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The non-competitive acetylcholinesterase inhibitor APS12-2 is a potent antagonist of skeletal muscle nicotinic acetylcholine receptors

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Introduction

APS12-2 is a synthetic polymeric 1,3-dodecylpyridinium salt with 14.7 kDa of molecular mass structurally related to natural polymeric alkylpyridinium salts (poly-APS) isolated from the marine sponge Reniera sarai. In the present work the effects of APS12-2 were studied on isolated mouse phrenic nerve–hemidiaphragm muscle preparations, using twitch tension measurements and electrophysiological recordings. APS12-2 in a concentration-dependent manner blocked nerve-evoked isometric muscle contraction (IC50 = 0.74 μM), without affecting directly-elicited twitch tension up to 2.72 μM. The compound (0.007–3.40 μM) decreased the amplitude of miniature endplate potentials until a complete block by concentrations higher than 0.68 μM, without affecting their frequency. Full size endplate potentials, recorded after blocking voltage-gated muscle sodium channels, were inhibited by APS12-2 in a concentration-dependent manner ([IC50 = 0.36 μM]) without significant change in the resting membrane potential of the muscle fibers up to 3.40 μM. The compound also blocked acetylcholine-evoked inward currents in Xenopus oocytes in which Torpedo (α1β1γδε) muscle-type nicotinic acetylcholine receptors (nAChRs) have been incorporated ([IC50 = 0.162 μM]), indicating a higher affinity of the compound for Torpedo (α1β1γδε) than for the mouse (α1β1γδε) nAChR. Our data show for the first time that APS12-2 blocks neuromuscular transmission by a non-depolarizing mechanism through an action on postsynaptic nAChRs of the skeletal neuromuscular junction.
action on the tissue and at the cellular level. APS12-2 and structurally related compounds are promising new chemotherapeutic agents that exhibit very low in vivo toxicity in experimental animals (Grandič et al., 2011). In view of their putative use to treat lung cancer, it is very important to evaluate other possible adverse effects of these compounds. In particular, due to the structural similarity between APS12-2 and quaternary ammonium compounds that have been reported to block muscle nAChRs, the aim of this study was to investigate the effects and the underlying mechanisms of APS12-2 on neuromuscular transmission.

Materials and methods

Materials

Drugs and solutions. Tricaine, kanamycin, collagenase type II, acetylcholine chloride, neostigmine methyl sulfate and 3,4-diaminopyrididine were from Sigma–Aldrich (Saint Quentin Fallavier, France). Atropine sulfate was purchased from Belupo, Croatia and μ-conotoxin GIIIB was from Bachem, Switzerland. All chemicals were of the highest grade available.

APS12-2 was synthesized using a microwave-assisted polymerization procedure (Houssen et al., 2010). In addition, it was structurally characterized by nuclear magnetic resonance (NMR), and its molecular weight determined by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and electrospray ionization mass spectrometry (ESI-MS). APS12-2 was found to be a 14.7 kDa polymer composed of sixty 1,3-dodecylpyridinium monomer units. Before use, the compound was dissolved in distilled water.

Experimental animals. Adult male Balb/C mice (24–28 g body weight) were purchased from the animal breeding house in Veterinary Faculty, University of Ljubljana. The experiments followed ethical standards, and were approved by Veterinary Administration of the Republic of Slovenia (permit no. 34401-20/2009/30). Adult female Swiss-Webster mice (31.6 ± 2.2 g body weight, n = 6) were purchased from Janvier Elevage (Le Genest-Saint-Isle, France) and housed at the Gif-sur-Yvette campus animal facility. Adult Xenopus laevis female frogs were obtained from the Centre de Ressources Biologiques Xenopos (Rennes, France). The Xenopus were housed in groups of 8 at the Gif-sur-Yvette campus animal facility.

Methods

Muscle twitch recordings from isolated mouse hemidiaphragms. Adult male Balb/C mice were euthanized by cervical dislocation followed by immediate exsanguination. The diaphragm muscle with corresponding phrenic nerves was isolated and mounted in a 3 mL Rhodorsil-lined organ bath containing the following oxygenated physiological solution composed of (in mM): 154 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 5 HEPES and 11 g-glucose, pH 7.4, at 22–24 °C. The lateral side of the hemidiaphragm preparation was tightly pinned to the Rhodorsil-lined bath. The medial side with tendon was attached with a silk thread via a stainless steel hook to an isometric mechanoh-electrical transducer (lits, ljubljana, slovenia). The motor nerve of isolated neuromuscular preparations was stimulated via a suction electrode with pulses of 0.1 ms duration, 0.1 Hz stimulation rate and supramaximal voltage of 5–10 V, using a square pulse S-48 stimulator (Grass Instruments, West Warwick, RI, USA). For direct muscle stimulation electrical field stimulation was carried out (pulses of 60–100 V, 150 μs in duration) by means of a platinum electrode assembly placed along the length of the hemidiaphragm and connected to the isolation unit of the S-48 Grass stimulator. In order to directly or indirectly evoke tetanic muscle contraction, trains of pulses (500 ms duration at 70 Hz) were used. The resting tension for each muscle preparation (typically 1.5–2.5 g) was adjusted in order to achieve maximal contractile response upon nerve-evoked muscle stimulation.

Each hemidiaphragm preparation was allowed to equilibrate for 20–30 min before starting experiments to achieve maximal contractile response and stable resting tension. Muscle twitch tension was measured using a Grass FT03 force transducer. Electrical signals were amplified by P22 strain gage amplifier (Grass Instruments, West Warwick, RI, USA), and continuously digitized at a sampling rate of 1 kHz using a data acquisition system (Digidata 1440A; Molecular Devices, Sunnyvale, CA, USA). The inhibitory effect of APS12-2 on nerve-evoked contraction was measured 60–90 min after APS12-2 application. Muscle twitch tension blockade produced by APS12-2 was expressed as percentage of the maximal twitch response before exposure to APS12-2. Concentration–response curves of APS12-2 were plotted 60–90 min after drug perfusion.

Membrane potential, miniature endplate potential and endplate potential recordings from mouse hemidiaphragm. Resting membrane potential, miniature endplate potentials (MEPPs) and endplate potentials (EPPs) were obtained from superficial muscle fibers using intracellular borosilicate glass microelectrodes with an Axoclamp 900A microelectrode amplifier (Molecular Devices, Sunnyvale, CA, USA). Microelectrodes were pulled with a P-97 Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA, USA) and filled with 3 M KCl. Microelectrodes with 10–20 MΩ resistance were used. EPPs were recorded in the endplate region, indicated by the presence of MEPPs. EPPs were evoked by stimulating the phrenic nerve via a bipolar suction electrode with supramaximal square pulses of 0.1 ms duration at 0.5 Hz using an S-48 stimulator (Grass Instruments, West Warwick, RI, USA). EPPs and MEPPs were digitized (Digidata 1440A and pCLAMP 10, Axon Instruments) at 25 kHz and stored for later analysis using pCLAMP Clampfit 10 software (Axon Instruments). All experiments were performed at 22–24 °C. Muscles were pretreated for 30 min with 2 μM μ-conotoxin GIIIB (Chevessier et al., 2008). Thereafter, EPPs were measured in the presence of 2 μM μ-conotoxin GIIIB which preferentially blocks muscle type voltage-gated sodium channels and allows measuring the full-size EPP amplitude (Cruz et al., 1985; Hong and Chang, 1989). Amplitudes of MEPPs and EPPs were normalized to a membrane potential of −70 mV using the formula Vm = Vpm × (70−E)/E, where Vpm is corrected amplitude of EPPs or MEPPs, Vm is corrected amplitude and E is resting membrane potential (Pardo et al., 2006).

Electrophysiological recordings from Xenopus oocytes having incorporated the Torpedo nAChR. The nAChRs expressed in the electric organ of Torpedo marmorata fish are similar in subunit composition and functional properties to those of fetal vertebrate neuromuscular junctions (reviewed by Sine, 2012). Therefore, in order to have additional information on the effects of APS12-2 on this muscle-type nAChR we performed micro-transplantation of electricocyte purified Torpedo membranes to Xenopus oocytes to study the α1β1δ1γ16 nAChR incorporated to the oocyte membrane. T. marmorata electricocyte membranes enriched with α1β1δ1γ16 nAChR were purified, as previously described (Krieger et al., 2008) and aliquots (2.7 mg/mL total protein) in 5 mM glycine were kept at −80 °C until use.

Adult X. laevis frogs were anaesthetized by immersion in a water solution containing 1.5 g/L tricaine for 20 min. Frogs were operated and oocytes were prepared as described (Krieger et al., 2008). Oocytes were kept at 18 °C in Barth’s medium containing: 88 mM NaCl, 1 mM KCl, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 2.5 mM Ca(NO₃)₂, 7.5 mM HEPES, and pH 7.6, supplemented with kanamycin (2 mg/mL) to prevent bacterial contamination.

Stage V and VI oocytes were microinjected each with 50 nl of purified Torpedo membranes using a Nanoliter 2000 Micro4 Controller (World Precision Instruments Inc., Stevenage, Herts, U.K.) and incubated at 18 °C. Oocytes were used 1–2 days after microinjection with Torpedo membranes. Viability of oocytes was checked, and Barth’s medium was replaced daily. Membrane current recordings were performed at 20 °C using the two-microelectrode voltage-
clamp technique and an OC-725B amplifier (Warner Instruments, LLC, Hamden, USA). Intracellular borosilicate glass microelectrodes were filled with 3 M KCl and had resistances of 2–5 MΩ. Each oocyte was placed in the recording chamber (300 μL capacity), equilibrated for about 5 min and then continuously superfused (8–12 mL/min) with a modified Ringer’s solution containing (mM): 100 NaCl, 2.8 KCl, 1 MgCl₂, 0.3 BaCl₂ and 5 HEPES, where BaCl₂ substitution to CaCl₂ prevents secondary activation of a Ca²⁺-dependent Cl-current (Sands et al., 1993).

The holding membrane potential was maintained at −60 mV. Experimental data were digitized with a Digidata-1322A A/D converter, and later analyzed with pCLAMP-9 software (Molecular Devices, Union City, CA, USA). Stock solutions of ACh and APS12-2 were prepared in distilled water. All stock solutions were diluted to the final concentrations in the modified Ringer solution.

The half maximal effective ACh concentration (EC₅₀) was 25 μM, as determined by a concentration–response curve in oocytes having incorporated the Torpedo muscle-type nAChR and voltage-clamped at −60 mV. We used 50 μM ACh (2 times the EC₅₀) which activated about 80% of the nAChR. ACh (50 μM) was usually applied twice in order to record control current amplitudes. ACh applications were followed by APS12-2 perfusion which was immediately followed by perfusion with the mixture of APS12-2 and ACh. Finally, each oocyte was washed-out and again exposed twice to ACh to record the

Fig. 1. Effects of APS12-2 on both nerve-evoked or directly-elicited single twitch and tetanic contractions in isolated mouse hemidiaphragm neuromuscular preparations. (A) Representative muscle contraction recordings showing the partial block of nerve-evoked muscle twitch and tetanic contraction by APS12-2 at a concentration of 1.02 μM. N — denotes nerve-evoked muscle contraction; D — denotes directly-elicited muscle contraction; Tn — denotes nerve-evoked tetanic contraction; Td — denotes directly-elicited tetanic contraction; W — wash-out. (B₁) Representative recordings of nerve-evoked single twitch before (N1), and 90 min after application of APS12-2 (N2), data recorded from the same muscle showing the blocking effects of APS12-2 (1.02 μM). (B₂) Representative recordings of tetanic contraction (at 70 Hz) before (Tn1), and 90 min after application of APS12-2 (Tn2), data recorded from the same muscle showing its blocking effects (1.02 μM). (C₁) Representative recordings showing tetanic contractions elicited by direct muscle stimulation before (D1), and 90 min after application of APS12-2 (D2). (D) Concentration-dependent inhibition curve for directly-elicited and nerve-evoked contraction for APS12-2 on mouse hemidiaphragms, expressed as percent of the maximal twitch response. Values are expressed as the mean ± SE (n=5 different muscles).

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recovery of current amplitudes. A computer-controlled solution-exchange system (VC-6, Warner Instruments) allowed limiting the duration of each drug exposure to 15 s. Recovery time between each application was 150 s. Amplitudes of the currents recorded in response to each drug concentration were normalized to the maximum amplitude of the current evoked by the control ACh.

Local in vivo effects on mouse neuromuscular system. The multimodal excitability properties of the mouse neuromuscular system were studied in vivo on adult mice, by means of minimally-invasive electrophysiological methods, using the Qtrac© software written by Prof. Hugh Bostock (Institute of Neurology, London, England). The experiments were performed in accordance with the guidelines established by the French Council on animal care “Guide for the Care and Use of Laboratory Animals”; EEC86/609 Council Directive-Decree 2001-131, on mice under isoflurane (AErrane®) anesthesia. The experimental protocols were approved by the French Departmental Direction of Animal Protection (no. A91–453 to E.B.).

Briefly, electrical stimulations were delivered to the caudal motor nerve by means of surface electrodes, and the compound muscle action potential (CMAP) was recorded using needle-electrodes inserted into the tail muscle. To study the local action of APS12-2, intramuscular injections of the compound [1.1–2.2 μmol/kg mouse in 100 μL phosphate buffer saline (PBS) solution] were delivered with a 100 μL micro-syringe at the base of the mouse tail, between stimulation and ground electrodes. Similar injections were also done with only PBS solution. Immediately after a given injection, on-line recordings were initiated to determine the effects of APS12-2 on selected excitability parameters, such as the excitability threshold and CMAP amplitude recorded continuously as a function of time.

Data analysis and statistics
The data were statistically analyzed using Sigma Plot for Windows version 11.0 (Systat Software Inc., USA). The Student two-tailed test was used for statistical analysis of data. The results are presented as mean ± S.E. The statistical significances were set at the P value ≤0.05. Concentration–response curves were fitted using the four parameter nonlinear regression model.

Results

APS12-2 blocks nerve-evoked muscle contraction in vitro

One of the goals of this study was first to determine the effects of the non-competitive AChE inhibitor APS12-2 on directly- and indirectly-evoked isometric muscle twitch and tetanic contraction in the mouse hemidiaphragm. The following APS12-2 concentrations were used: 0.07, 0.34, 0.68, 0.82, 1.02, and 2.72 μM. An example of the time-course of APS12-2 effects on muscle contraction is shown in Fig. 1A. APS12-2 blocked nerve-evoked single twitch (Fig. 1B1) and tetanic contraction (Fig. 1B2) in a concentration-dependent manner (Fig. 1D). From the concentration–response curve it was calculated an IC₅₀ = 0.74 μM for APS12-2.

No drop in the amplitude of muscle twitch was observed in control experiments during 90 min (record and data not shown). After concentration-dependent blockade of indirectly-evoked muscle twitch, thorough washing of the preparation with standard physiological solution partially restored indirectly-evoked muscle twitch and tetanic contractions (Fig. 1A). In all preparations exposed to different APS12-2 concentrations we found that single twitch, as well as tetanic contraction of about the same amplitude as before the application of APS12-2, could still be produced by direct electrical muscle stimulation (Figs. 1C1 and C2, respectively). With most APS12-2 concentrations used, the drug did not reduce the amplitude of directly-elicited muscle contraction except at 2.72 μM which was the highest concentration used (Fig. 1D). During the development of the contraction block, obtained 30 min after application of 0.82 μM APS12-2, the reversible AChE inhibitor neostigmine (1 μM; Alderdice, 1982; Goodman Gilman, 2001) did not augment single twitch and tetanic muscle contraction (not shown). In contrast, 30 min after application of APS12-2, when twitch responses were reduced to about 30%, 300 μM 3,4-diaminopyridine transiently reversed the blocking effect of APS12-2 on single twitch and tetanic contraction by about 86% (n = 3; not shown).

Effects of APS12-2 on the resting membrane potential of muscle fibers

At the concentration of 3.40 μM, APS12-2 completely blocked nerve-evoked muscle contraction. APS12-2 in all the concentrations

![Fig. 2. Concentration- and time-dependent lack of effect of APS12-2 on the resting membrane potential (νM) of muscle fibers. Recordings were performed at the end-plate region of muscle fibers in mouse hemidiaphragms. Muscles were exposed to 0.007, 0.14, 0.34, 0.68, 1.02 and 3.40 μM APS12-2, before (time 0), after 45 and 50 min of APS12-2 exposure, and after wash-out of the drug from the medium for 15 min. Each column represents the mean ± SE of νM records from 3 different left hemidiaphragms (by sampling 6–8 fibers from each muscle).](image-url)
used (0.007, 0.14, 0.34, 0.68, 1.02 and 3.40 μM) did not significantly affect the resting membrane potential recorded in the endplate region of muscle fibers (P > 0.05; Fig. 2). The average membrane potentials measured from 6 to 8 fibers were −67.40 ± 0.70 mV, −67.24 ± 1.35 mV and −69.14 ± 0.52 mV before application, 45 min and 90 min after exposure to the highest concentration of APS12-2 (3.40 μM), respectively (Fig. 2).

Effects of APS12-2 on MEPP and EPP amplitude

The effects of APS12-2 on MEPP amplitude and frequency are shown in Fig. 3. At concentrations of 0.007, 0.14 and 0.34 μM, APS12-2 significantly (P ≤ 0.05) reduced the amplitude of MEPPs measured in superficial muscle fibers of the mouse hemidiaphragm (Figs. 3A and B). The mean MEPP frequency was not significantly (P > 0.05) affected by APS12-2 at concentrations that affected MEPP amplitude (Fig. 3C). Above 0.68 μM, APS12-2 completely blocked MEPP amplitudes. However, when MEPP amplitude was completely blocked, nerve stimulation still evoked EPPs of low amplitude (Fig. 4A).

Electrophysiological recordings from endplates of the mouse hemidiaphragm, exposed to APS12-2 (from 0.007 to 3.40 μM) for 45 and 90 min (Fig. 4A), revealed that APS12-2 significantly reduced the amplitude of full size EPPs in a concentration-dependent manner (Fig. 4B) with a calculated IC50 of 0.36 μM. No significant changes in the EPP half-decay time (P > 0.05) were observed with APS12-2 in the range of concentration studied (from 0.007 to 3.40 μM). The effects of APS12-2 on MEPPs and EPPs were fully reversible at the concentration of 0.007 μM, and partially reversible at higher drug concentrations (not shown).

APS12-2 blocks Torpedo α1β γδ nAChRs incorporated to Xenopus oocytes

APS12-2 alone at all concentrations used (0.001 to 2.5 nM) did not change the oocyte resting membrane conductance and membrane potential. When oocytes were exposed to ACh (50 μM) and a series of concentrations of APS12-2, a blockade of ACh-induced current was evident. ACh-evoked currents, recorded at a holding potential of −60 mV were inhibited by APS12-2 (0.001 to 2.5 nM) in a concentration-dependent manner as shown in Fig. 5A, and reversibly. Examples of the ACh-evoked currents are shown in Fig. 5A, either in the presence of ACh before, and after APS12-2 was applied together with ACh. APS12-2-induced ACh current inhibition on nAChRs expressed in Xenopus oocytes, showed no voltage-dependence (data not shown). The normalized concentration-dependent data were fitted by the four parameter non-linear ordinary fit method producing an IC50 of 0.55 nM (Fig. 5B). The Hill coefficient of the dose-response curve was 3.82, indicating stoichiometry of 4:1 interaction between T. marmorata nAChRs and APS12-2.

APS12-2 blocks in vivo the compound muscle action potential in mice

The major effect of an intramuscular injection of APS12-2 on the multimodal excitability properties of the mouse neuromuscular system was a time- and dose-dependent decrease of CMAP maximal amplitude, as exemplified in Fig. 6 for 2.2 μmol/kg mouse of compound injected, without significant modification of the other excitability parameters studied. This effect occurred within 15–30 min, depending on the APS12-2 dose injected, and was completely reversed within 2–3 h after compound injections. It is worth noting that the CMAP maximal amplitude remained stable before APS12-2 injections (Fig. 6B), or before and after PBS solution injections (Figs. 6A and B). This latter result indicates that the injection of the vehicle in which APS12-2 was dissolved had, by itself, no significant effect on the CMAP maximal amplitude.

Discussion

In a previous study we reported that APS12-2 toxicity to mice was low (LD50 = 11.5 mg/kg). The rapid death of experimental animals, following intravenous injection, is the result of cardiopulmonary arrest due to APS12-2 hemolytic activity (Grandić et al., 2011). The data obtained in the present work on isolated neuromuscular preparations revealed that APS12-2 causes concentration-dependent block of nerve-evoked twitches and tetanic contractions. The neuromuscular block produced by the APS12-2 in the mouse hemidiaphragm occurs at lower equimolar concentrations than that reported for o-tubocurarine (oTC), a non-depolarizing antagonist of muscle type nAChRs (IC50 = 0.74 μM vs. oTC 1.1 or 1.42 μM, respectively; Nguyen-Huu et al., 2005; Ridtitid et al., 1998). APS12-2 in low concentrations, without affecting frequency, decreased MEPP amplitude measured in superficial muscle fibers without a significant decrease of muscle fiber resting membrane potential indicating the postsynaptic activity of the compound. The neuromuscular block was not antagonized by the low concentration of neostigmine (1 μM) used, which does not inhibit completely AChE activity. If APS12-2 would exert...
an inhibitory action on endplate acetylcholinesterases it would be expected to prevent further neostigmine action. However, no evidence was obtained in the present study of an eventual anticholinesterase effect of APS12-2 which would result in an enhancement of twitch amplitude, and a prolongation of the decay phase of synaptic potentials (MEPPs and full-size EPPs), since the lack of AChE activity allows ACh to persist in the synaptic cleft and to activate repetitively nAChRs of the endplate (Van der Kloot et al., 1994), unless such an action is masked by the APS12-2-induced block of nAChRs which reduces availability of receptors for repetitive binding. The absence of evidence of an APS12-2-induced AChE inhibition, in contrast to previous studies (Houssen et al., 2010), may be due to the polycationic nature of APS12-2 leading to limited capacity of the compound to reach its targets. The muscle twitch blockade produced by APS12-2 was almost completely reversed (86%) by the potassium channel blocker 3,4-diaminopyridine, which increases ACh release from motor terminals (Hong and Chang, 1990; Molgá et al., 1980) and prolongs the duration of action potentials in skeletal muscle, thereby greatly improving skeletal muscle contractile performance (Khan and Lemeignan, 1983; Van Lunteren et al., 2008).

In order to determine possible direct effects of APS12-2 on sarclemma or muscle excitation–contraction coupling processes, single twitch and tetanic responses of muscle with direct electrical stimulation were performed. After partial or complete block of nerve-evoked muscle contraction produced by APS12-2, muscle contraction of the same amplitude as before APS12-2 application could be evoked by direct electrical stimulation of muscle fibers. A direct effect of APS12-2 on muscle membrane excitability is not likely, since direct electrical stimulation produced muscle twitch responses similar in amplitude to those obtained before the application of APS12-2 in the same preparations. These results clearly indicate that APS12-2 has no direct effect on muscle fibers. Therefore, based on the described results from direct muscle stimulation experiments, a direct effect of APS12-2 on the sarclemma or physiological processes (excitation–contraction coupling) leading to the block of contraction is unlikely. The muscle response to the direct electrical stimulation was reduced only at the highest concentration of APS12-2 used (2.72 μM; Fig. 1D). We anticipated that above 2.72 μM, APS12-2 could depolarize and inactivate voltage-dependent sodium channels in the muscle fibers, since it was shown that APS12-2 may permeabilize natural and artificial membranes in nanomolar concentrations (Grandič et al., 2012). This assumption was rejected in this study by the intracellular recordings of membrane potential in the muscle preparations exposed to different concentrations of APS12-2 and different times of exposition to the compound, clearly showing that APS12-2 has no effect on the resting membrane potential at all the concentrations used. In the following experiments, we showed that APS12-2 reduces MEPP and full-size EPP amplitudes, indicating a postsynaptic nAChR effect, since changes of postsynaptic potentials are proportional to the number of nAChR open channels. In concentrations insufficient to block neuromuscular transmission (Fig. 4A), APS12-2 significantly decreased MEPP amplitude (Fig. 3B) without affecting the resting membrane potential (Fig. 2), the rate of MEPP frequency (Fig. 3C) and their decay suggesting that this compound reduces the number of open nAChRs not by altering either muscle fiber passive membrane properties or neurotransmitter ACh release. Subsequent electrophysiological recordings demonstrated the high potency of APS12-2 to inhibit functional responses (nicotinic current) of muscle-type Torpedo nAChRs incorporated to Xenopus oocytes (Fig. 5). Differences in the calculated IC50 concentrations of APS12-2 on muscle contraction (IC50 = 0.74 μM) and full-sized EPPs (IC50 = 0.36 μM) are expected and may be related to the safety margin of neuromuscular transmission.
In vivo effects of intramuscular injections of APS12-2 (2.2 μmol/kg mouse) and PBS solution on the multimodal excitability properties of mouse neuromuscular system. (A) Superimposed traces of the CMAP recorded from tail muscle following increasing intensities of caudal motor nerve stimulation, before and after injections of APS12-2 or PBS solution. (B) Time-course of the effects of APS12-2 or PBS solution injections on the CMAP maximal amplitude recorded continuously as a function of time. Values are expressed relatively to those before injections. The arrow indicates the time of injections.


