gundersen *et al.*, 1982; Mallart, 1984). At the perineural space the signals generated at the endings upon invasion of nerve impulses are recorded with inverse polarity (Mallart, 1984). In all cases positivity at the recordings electrode is signalled by upward deflections. In all perineural recordings (+)-tubocurarine (30 μ M) and procaine (50–100 μ M) were added to the physiological solution to abolish completely postsynaptic activity and to prevent spontaneous repetitive nerve firing in the presence of tetraethylammonium (TEA). Superficial nerves and endplates were observed at a magnification $\times$ 400 with a microscope fitted with a water immersion $\times$ 40 Zeiss objective (working distance 1.6 mm) and interference contrast (Nomarski) optics.

Electrical signals were, after conventional amplification, digitized, displayed on a digital oscilloscope and simultaneously recorded on video tape with the aid of a modified digital audio processor (Sony PCM 701 ES) and a video cassette recorder (Sony SLC 9F). Data were collected and analysed with the aid of an IBM-AT microcomputer equipped

with a TM-100 Labmaster analogue and digital interface board (Scientific solutions, U.S.A.) and modified pClamp software (Axon Instruments, U.S.A.). An event detector (AI 2020, Axon Instruments) was used to detect synaptic events.

Drugs used were: formamide, (+)-tubocurarine, tetrodotoxin, 3,4-diaminopyridine, ethyleneglycol-bis-(β -aminoethylether) N,N',-tetraacetic acid (EGTA), ethylenediaminetetraacetic acid (EDTA), (Sigma, St. Louis, MO, U.S.A.); procaine hydrochloride (Merck, Darmstadt, Germany); tetraethylammonium bromide (Koch-Light, Haverhill, U.K.) and gadolinium chloride (Ventron, Karlsruhe, Germany). Since the supplier of gadolinium did not specify the moles of water content present in the GdCl₃ sample, the chloride concentration was determined in every stock solution made. All salts were of analytical grade.

Statistical analysis of data was performed with Student's *t* test (two tailed). Values are expressed as mean $\pm$ s.e.mean. Data were considered significant at $P < 0.05$.

Results

Effect of Gd³⁺ on neuromuscular transmission

Nerve stimulation of cutaneous pectoris muscles, previously treated with formamide (see Methods), elicited at junctional areas action potentials without contraction triggered by e.p.ps (Figure 1a). Under these conditions, addition of 450 μ M Gd³⁺ to the normal Ringer solution reduced, in a few minutes, the amplitude of e.p.ps which could no longer reach the threshold for action potential generation in the muscle fibre. Finally Gd³⁺ completely blocked neuromuscular transmission

Figure 1 Effects of Gd³⁺ on neuromuscular transmission in three different neuromuscular preparations. (a) Action potential elicited by nerve stimulation (0.5 Hz) in a junction perfused with standard Ringer solution in which excitation-contraction was uncoupled by formamide. (b) Superimposed recordings obtained on the same junction 1 and 3 min after the addition of Gd³⁺ (0.45 mM) to the standard Ringer solution. Note that at this concentration, Gd³⁺ completely suppressed the evoked e.p.p. Resting membrane potential during measurements in (a) and (b) –81 mV. Same calibration for (a) and (b). (c) Extracellular focal recordings obtained from a junction perfused with low Ca²⁺ (0.6 mM) high Mg²⁺ (6 mM) Ringer solution showing that upon nerve stimulation, presynaptic currents (▲) can be recorded in the presence of Gd³⁺ (40 μ M) even when transmitter release is greatly inhibited. (d) Spontaneous miniature endplate current recorded on the same synaptic site as (c). Note that the evoked and spontaneous postsynaptic currents have similar amplitudes and time courses. (e) and (f) Intracellular m.e.p.ps recorded in a junction perfused with the same Ringer as in (c) showing that in the presence of Gd³⁺ (100 μ M) the blockade of evoked transmitter release is followed by an increase in m.e.p.p. frequency.

Figure 2 Dose-dependent reduction of the mean quantal content of e.p.ps by Gd^{3+} . The extracellular medium contained low Ca^{2+} (0.5 mM) and high Mg^{2+} (6 mM). Each point represents the percent reduction $\pm$ s.e. as compared to respective control obtained in a single junction. Data were obtained from 4–8 different preparations.

(Figure 1b). The effect of Gd^{3+} was reversible, provided preparations were exposed for 10–15 min and washed with Gd^{3+} -free solution. Gd^{3+} in the range of concentrations used (5–450 μM) had no consistent effect on the resting membrane potential of the muscle fibres. A similar blockade of neuromuscular transmission by Gd^{3+} was observed at junctions in which excitation-contraction coupling was unaffected. The inhibitory effect of Gd^{3+} on neuromuscular transmission raised the possibility that the cation may have pre- and/or postsynaptic actions at the neuromuscular junction.

Gd^{3+} on evoked quantal release

In preparations in which the normal release of transmitter was reduced by a medium containing low Ca^{2+} (0.5 mM) and high Mg^{2+} (6 mM), the addition of Gd^{3+} (5–40 μM) produced a dose-dependent increase in the number of failures of release, during trains of nerve stimulation at 0.5 Hz, and a decrease in the mean quantal content of e.p.ps (Figure 2). Extracellular focal recordings from endplate regions revealed that blockade of quantal release caused by Gd^{3+} was neither due to an impairment of the presynaptic action potential to reach the nerve terminal (Figure 1c), nor the result of a postsynaptic blockade, since miniature endplate currents could be recorded from the same synaptic sites (Figure 1d) and had a similar amplitude and time course to unitary evoked responses. These results indicate that Gd^{3+} , in the range of concentrations studied, reduces the number of transmitter quanta released by each nerve impulse.

A notable feature in Gd^{3+} -blocked junctions was the observation that nerve stimulation at low rates (0.2–0.5 Hz) evoked a period of high frequency m.e.p.ps after each stimulus, even when failures of evoked transmitter release occurred as shown in representative recordings (Figure 1e, f).

3,4-Diaminopyridine (3,4-DAP) (5–10 μM), which by blocking voltage-sensitive K^+ channels in motor endings prolongs the duration of the presynaptic depolarization, enhancing Ca^{2+} influx and consequently evoked transmitter release (Molgó, 1982), consistently reversed the depression of nerve-impulse evoked transmitter release caused by Gd^{3+} in the low Ca^{2+} -high Mg^{2+} medium as shown in Figure 3.

Effects of Gd^{3+} on presynaptic Ca^{2+} currents

The possibility that blockade of evoked transmitter release in Gd^{3+} -treated junctions reflects inhibition of Ca^{2+} entry in presynaptic terminals was directly investigated by use of perineurial current recordings. The calcium current entering the nerve terminals is not readily detectable in recordings performed in standard Ringer solution but can be revealed after

Figure 3 Reduction in quantal content of e.p.ps produced by 10 μM Gd^{3+} (solid column) over controls (open column) and its reversal by 10 μM 3,4-diaminopyridine (hatched column). Preparation bathed in low Ca^{2+} (0.5 mM)-high Mg^{2+} (6 mM) Ringer solution. Each column represents the mean of 3 values obtained in different preparations; s.e. shown by vertical bars. The quantal content was estimated by the direct method. Gd^{3+} was applied for 20 min, while 3,4-diaminopyridine was applied for 15 min in the continuous presence of Gd^{3+} .

blocking outward K^+ currents (Gundersen *et al.*, 1982; Mallart, 1984).

To study the effects of Gd^{3+} on presynaptic Ca^{2+} currents experiments were performed in the presence of TEA, which blocks voltage- and calcium-dependent K^+ currents in motor terminals (Mallart, 1984; Hevron *et al.*, 1986), together with elevated extracellular Ca^{2+} levels. Figure 4a, shows typical perineurial recordings obtained in preparations perfused with

Figure 4 Presynaptic currents recorded from the perineurial space of a small preterminal nerve bundle. (a) Control (average of 5 tracings). (b) (i) Single trace obtained on the same recording site 20 min after the addition of 10 mM tetraethylammonium. The upward deflection signals the prolonged calcium current. (ii) and (iii) show the progressive blockade of the calcium current after the addition of 0.5 mM Gd^{3+} . Note in (iii) the presence of an inward current (presumably carried by Na^+) entering the terminals that was unaffected by Gd^{3+} . Recordings obtained in Ringer solution containing 8 mM Ca^{2+} and 30 μM (+)-tubocurarine. In (b), procaine (100 μM) was added to avoid repetitive nerve firing. Stimulation rate was 0.5 Hz in (a) and 0.008 Hz in (b).

Figure 5 (a) Time course of the increase in m.e.p.p. frequency caused by $40\mu\text{M}$ Gd^{3+} at two different neuromuscular junctions equilibrated for respectively 1 h in a nominally calcium-free solution containing 2 mM Mg^{2+} and $0.7\mu\text{M}$ tetrodotoxin ($\blacktriangle$) and in standard Ringer solution containing 2 mM Mg^{2+} ($\bullet$). (b) Time course of m.e.p.p. frequency at a single junction previously exposed for 30 min to $40\mu\text{M}$ Gd^{3+} and then washed with a nominally calcium-free solution containing 1 mM EGTA. In (a) and (b) each point represents the relative increase in m.e.p.p. frequency as compared to respective controls.

Ringer solution containing 8 mM Ca^{2+} . Two current components can be distinguished. The early component has been ascribed to the sodium current which flows into the motor axons at the nodes of Ranvier and promotes depolarization of their terminals. The second component corresponds to the potassium current which underlies spike repolarization in the terminals (Mallart, 1984; Hevron *et al.*, 1986). Under these conditions addition of 10 mM TEA to the solution allowed the development of a long-lasting plateau current (Figure 4b(i)).

Several lines of evidence identified the plateau current as an inward Ca^{2+} current in the terminals (I_{Ca}): (1) I_{Ca} was not present or could be suppressed in a calcium-free medium; (2) divalent cations like Co^{2+} (10 mM) and Cd^{2+} (0.2 mM), well known calcium channel blockers, abolished I_{Ca} . Calcium plateaus of long duration (several hundred ms) could be evoked only when low frequency stimulation ($<0.03\text{ Hz}$) was used. At higher rates, shortening of the plateau duration occurred probably because of calcium-channel inactivation by intraterminal Ca^{2+} accumulation. Using low rates of nerve stimulation we found that $0.2\text{--}0.5\text{ mM}$ Gd^{3+} suppressed I_{Ca} , as shown in Figure 4(b(ii), (iii)). Although the positive current component which remains after Gd^{3+} action was not fully investigated, it is likely that it may be due to Na^{+} ions entering the terminals, since it was also observed after Co^{2+} - and Cd^{2+} -blockade of calcium currents (results not shown).

Gd^{3+} on spontaneous quantal release

Addition of Gd^{3+} to the standard Ringer solution in concentrations of $40\text{--}200\mu\text{M}$ caused, within a few min, a significant rise in spontaneous quantal release recorded as m.e.p.p. frequency. Simple withdrawal of Gd^{3+} from the medium did not interrupt the marked increase in m.e.p.p. frequency. With continued application of Gd^{3+} , the frequency reached a

Figure 6 Amplitude distribution of spontaneous m.e.p.ps recorded at the same endplate in standard Ringer solution (a), after 20 min exposure to $200\mu\text{M}$ Gd^{3+} added to the Ringer solution (b) and after washing out Gd^{3+} for 15 min from the external medium. Mean resting membrane potential during measurements was -82.6 mV .

maximum usually in the range of $100\text{--}150\text{ Hz}$, and subsequently subsided slowly. A similar effect was observed in junctions bathed in a nominally Ca^{2+} -free medium containing 2 mM Mg^{2+} supplemented with $0.7\mu\text{M}$ tetrodotoxin (Figure 5a). As shown in Figure 5b, removal of extracellular Gd^{3+} by continuous perfusion with a Gd^{3+} -free and Ca^{2+} -free solution containing 1 mM EGTA (Figure 5b) or 1 mM EDTA (not shown) to chelate extracellular traces of Gd^{3+} did not completely reverse the increase in m.e.p.p. frequency caused by Gd^{3+} . These experiments indicate that Gd^{3+} can itself act to increase quantal release in the nominal absence of external Ca^{2+} . This effect presumably depends upon entry of Gd^{3+} into the nerve endings, since it is slowly, if at all, reversed upon removal of extracellular Gd^{3+} .

Gd^{3+} , in addition to increasing the frequency of m.e.p.ps, also caused at concentrations equal or higher than $100\mu\text{M}$ a reduction in m.e.p.p. amplitude. Thus, a 20 min exposure to $100\mu\text{M}$ or $200\mu\text{M}$ Gd^{3+} depressed the amplitude of m.e.p.ps to 0.70 ± 0.12 ($n = 3$) and 0.36 ± 0.05 ($n = 3$) respectively of control values (mean $\pm$ s.e.). These effects of Gd^{3+} , in contrast to those on the frequency of m.e.p.ps were reversible upon 15–20 min washing with a Gd^{3+} -free medium. Figure 6 shows an example of the amplitude distribution of m.e.p.ps and the shift of the modal value to lower amplitude levels during the action of Gd^{3+} and, to control levels during the washing out of Gd^{3+} from the medium. With $450\mu\text{M}$ Gd^{3+} , m.e.p.ps were no longer detectable by intracellular recordings at junctional areas of the muscle fibres.

Discussion

The present results show that Gd^{3+} has a dual action on quantal transmitter release from frog motor nerve terminals since it blocks nerve-impulse evoked transmitter release and enhances spontaneous quantal release recorded as m.e.p.p. frequency. In that respect the action of Gd^{3+} is similar to that previously observed with other trivalent lanthanide cations like lanthanum (Blioch *et al.*, 1968; Heuser & Miledi, 1971), praseodymium (Alnaes & Rahamimoff, 1974) and erbium (Metral *et al.*, 1978).

Gd^{3+} was previously found to inhibit twitch tension evoked by nerve stimulation on the frog isolated sartorius nerve-muscle preparation (Bowen, 1972) and to block skeletal muscle contraction (Hambly & Dos Remedios, 1977). The present results indicate that the block of evoked quantal transmitter release caused by Gd^{3+} can be ascribed to its ability to block the calcium current entering motor endings, as shown by perineural recordings. The results support the view that Gd^{3+} blocks N-type Ca^{2+} channels (Docherty, 1988) and suggest that such channels are the major type involved in nerve-impulse evoked transmitter release from frog motor nerve terminals. These findings agree with previous studies showing that Gd^{3+} inhibits [3H]-noradrenaline and vasopressin release (Bourne & Trifaró, 1982; Muscholl *et al.*, 1985) and effectively reduced depolarization-induced $^{45}Ca^{2+}$ uptake into cultured chromaffin cells and rat brain synaptosomes (Nachshen, 1984).

The calcium current entering frog motor nerve terminals differed from the one recorded at mouse preparations by the fact that long-lasting plateau currents could not be obtained in the latter preparations in the presence of TEA alone, but only after the combined action of TEA and 3,4-DAP (Dreyer & Penner, 1987; Anderson & Harvey, 1987; Tabti *et al.*, 1989). This was taken as evidence for the presence of a TEA-resistant potassium current in mouse motor nerve terminals which develops with a relatively slow time course and which is sensitive to 3,4-DAP (Dreyer & Penner, 1987; Tabti *et al.*, 1989). Since calcium plateau magnitude and duration depend on the balance between Ca^{2+} and K^+ permeabilities (Katz & Miledi,

1971), we suggest that frog motor nerve terminals lack the slowly activating, TEA-insensitive potassium current, reported in mammalian terminals allowing therefore the development of calcium plateaus after blocking both the voltage-dependent and the calcium-activated potassium currents.

In addition to its blocking action on nerve-impulse-evoked transmitter release, Gd^{3+} was found to enhance asynchronous quantal release from motor terminals. On isolated adrenal medullary cells and in the rat neurohypophysis no effect of Gd^{3+} was detected on the basal release of [3H]-noradrenaline and vasopressin (Bourne & Trifaró, 1982; Muscholl *et al.*, 1985) while in striatal synaptosomal preparations Gd^{3+} slightly increased basal [3H]-dopamine release (Sheer, 1989). The increase in spontaneous quantal release caused by Gd^{3+} is most probably the result of its entry into motor nerve terminals and its intracellular action is reminiscent of the action of La^{3+} (Curtis *et al.*, 1986). The action of Gd^{3+} in enhancing m.e.p.p. frequency may be either secondary to an increase in intraterminal Ca^{2+} concentration, caused by the interference of energy-dependent Ca^{2+} sequestering systems within the motor terminals or may be due to a direct intracellular action at a site that governs quantal transmitter release.

In addition to the presynaptic effects discussed here, Gd^{3+} was found to reduce reversibly m.e.p.p. amplitude by what seemed to be a reduction in the sensitivity of the postsynaptic membrane to the transmitter. Further experiments will be necessary to understand the action(s) of Gd^{3+} on the endplate acetylcholine receptor-channel complex. Thus, both pre- and postjunctional actions seem to be implicated in the effects of Gd^{3+} at the neuromuscular junction.

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References

- ALNAES, E. & RAHAMIMOFF, R. (1974). Dual action of Praseodymium (Pr^{3+}) on transmitter release at the frog neuromuscular synapse. *Nature*, **274**, 478–479.
- ANDERSON, A.J. & HARVEY, A.L. (1987). ω -Conotoxin does not block the verapamil-sensitive calcium channels at mouse motor nerve terminals. *Neurosci. Lett.*, **82**, 177–180.
- AUGUSTINE, G.J., CHARLTON, M.P. & SMITH, S.J. (1987). Calcium action in synaptic transmitter release. *Annu. Rev. Neurosci.*, **10**, 633–693.
- ARNON, A., DAVID, G., HEVRON, E. & YAARI, Y. (1988). Calcium currents in frog motor nerve terminals. *Soc. Neurosci. Abstr.*, **14**, 68.
- BLIOCH, Z.L., GLAGOLEVA, I.M., LIBERMAN, E.A., & NENASHEV, V.A. (1968). A study of the mechanism of quantal transmitter release at a chemical synapse. *J. Physiol.*, **199**, 11–35.
- BOURNE, G.W. & TRIFARÓ, J.M. (1982). The gadolinium ion: a potent blocker of calcium channels and catecholamine release from cultured chromaffin cells. *Neuroscience*, **7**, 1615–1622.
- BOWEN, J.M. (1972). Effects of rare earths and yttrium on striated muscle and the neuromuscular junction. *Can. J. Physiol. Pharmacol.*, **50**, 603–611.
- BROWN, D.A., DOCHERTY, R.J. & McFADZEAN, I. (1989). Calcium channels in vertebrate neurons. *Ann. New York Acad. Sci.*, **560**, 358–372.
- CURTIS, M.J., QUASTEL, D.M.J. & SAINT, D.A. (1986). Lanthanum as a surrogate for calcium in transmitter release at mouse motor nerve terminals. *J. Physiol.*, **373**, 243–260.
- DEL CASTILLO, J. & ESCALONA DE MOTTA, G. (1978). A new method for excitation-contraction uncoupling in frog skeletal muscle. *J. Cell Biol.*, **78**, 782–784.
- DOCHERTY, R.J. (1988). Gadolinium selectively blocks a component of calcium current in rodent neuroblastoma $\times$ glioma hybrid (NG108-15) cells. *J. Physiol.*, **398**, 33–47.
- DREYER, F. & PENNER, R. (1987). The action of presynaptic snake toxins on membrane currents of the mouse motor nerve terminals. *J. Physiol.*, **386**, 455–463.
- ENOMOTO, K., SANO, K., SHIBUYA, Y. & MAENO, T. (1986). Blockade of transmitter release by a synthetic venom peptide, ω -conotoxin. *Proc. Jpn. Acad., Ser. B*, **62**, 267–270.
- GOTGILF, I.M. & MAGAZANIK, L.G. (1977). Action of calcium channels blocking agents (verapamil, D-600 and manganese ions) on transmitter release from motor nerve endings of frog muscle. *Neurofiziologia*, **9**, 415–422. (in Russian)
- GUNDERSEN, C.B.S., KATZ, B. & MILEDI, R. (1982). The antagonism between botulinum toxin and calcium in motor nerve terminals. *Proc. R. Soc. B*, **216**, 369–376.
- HAMBLY, B.D. & DOS REMEDIOS, C.G. (1977). Responses of skeletal muscle fibres to lanthanide ions. Dependence of the twitch response on ionic radii. *Experientia*, **33**, 1042–1044.
- HEUSER, J. & MILEDI, R. (1971). Effect of lanthanum ions on function and structure of frog neuromuscular junctions. *Proc. R. Soc. B*, **179**, 247–260.
- HEVRON, E., DAVID, G., ARNON, A. & YAARI, Y. (1986). Acetylcholine modulates two types of presynaptic potassium channels in vertebrate motor nerve terminals. *Neurosci. Lett.*, **72**, 87–92.
- KATZ, B. & MILEDI, R. (1971). The effect of prolonged depolarization on synaptic transfer in the stellate ganglion of the squid. *J. Physiol.*, **216**, 503–512.
- KERR, L.M. & YOSHIKAMI, D.C. (1984). A venom peptide with a novel presynaptic blocking action. *Nature*, **308**, 282–284.
- MALLART, A. (1984). Presynaptic currents in frog motor endings. *Pflügers Arch.*, **400**, 8–13.
- METRAL, S., BONNETON, C., HORT-LEGRAND, C. & REYNES, J. (1978). Dual action of erbium on transmitter release at the frog neuromuscular synapse. *Nature*, **271**, 773–775.

- MILLER, R.J. (1987). Multiple calcium channels and neuronal function. *Science*, **235**, 46–52.
- MOLGO, J. (1982). Effects of aminopyridines on neuromuscular transmission. In *Aminopyridines and Similarly Acting Drugs: Effects on Nerves, Muscles and Synapses*. ed. Lechat, P., Thesleff, S. & Bowman, W.C. *Advances in the Biosciences*, Vol. 35, pp. 95–116. Oxford: Pergamon Press.
- MUSCHOLL, E., RACKE, K. & TRAUT, A. (1985). Gadolinium ions inhibit exocytotic vasopressin release from the rat neurohypophysis. *J. Physiol.*, **365**, 419–434.
- NACHSHEN, D.A. (1984). Selectivity of the Ca binding site in synaptosome Ca channels. *J. Gen. Physiol.*, **83**, 941–967.
- NACHSHEN, D.A. & BLAUSTEIN, M.P. (1979). The effect of some organic 'calcium antagonists' on Ca influx in presynaptic nerve terminals. *Mol. Pharmacol.*, **16**, 579–586.
- SANO, K., ENOMOTO, K. & MAENO, T. (1987). Effects of synthetic ω -conotoxin, a new type Ca^{2+} antagonist, on frog and mouse neuromuscular transmission. *Eur. J. Pharmacol.*, **141**, 235–241.
- SCHEER, H.W. (1989). Interactions between α -latrotoxin and trivalent cations in rat striatal synaptosomal preparations. *J. Neurochem.*, **52**, 1590–1597.
- TABTI, N., BOURRET, C. & MALLART, A. (1989). Three potassium currents in mouse motor nerve terminals. *Pflügers Arch.*, **413**, 395–400.

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