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SNX482 selectively blocks P/Q Ca^{2+} channels and delays the inactivation of Na^+ channels of chromaffin cells

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Abstract

The effects of the toxin SXN482 on Ca^{2+} channel currents (I_{Ca}), Na^+ currents (I_{Na}), and K^+ currents (I_K) have been studied in bovine adrenal medullary chromaffin cells voltage-clamped at −80 mV. Currents were elicited by depolarising pulses to 0–10 mV (I_{Ca} and I_{Na}) or to +60 mV (I_K). SNX482 blocked I_{Ca} in a concentration-dependent manner. The inhibition curve exhibited two phases. The first high-affinity phase comprised 28% of the whole-cell current and exhibited an IC_{50} of 30.2 nM. The second low-affinity phase comprised over 70% of I_{Ca} and had an IC_{50} of 758.6 nM. Blockade was rapid and fully reversible upon washout of the toxin. Occlusion experiments showed additivity of blockade exerted by nifedipine plus SNX482 (0.3 μM) and by ω-conotoxin GVIA plus SNX482. In contrast, blockade exerted by combined ω-agatoxin IVA plus SNX482 (about 50% of the whole cell) did not show additivity. At 0.3 μM and higher concentrations, SNX482 delayed the inactivation of I_{Na}. The time constant (τ) for inactivation of I_{Na} in control conditions doubled in the presence of 0.5 μM SNX482. At 0.3 μM, SNX482 did not affect I_K. Our data demonstrate that: (i) SNX482 selectively blocks P/Q Ca^{2+} channels at submicromolar concentrations; (ii) the toxin partially blocks Na^+ channels; (iii) SNX482 delays the inactivation of Na^+ channels. These results reveal novel properties of SNX482 and cast doubts on the claimed selectivity and specificity of the toxin to block the R-type Ca^{2+} channel.

Keywords: Ca^{2+} current; Na^+ current; Ion channel; Toxin; Catecholamine cell

1. Introduction

Various toxins isolated from natural venom extracts have been fundamental for the pharmacological separation and characterisation of Ca^{2+} channel subtypes. Furthermore, these toxins have also been essential for the elucidation of the role of L-, N- and P/Q-types of Ca^{2+} channels in the control of Ca^{2+}-dependent cell functions (Olivera et al., 1994; García et al., 2000).

A toxin-resistant R-type channel was first described electrophysiologically (Randall and Tsien, 1995). Several subsequent reports have depicted this R-type current in various neuronal cell types (Magnelli et al., 1998; Neelands et al., 2000; Tottene et al., 2000; Wilson et al., 2000). When expressed, the cloned α_{1E} Ca^{2+} channel possesses electrophysiological and pharmacological properties similar to the native R channel, i.e. activation at higher transmembrane voltages and resistance to agents that block N, P/Q and L Ca^{2+} currents (Forti et al., 1994; Williams et al., 1994; Albillos et al., 2000). This similarity was strengthened by the finding that antisense oligonucleotides against α_{1E} channels produced a partial reduction of R-type currents in cerebellar granule cells (Piedras-Rentería and Tsien, 1998). However, the lack of a toxin to block selectively R-type channels prevented studies to clarify their functional role. In 1998, Newcomb et al. described the first selective R channel blocker, SNX482, a 41 amino acids peptide purified from the venom of an African tarantula, *Hysterocrates gigas*. This discovery opened the way to further characterisation of R-type Ca^{2+} channel functions in various cell systems.

For the last two decades, our laboratory has been studying the different Ca^{2+} entry pathways that regulate the exocytotic catecholamine release in adrenal medullary chromaffin cells. At the beginning, we thought that the secretory response was modulated mostly by L-type Ca^{2+} channels

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(Ceña et al., 1983). However, as new toxins became available to study new Ca^{2+} channel subtypes we found that as neurones (Olivera et al., 1994), chromaffin cells of various species (human, bovine, cat, rat, mouse) expressed different proportions of L, N, and P/Q subtypes of Ca^{2+} channels (see García et al., 2000 for a review). More recently, measuring the expression of mRNAs in bovine chromaffin cells we have identified an mRNA for α_{1E} channels (García-Palomero et al., 2000). Furthermore, using the perforated configuration of the patch-clamp technique we have also found a toxin-resistant component that accounts for 22% of the whole-cell current and 55% of the rapid secretory response evoked by short depolarising pulses in mouse adrenal slice chromaffin cells (Albillos et al., 2000).

These observations suggest that chromaffin cells also express R-type channels. To know the role of R channels in controlling Ca^{2+} signals and exocytosis, we thought that SNX482 could be a valuable pharmacological tool. Before getting involved in these studies, we found of interest to explore first the effects of the toxin on L, N and P/Q Ca^{2+} channels as well as on Na^{+} and K^{+} channels in bovine chromaffin cells. To our surprise, we found that SNX482 behaved as a selective blocker of P/Q channels at submicromolar concentrations. Furthermore, the toxin caused a pronounced delay of the inactivation of the Na^{+} current, leaving untouched K^{+} currents. We report here these findings.

2. Materials and methods

2.1. Isolation and culture of chromaffin cells

Bovine adrenal glands were obtained from a local slaughterhouse. Chromaffin cells were isolated by adrenal medulla digestion with collagenase, according to the classical procedure of Livett (1984) with some modifications (Moro et al., 1990). Our preparations were enriched in adrenaline-contain-

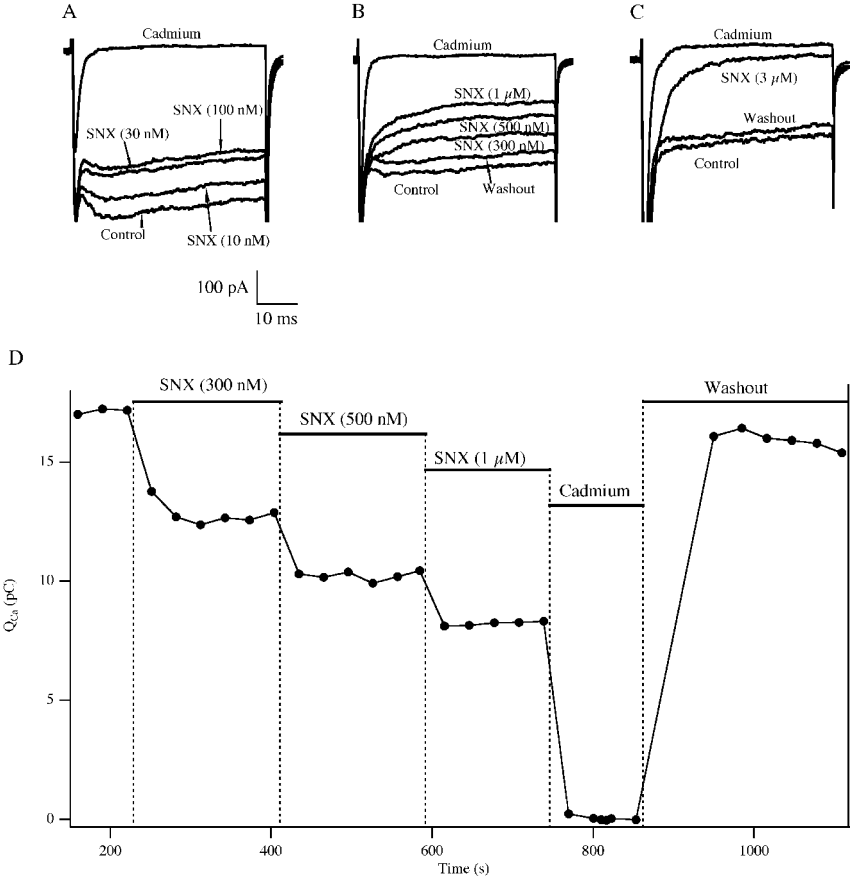

Fig. 1. Whole-cell voltage-clamp experiments performed to study the blocking effects of SNX482, on inward whole-cell Ca^{2+} channel currents (I_{Ca}) in bovine chromaffin cells. Cells voltage-clamped at −80 mV were continuously and locally superfused with an extracellular solution containing 5 mM Ca^{2+} (see Materials and methods). Depolarising 50-ms pulses to the voltage steps of 0 mV were applied at 30-s intervals. Panels A, B and C show families of current traces obtained in three different cells, and the blockade exerted by application of different concentrations of SNX482 (SNX), as indicated in each trace. Panel D shows the time course of Q_{Ca} (measured as current area; see Materials and methods) obtained with depolarising pulses to 0 mV, and the blocking effect of increasing concentrations of SNX482 on such currents. The toxin was applied during the length of time indicated by the top horizontal bars. Note that cadmium 200 μM suppressed the current and that recovered quickly after washout. The current is expressed in the ordinate as the area of each trace (pC). It was calculated in the time range 5–50 ms of the depolarising pulse.

ing cells. Cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% foetal calf serum, 10 μM cytosine arabinoside, 10 μM fluorodeoxyuridine, 50 IU ml^{-1} penicillin, and 50 μg ml^{-1} streptomycin. For electrophysiological-recording experiments, cells were plated on 1-cm diameter glass coverslips at density of 5×10^5 cells/ml^{-1}. Media were replaced by serum-free DMEM 24 h later, and then every 2 days.

2.2. Patch-clamp measurements and analysis of whole-cell currents

Ion channel currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Coverslips containing the cells were placed on an experimental chamber mounted on the stage of an up-right Olympus microscope. The chamber was continuously perfused with a control Tyrode solution containing (in mM): 145 NaCl, 5.5 KCl, 1 $MgCl_2$, 10 HEPES, 2 $CaCl_2$, 10 glucose, pH 7.3. To record Ca^{2+} currents, the extracellular solution contained (in mM): 145 NaCl, 5.5 KCl, 1 $MgCl_2$, 10 HEPES, 5 $CaCl_2$, 10 glucose, pH 7.3. In some experiments to record Na^+ currents, $CaCl_2$ was substituted by 3 mM $MgCl_2$. External solutions were exchanged using electronically driven miniature solenoid valves coupled to a multi-barrel concentration-clamp device, the common outlet of which was placed within 100 mm of the cell to be patched. The flow rate was low (0.5–1 ml min^{-1}) and regulated by gravity.

For recording of inward currents through voltage-dependent Na^+ and Ca^{2+} channels, the composition of the intracellular solution was (in mM): 10 NaCl, 100 CsCl, 20 tetraethylammonium chloride, 5 Mg·ATP, 14 EGTA, 0.3 Na·GTP and 20 HEPES, pH 7.2. For recording of outward currents through voltage-dependent K^+ channels, CsCl and tetraethylammonium chloride were substituted by 130 mM KCl.

To elicit Na^+, Ca^{2+}, and K^+ currents, step depolarisations to the voltage that generated the peak current, from a holding potential of −80 mV, were applied every 30 s. The access resistance was in the range of 4.0–8.5 MΩ. A ramp protocol was first performed to get the voltage that elicited the peak current in each case.

Electrophysiological measurements were carried out using an EPC-9 amplifier (HEKA Electronics Lambrecht/Pfalz, Germany) and PULSE software running on an Apple Macintosh. Pipettes of 2–3 MΩ resistance were pulled from borosilicate glass capillary tubes, partially coated with wax and fire polished, mounted on the headstage of the EPC-9 amplifier, allowing cancellation of capacitative transients and compensation of series resistance. Ca^{2+} current charge was calculated as the integral of the Ca^{2+} current minus the current charge in the presence of Cd^{2+} to avoid Na^+, leak and capacitative current charges. K^+ current charge was calculated as the integral of the K^+ current minus the current charge in the presence of tetraethylammonium chloride, to avoid leak and capacitative current charges.

2.3. Statistical analysis

Results are expressed as means ± S.E.M. The statistical differences between means of two experimental results were assessed by Student's *t*-test. A value of *P* equal to or smaller than 0.05 was taken as the limit of significance. The dose–response curve was fitted according to the empirical Hill equation:

$$y = y_{max}/[1 + (IC_{50}/x)^{n_H}]$$

where: y = the percentage inhibition of Q_{Ca}, y_{max} = the maximum percentage inhibition, IC_{50} = mid-point of response, x = the agonist concentration and n_H = the Hill coefficient.

To calculate the time constant (τ) for current inactivation of Na^+ channels, the recordings were fitted to a single exponential curve.

3. Results

3.1. Characteristics of the inward Ca^{2+} channel current (I_{Ca}) of bovine chromaffin cells: effects of SNX482

An *I–V* curve was initially performed in several voltage-clamped cells, in order to select the experimental conditions

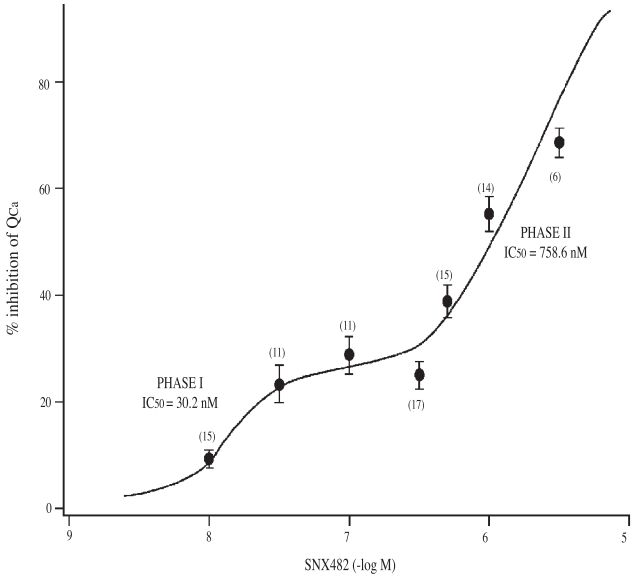

Fig. 2. Concentration–response curve for the blockade of Q_{Ca} by SNX482, in voltage-clamped bovine chromaffin cells. Pooled data were taken from experiments done according to the protocols shown in Fig. 1 (three concentrations of toxin were tested in each individual cell). Data points are means ± S.E.M. of the number of cells from at least three different cultures, shown in parentheses. Area of the current traces were calculated and normalised as % of the fraction of current blocked by each toxin concentration (see Materials and methods).

adequate to study the effects of SNX482 on I_{Ca}. Usually, the currents started to activate at −40 mV, peaked at 0 or +10 mV, and had a reversal potential at +60 mV (not shown). As a consequence, the voltage-depolarising steps used to study the effects of SXN482 in subsequent experiments were 0 or +10 mV.

Panels A, B and C in Fig. 1 show three families of I_{Ca} curves obtained in three different cells, using 5 mM Ca^{2+} as charge carrier. Once the control current stabilised, the cells were superfused for 30 s with each of three concentrations of toxin, as indicated in the traces. Cadmium (200 μM) was given after the last concentration of toxin, and then I_{Ca} were recorded again in the absence of blockers.

The traces show an initial peak current that corresponds to the Na^+ current (I_{Na}), because tetrodotoxin was not used to see the effects of SNX482 simultaneously on I_{Na} and I_{Ca}. I_{Na} tended to relax quickly and then, I_{Ca} developed. A slow tendency to suffer inactivation of I_{Ca} was observed along the 50-ms depolarising pulse. SNX482 caused a blockade of I_{Ca} in the full wide range of concentrations used (10 nM to 3 μM). The blockade was quickly reversed upon washout of the toxin, (panels B, C and D in Fig. 1); current recovery was near total in most cases.

An example of the time course of I_{Ca} blockade by SNX482, measured as current areas, is shown in the experiment in Fig. 1D (current areas are expressed in the ordinate in pC). Initially, the currents had an area of around 16.5 pC that decreased in a step-wise manner upon the sequential addition of 0.3, 0.5 and 1 μM SNX482. The time course of the blockade was faster as higher the concentration of toxin; for instance, compare how the blockade developed with 0.3 μM SNX482 and with 0.5 or 1 μM, that was completed at the first depolarising pulse applied after giving the toxin.

Fig. 2 shows the concentration–inhibition relationship for the blockade of Q_{Ca} elicited by increasing concentra-

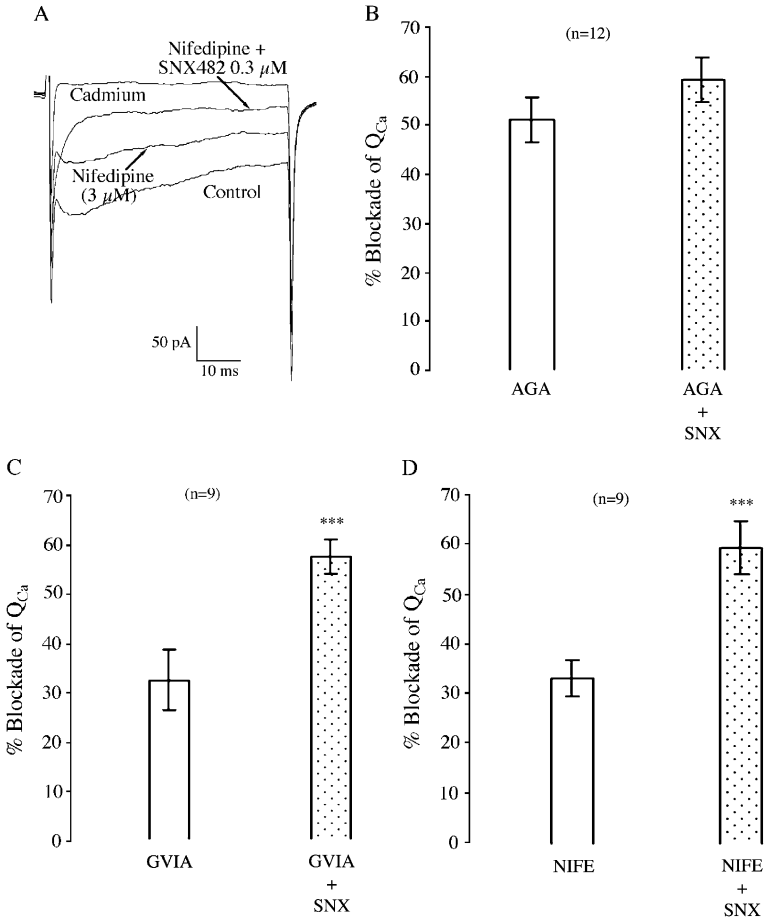

Fig. 3. Occlusion experiments performed to study the effects of SNX482 on I_{Ca}, when added on top of selective blockers for Ca^{2+} channel subtypes. Currents were elicited in voltage-clamped cells (−80 mV), at 30-s intervals. Panel A shows the original traces obtained in a prototype cell. Control current was obtained in the absence of blocker. The trace labelled "Nifedipine" was obtained 30 s after local superfusion of the cell with 3-μM nifedipine. Then, the trace "Nifedipine + SNX482" was obtained 30 s after superfusion with 3 μM nifedipine and 0.3 μM SNX482. The last trace, labelled "Cadmium", was obtained 30 s after superfusion with 200 μM cadmium. Panel D shows pooled results of nine cells, using this protocol. Panel C shows the % blockade of Q_{Ca} elicited by ω-conotoxin GVIA alone (GVIA, 1 μM, 30 s) or combined GVIA plus SNX (0.3 μM). Panel B shows the % blockade of Q_{Ca} elicited by ω-agatoxin IVA alone (AGA, 1 μM, 30 s) or combined AGA plus SNX (0.3 μM for 30 s). Data are means ± S.E.M. of the number of cells shown in parentheses, taken from at least three different cultures. ***$P < 0.001$, compared with blockade produced in the absence of SNX482.

tions of SNX482. Phase I relates to a current component that is sensitive to nanomolar concentrations of SNX482; phase II belongs to a current component carried by Ca^{2+} channel subtypes that are blocked by micromolar concentrations of the toxin. The application of the Hill equation to the two components of the curve provided values of IC_{50}s of 30.2 and 758.6 nM, with n_H coefficients of 1.72 and 1.73 for the high and the low affinity phases, respectively. The phase II of the curve suggests that SNX482 might eventually block all the current at concentrations of 10 μM and above; however, these experiments were economically prohibitive.

3.2. Occlusion experiments performed with the combination of SNX482 with selective blockers of Ca^{2+} channel subtypes

The idea behind these occlusion experiments rested in knowing whether SNX482 blocked selectively some of the main three Ca^{2+} channel subtypes expressed by bovine chromaffin cells. Hence, cells were first superfused with a supramaximal concentration of a given blocker (1 μM ω-agatoxin IVA to block P/Q channels, 1 μM ω-conotoxin GVIA to block N channels, and 3 μM nifedipine to block L channels of bovine chromaffin cells; García et al., 2000). Then, on top of each channel blocker, SNX482 was added to test whether an additional blockade was obtained; 0.3 μM of toxin was used since this was the concentration separating phase I from phase II of the concentration–inhibition curve for SNX482 (Fig. 2).

Fig. 3A shows an example of one of these occlusion experiments. To a cell voltage-clamped at −80 mV, depolarising pulses to 0 mV were applied at 30 s intervals to elicit inward I_{Ca}, as in Fig. 1. After 30 s of superfusion with nifedipine, the control Q_{Ca} was reduced by 34%. Combined nifedipine plus SNX482 reduced further I_{Ca}, suggesting a blockade of different current fractions. Pooled experiments performed in nine cells showed that nifedipine blocked Q_{Ca} by 33.9 ± 3.6%; with the combination of nifedipine plus SNX482 the blockade rose to 60.2 ± 5.3% (Fig. 3D).

Fig. 3C shows an experiment performed in cells that were sequentially superfused first with ω-conotoxin GVIA that, after 30 s of superfusion blocked a fraction of current of 34.5 ± 4.1%. Subsequently, the same cells were superfused with combined ω-conotoxin GVIA plus SNX482; now, the fraction of current blocked increased to 61 ± 6.4%. Fig. 3B shows the blockade of Q_{Ca} exerted by ω-agatoxin IVA alone (50.8 ± 4.6%) or by the combination of ω-agatoxin IVA plus SNX482 (59 ± 4.5%). These occlusion experiments suggest that ω-agatoxin IVA and SNX482 are blocking the same current component; however, nifedipine and ω-conotoxin GVIA are blocking current components that are not targeted by 0.3 μM SNX482.

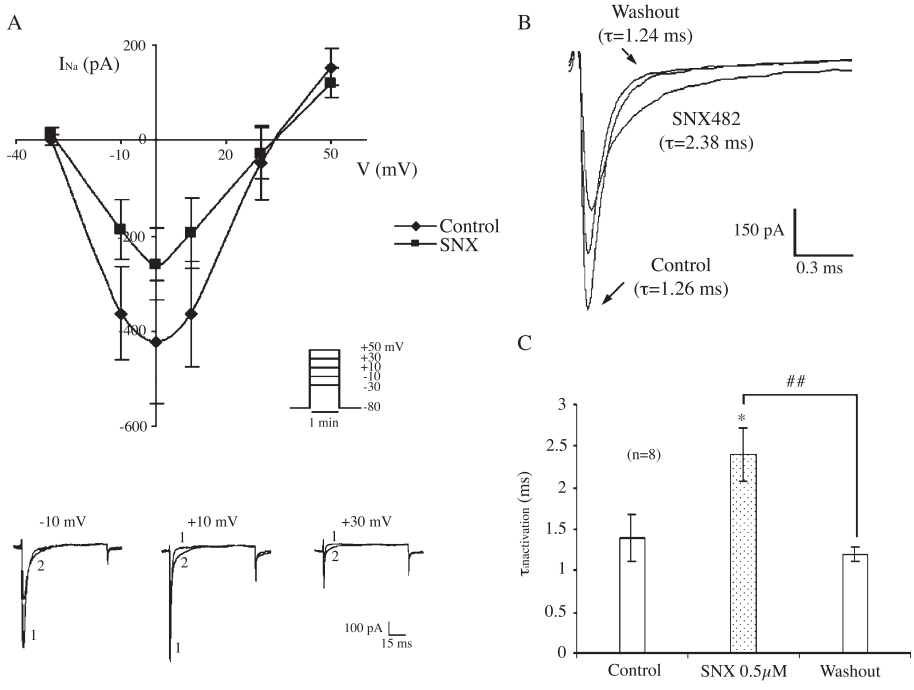

Fig. 4. Effects of SNX482 on I_{Na}. In top panel A, a current–voltage relationship was elicited by a voltage-step protocol in the absence (Control) and presence of SNX482 (0.5 μM). This protocol delivered a series of 1-min voltage steps ranging from −30 to +50 mV from a holding potential of −80 mV at 20-mV increments. Data were pooled from seven cells; they are means ± S.E.M. Bottom traces show the blockade and the inactivation effect, at three different potentials, of SNX482 (traces 2) on control I_{Na} (traces 1). In panel B, the traces obtained with the pulse to 0 mV are shown. The time scale was expanded to see better the inactivation phase of I_{Na}. Such inactivation was fitted to a single exponential, with calculated τ of 1.26 ms (control) and 2.38 ms (SNX482). Panel C shows pooled data of the inactivation τ (ordinate) obtained before, during and after washout of SNX482, using the protocol of panel B (inset); they are means ± S.E.M. of eight cells from at least three different cultures. *$P<0.05$ compared with control; ##$P<0.01$ compared with wash out.

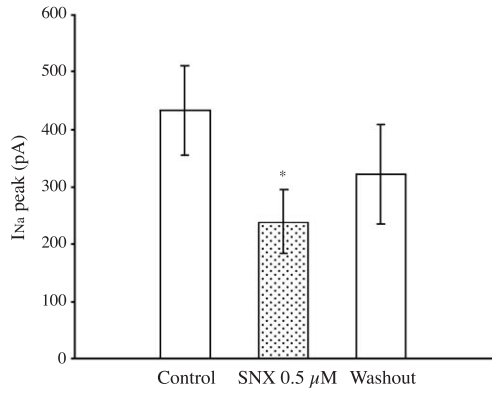

Fig. 5. Blockade of 0.5 μM SNX482 on the peak Na^+ current in cells voltage-clamped at −80 mV. The figure shows pooled data of the I_{Na} inhibition using the protocol in Fig. 4B; they are means ± S.E.M. of 13 cells (Control and SNX482 0.5 μM) and eight cells (Washout) from at least three different cultures. *$P<0.05$ compared with control.

3.3. Effects of SNX482 on sodium currents (I_{Na})

In some of the pioneering experiments of this study, we observed that the inactivation of I_{Na} seemed to slow down in the presence of SNX482. This was particularly visible when using the highest concentration of SNX482, 3 μM, as shown in Fig. 1C, which did not modify the inactivation of I_{Ca} (Fig. 1A,B,C).

Hence, it seems that the effect of the toxin on current inactivation is due to Na^+ channels. To reinforce this view, an experiment was done in the absence of external Ca^{2+} (nominal 0 Ca^{2+}, about 10–20 μM Ca^{2+}; 3 mM Mg^{2+} was added to this extracellular solution), to suppress the Ca^{2+} channel current component in the recorded overall current. Fig. 4A shows current–voltage relationship of Na^+ channel currents obtained with a voltage-step from −30 to +50 mV in the presence and the absence of SNX482 (0.5 μM). In the absence of SNX482 (Control), I_{Na} began to activate at potentials around −30 mV, reached maximal amplitude at 0 mV and reversed at about +35 mV. These parameters were not significantly changed by SNX482, indicating that the toxin did not affect the current–voltage relationship of Na^+ channel currents.

To quantitate the delay of I_{Na} inactivation caused by SNX482, experiments following the protocol in Fig. 4B were made. Cells were voltage-clamped at −80 mV and 50 ms depolarising pulses to 0 mV were applied at 30-s intervals before, during and after washout of the toxin. In this example, the inactivation constant for the control I_{Na} was 1.26 ms; in the presence of SNX482 τ rose to 2.38 ms. After washout, τ recovered its control initial value, 1.24 ms. Pooled results from eight cells are shown in Fig. 4C. SNX482 reversibly increased the τ for the inactivation of I_{Na} by 53.3%.

SNX482 (0.5 μM) had an additional effect on I_{Na}. As shown in Fig. 4A and B, the peak I_{Na} was reduced by 45% in the presence of SNX482. The blockade was reversible upon washout of the toxin. In eight cells (Fig. 5), I_{Na} peak was reduced by 45.5 ± 7.0%. After washout, the current recovered only partially (64.1 ± 6.4% of the initial peak I_{Na}).

3.4. Effects of SNX482 on K^+ currents (I_K)

These experiments were performed to test the possibility that SNX482 was also affecting K^+ channel currents. Fig. 6A shows I_K traces obtained following the protocol shown on top. The control outward I_K (trace 1) was obtained after current stabilisation. After 30 s of cell superfusion with 0.3 μM SNX482, I_K was slightly decreased (trace 2). After washout, I_K recovered its initial amplitude. Tetraethylammonium chloride (20 mM) drastically reduced the current (not shown).

Fig. 6B shows the results of 11 pooled cells. The areas of I_K were calculated and expressed in μC (ordinate). Note that

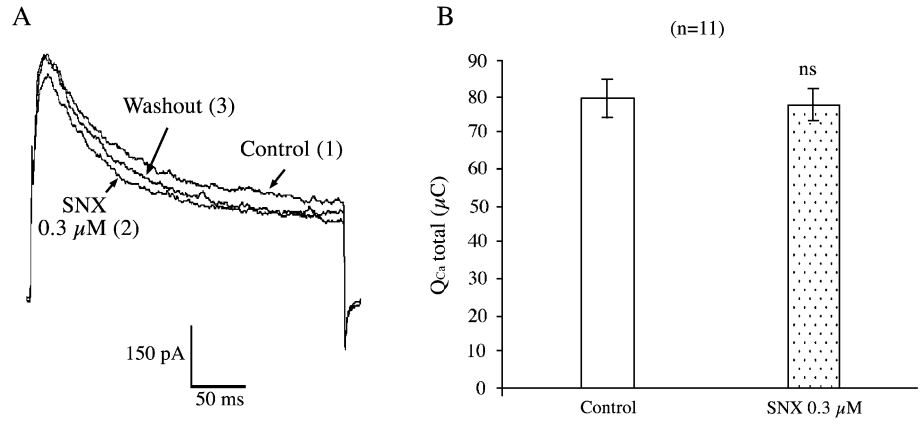

Fig. 6. SNX482 is not affecting the outward K^+ currents (I_K) of chromaffin cells. Panel A shows I_K traces obtained in a cell voltage-clamped at −80 mV and stimulated with pulses to +70 mV applied at 30-s intervals. Trace 1 (control) was obtained once the I_K stabilised after braking into the cell with the patch pipette. Trace 2 was obtained after 30 s of cell superfusion with 0.3 μM SNX482. Trace 3 was obtained after 30-s washout. Panel B shows pooled data on I_K areas (expressed in μC in the ordinate), averaged from experiments using the protocol of panel A; data are means ± S.E.M. of 11 cells.

the K^+ currents were slightly, but nonsignificantly reduced by SNX482.

4. Discussion

We have discovered in this study that SNX482, that is reputed to behave as a selective blocker of R-type Ca^{2+} channels (Newcomb et al., 1998), is also blocking other Ca^{2+} channel subtypes as well as Na^+ channels; additionally, the toxin is delaying the inactivation of I_{Na}. Our data cause surprise if consideration is made that at concentrations behaving as a selective R-type Ca^{2+} channel blocker, SNX482 is a quickly reversible P/Q Ca^{2+} channel blocker, leaving unblocked L- as well as N-type Ca^{2+} channels.

The *Conus magus* toxin ω-conotoxin MVIIC (Hylliard et al., 1992) and the *Agelenopsis aperta* spider toxin ω-agatoxin IVA (Mintz et al., 1992) have been widely used for the last decade as "irreversible" blockers of P/Q Ca^{2+} channels (Olivera et al., 1994; García et al., 2000). We have seen here that at submicromolar concentrations, SNX482 blocks the fraction of current targeted by ω-agatoxin IVA. Contrary to the blockade exerted by ω-agatoxin IVA in bovine chromaffin cells that is long-lasting (Albillos et al., 1993, 1996), the blockade of this current fraction by SNX482 was quickly reversible. Also, the fraction of Ca^{2+} channel current blocked by ω-conotoxin MVIIC in bovine chromaffin cells (about 60%) is long lasting (Albillos et al., 1996; Gandía et al., 1997); in addition, ω-conotoxin MVIIC also blocks the N-type current component of bovine chromaffin cells (López et al., 1994; Gandía et al., 1997). In contrast to ω-conotoxin MVIIC, the occlusion experiments presented here show that SNX482 did not block the N-component of Ca^{2+} currents in these cells. Thus, SNX482 seems to be a more selective blocker of the P/Q-type of Ca^{2+} channels, as compared to ω-conotoxin MVIIC. Furthermore, the rapid reversibility of its blockade makes SNX482 a tool that may be potentially more useful than ω-agatoxin IVA and ω-conotoxin MVIIC, to study the role of P/Q Ca^{2+} channels in controlling exocytosis and other Ca^{2+}-dependent functions of bovine chromaffin cells.

Our results in bovine chromaffin cells differ from those reported by Newcomb et al. (1998), showing: (i) a 5–10% blockade of I_{Ba} by 250 nM SNX482, in oocytes expressing α_{1A} Ca^{2+} channels; and (ii) less than 10% blockade of $^{45}Ca^{2+}$ uptake into cortical synaptosomes by 0.3 μM SNX482. They also differ from the results of Neelands et al. (2000) showing that 1.1 μM SNX482 have small effects on N and P/Q Ca^{2+} channels of human NT2-N teratocarcinoma cells. These differences might be due to the expression of different subtypes of P/Q Ca^{2+} channels in various excitable cells. For instance, we compared the binding of [^{125}I]ω-conotoxin MVIIC to bovine adrenal medullary membranes and to bovine brain membranes; we observed that ω-conotoxins MVIIC and MVIID have a high-affinity binding site in chromaffin cell membranes distinct to that found in the brain. On the basis of these and other functional experiments, we concluded that P/Q Ca^{2+} channels of bovine chromaffin cells might represent a subset of Q-type Ca^{2+} channels, or an entirely new subtype of voltage-dependent high-threshold Ca^{2+} channel (Gandía et al., 1997). In any case, caution should be exerted in studies that use SNX482 to characterise R-type Ca^{2+} channels, such as those of Wilson et al. (2000) and Tottene et al. (2000).

In the original report of Newcomb et al. (1998), where the SNX482 properties on ion channels were first described, it was stated that 0.5 μM of the toxin had no effects on Na^+ currents in retinal ganglion cells maintained in primary cultures (less than 4% blockade of peak current). We, however, have observed two effects of SNX482 on Na^+ channels of bovine chromaffin cells: at 0.5 μM the toxin halved the peak I_{Na}, and at 0.3–0.5 μM the toxin delayed the inactivation of I_{Na}. These effects might prove of value when using SNX482 as a probe to explore Na^+ channel properties in excitable cells. We found particularly interesting the slowing of I_{Na} inactivation caused by SNX482. This effect might be related to that of the alkaloid veratridine that increases the Na^+ permeability of cells by shifting the voltage-dependence for activation of Na^+ channels toward more negative values, and by decreasing their rate of inactivation (Ohta et al., 1973). We discovered an interesting synergism between veratridine and a toxin-γ purified from the Brazilian scorpion venom *Tityus serrulatus*. Toxin-γ shifted the current–voltage curve for Na^+ channel current to the left. The combination of toxin-γ with veratridine increased the shift of the $I–V$ curve to the left, resulting in a greater recruitment of Na^+ channels at more hyperpolarising potentials. This led to an enhanced and more rapid accumulation of Na^+ into bovine chromaffin cells, causing cell depolarisation, the opening of voltage-dependent Ca^{2+} channels, Ca^{2+} entry and catecholamine release (Conceiçao et al., 1998). It will be interesting to test the effects of SNX482, alone or combined with veratridine or toxin-γ, on those parameters in chromaffin cells. SNX482 might exert unexpected actions derived on its property to delay the inactivation of Na^+ channels.

In conclusion, SNX482 reveals as a new effective toxin to block selectively the high-voltage activated P/Q-type of Ca^{2+} channels in a readily reversible manner. In addition, the toxin delays the inactivation of Na^+ channels. These properties make SNX482 a potential tool to explore further the intricacies of the stimulus-secretion coupling process in chromaffin and other neurosecretory cell types.

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