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Identification of a cationic channel in synaptosomal membranes

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Abstract. Synaptosomal membranes were fused with liposomes using the 'hydration technique' to produce giant proteoliposomes amenable to patch clamp recordings. Single channel currents of a cationic channel with particular properties were detected. In a solution of 150 m*M* NaCl, the channel displayed a unit conductance of 136 pS and a mean open state lifetime of 1.1 ms. The gating of the channel was shown to be voltage as well as calcium dependent. Pharmacological studies revealed that the channel was insensitive to a variety of channel blockers, but was inactivated by ruthenium red. Presumably, this channel may play a role in regulating the evoked release of neurotransmitters.

Key words: Synaptosomes – Channel – Proteoliposomes – Patch clamp

Introduction

The nerve terminal is a specialized region of the neuron, releasing neurotransmitter upon activation by an electrical signal carried by the axon. Decades of research have established the following scheme for the electrical secretion coupling. In response to depolarisation of the presynaptic membrane, voltage-dependent Ca^{2+}-channels are activated and mediate the influx of Ca^{2+}-ions, which in turn trigger the exocytotic release of neurotransmitters from synaptic vesicles (Reichardt and Kelly 1983; Augustine 1987). For the most part, transmitter release appears to be regulated by Ca^{2+} entry and removal. Ca^{2+}-ions enter the cytosol through channels that are controlled by the membrane potential, which in turn reflects the conductance of channels that are selective for other ions, most notably sodium and potassium. To understand the regulation of transmitter release, therefore, it is most important to examine the regulation of ionic conductance in the nerve terminals. However, direct electrical recordings from nerve terminals are not feasible because of the small size and accessibility problems.

Synaptosomes, pinched-off nerve ending particles (Gray and Whittaker 1962), have greatly improved our knowledge of biochemical aspects of presynaptic function (Whittaker 1984). Isolated nerve endings have been used widely to determine membrane potentials of and ion fluxes across synaptosomal membranes (Blaustein and Goldring 1975; Nachshen and Blaustein 1980). Unfortunately, these structures are too small (≤ 1 μm diameter) to be penetrated with microelectrodes; the minute size of synaptosomes also prevented measurements of single channel currents by means of patch clamp techniques. The first microelectrode recordings were achieved when 'giant synaptosomes' were produced by fusing isolated nerve endings with red blood cells (Umbach et al. 1984). However, as these particles contain channels from both cells, they were not analysed with patch clamp approaches. Single channel currents in synaptosomal membranes have recently been recorded using a planar lipid membrane reconstituted by fusing synaptosomes into the phospholipid layer (Krueger et al. 1983; Nelson et al. 1986). However, the application of standard patch clamp techniques is supposed to allow measurements with higher time resolution and higher signal-to-noise ratio.

The advent of a novel fusion technique (Criado and Keller 1987) allows to produce giant proteoliposomes suitable for studies with the patch clamp technique. Using the dehydration-rehydration procedure, membranes can be assayed for channel activity which are otherwise not readily accessible for patch clamp approaches (Keller et al. 1988; Hirashima and Kirino 1988; Berrier et al. 1989).

In the present study, we have applied this new method to form giant proteoliposomes from insect synaptosomes and have examined a characteristic cation channel using patch clamp techniques.

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Materials and methods

Materials

All salts and solvents used were at least p.a. grade. Water was double quartz destilled. Asolectin (type II-S) was from Sigma; before use it was partially purified by the method described by Cook et al. (1986). Phenymethylsulfonylfluoride (PMSF), tetrodotoxin (TTX) and apamine were purchased from Sigma. Ruthenium red was purchased from Aldrich-Chemie (Steinheim), ryanodine, Bay K 8644 and verapamil from Calbiochem.

Preparation of synaptosomal membranes

Synaptosomes from the nervous tissue of *Locusta migratoria* were prepared as described previously (Breer 1981). Briefly, ganglia were prepared from cold-anaesthetized locusts and subsequently homogenized in isotonic buffered sucrose in a cooled microhomogenizer. The homogenate was differentially centrifuged and the suspended 15 000 *g* pellet (P_2) mixed with Ficoll solution. During centrifugation in microtubes at 10 000 *g* for 40 min, synaptosomes accumulated in the floating pellicle (P_L) whereas mitochondria were concentrated in the pellet fraction (P_H). Membrane vesicles were produced from synaptosomes as described previously (Breer and Lueken 1983). Synaptosomes were lysed in hypo-osmotic medium (1 m*M* EDTA, 5 m*M* *Tris*-HCl pH 7.4) and the suspension was centrifuged at 27 000 *g* for 20 min. The resulting pellet was resuspended in 1 m*M* $MgSO_4$, 50 m*M* sucrose, 0.1 *M* K^+-phosphate pH 7.4; after stirring for 30 min, the suspension was pelleted again (20 min, 27 000 *g*) and resuspended in hypo-osmotic medium. After repeating the lysis, revesiculation and centrifugation steps, the final pellet was resuspended in saline buffer (150 m*M* NaCl, 1 m*M* $CaCl_2$, 10 m*M* *Tris*-HCl pH 7.4). Freezing the suspension in liquid nitrogen produced no observable changes in subsequent electrophysiological measurements.

Formation of giant proteoliposomes

Three different reconstitution techniques were used to produce large proteoliposomes, accessible for patch-clamp analysis.

Dehydration–rehyration

Formation of proteoliposomes was performed in the cold (4 °C) using the dehydration–rehydration procedure (Criado and Keller 1987; Keller et al. 1988). Briefly, asolectin (from soybean, type II-S) was sonicated for 5 min at 100 mg/ml in 20 m*M* *Tris*-HCl, pH 7.4 containing 100 m*M* NaCl to yield small multilamellar liposomes. Synaptosomal membranes, prepared as described above, were resuspended in a medium containing 20 m*M* MOPS, pH 7.5, 150 m*M* NaCl, 0.1 m*M* PMSF, 5% glycerol, 0.08% asolectin and 1% CHAPS and mixed for 5 min (2 mg protein/ml). The suspension was centrifuged for 15 min at maximal speed in an Airfuge, and the supernatant combined with 0.2 vol of the liposome suspension. The suspension of liposomes and synaptosomal membranes was dialysed for 12 h against 100 m*M* NaCl, 1 m*M* $CaCl_2$, 20 m*M* *Tris*-HCl, pH 7.4 and subsequently for 24 h against 100 m*M* NaCl, 20 m*M* *Tris*-HCl, pH 7.4. The resulting liposomes were centrifuged for 60 min in the airfuge and the pellet resuspended in 10–20 µl of 10 m*M* MOPS, pH 7.4 containing 5% ethylene glycol. The suspension was deposited on a glass slide and dehydrated in a desiccator with $CaCl_2$ for 3 h at 4 °C. The partially dehydrated film was covered with saline buffer and incubated in a wet chamber overnight at 4 °C. Giant proteoliposomes (5–100 µm) were usually formed within a few hours.

Freeze-thaw liposomes

Giant liposomes were produced according to the procedure described by Tank et al. (1982). A 1 : 1 mixture of liposomes (20 mg/ml lipid) and synaptosomal membranes (see above) was frozen rapidly and stored in liquid nitrogen. The frozen sample was thawed on ice and frozen again. This cycle was repeated three times.

Liposome formation on glass beads

Large liposomes were produced at 4 °C without detergents from films of dried lipid using a procedure as described (Cohen et al. 1979; Schindler and Quast 1980). 10 mg of lipid was dissolved in 300 µl of chloroform in a round bottom flask, the mixture was dried under nitrogen using a rotary evaporator. After addition of 100 µl suspension of synaptosomal membranes (0.5 mg protein) and 400 µl saline buffer (supplemented with 50 m*M* sucrose) along with 5–10 glass beads (1–3 mm diam.), the flask was shaken for few minutes (Bangham et al. 1974). The resulting multilamellar proteoliposomes were freeze-thawed three times before the experiment.

Single channel recording

Experiments using excised patches in the inside-out configuration were performed with a standard patch-clamp set-up (Hamill et al. 1981) using a Zeiss microscope (IM 35) and a hydraulic Narishige manipulator. For patch clamp measurements of proteoliposomes, 2–5 µl of liposome suspension was dropped on a plastic tissue culture dish. The liposomes were diluted by addition of 20 µl bath solution (If not stated otherwise, bath and pipette solution contained 150 m*M* NaCl, 1 m*M* CaCl, 10 m*M* *Tris*-HCl, pH 7.4) and allowed to adhere to the culture dish. After 2–5 min, 1 ml bath solution was added and the bath intensely perfused to remove the non-settled liposome fraction. Seals with resistances between 3 and 40 Gohms were routinely obtained by using

thick-walled borosilicate glass electrodes with resistances of 5–20 Mohms. For exchange of the bath solution a perfusion system was used, which allowed the application of up to 8 different solutions to the patch. Using this equipment, different salt solutions and drug concentrations were applied to the patch.

Data analysis

A List EPC/7 amplifier was used as curent-to-voltage converter. Current fluctuation traces were stored on a PCM/video tape recorder (or FM tape recorder) and in parallel monitored on an oscilloscope. Data were later replayed from the tape, digitized using an A/D-converter and an IBM-AT computer and analysed by a single-channel analysis program.

Results

General properties

Reconstitution of synaptosomal membranes in liposomes using the dehydration/rehydration procedure as described by Criado et al. (1987) led to the formation of giant proteoliposomes (diameters of ≥ 10 μm); after osmotic swelling the particles occassionally showed 'bleb'-like structures protruding from the surface. Such giant vesicles attached to the bottom of a plastic dish were approached with a patch-pipette. Using techniques developed for recording of single channels in cell membrane patches (Hamill et al. 1981) high resistance 'giga seals' were obtained, especially on the protruding bleb-like regions of the liposomes. Discrete unit conductance changes were recorded in isolated patches (Fig. 1 a). The fluctuations represent the opening and closing of an individual reconstituted ion channel. When a holding potential of -60 mV was applied to the pipette interior, with the patch separating symmetrical salt solutions, the fluctuation showed a main current level of about 9 pA.

Conductance of the channel

The current-voltage relationship for the channel is shown in Fig. 2; it was linear over the entire voltage range, indicating an ohmic behavior of the channel current. Linear regression analysis of the data was performed to determine the slope of the straight line, which leads to a single channel conductance of 150 pS. Determining the current-voltage relation of 42 channels with similar properties gave a mean conductance value of 136 ± 24 pS. The conductance of the channel was determined in synaptosomal membranes reconstituted by different procedures in the presence of different anions (Table 1). Under all the various conditions very similar conductance values were estimated.

Figure 1 a shows a long, continuous trace showing the channel fluctuations recorded at $U = -60$ mV. It is evident that besides the predominant conductance of about

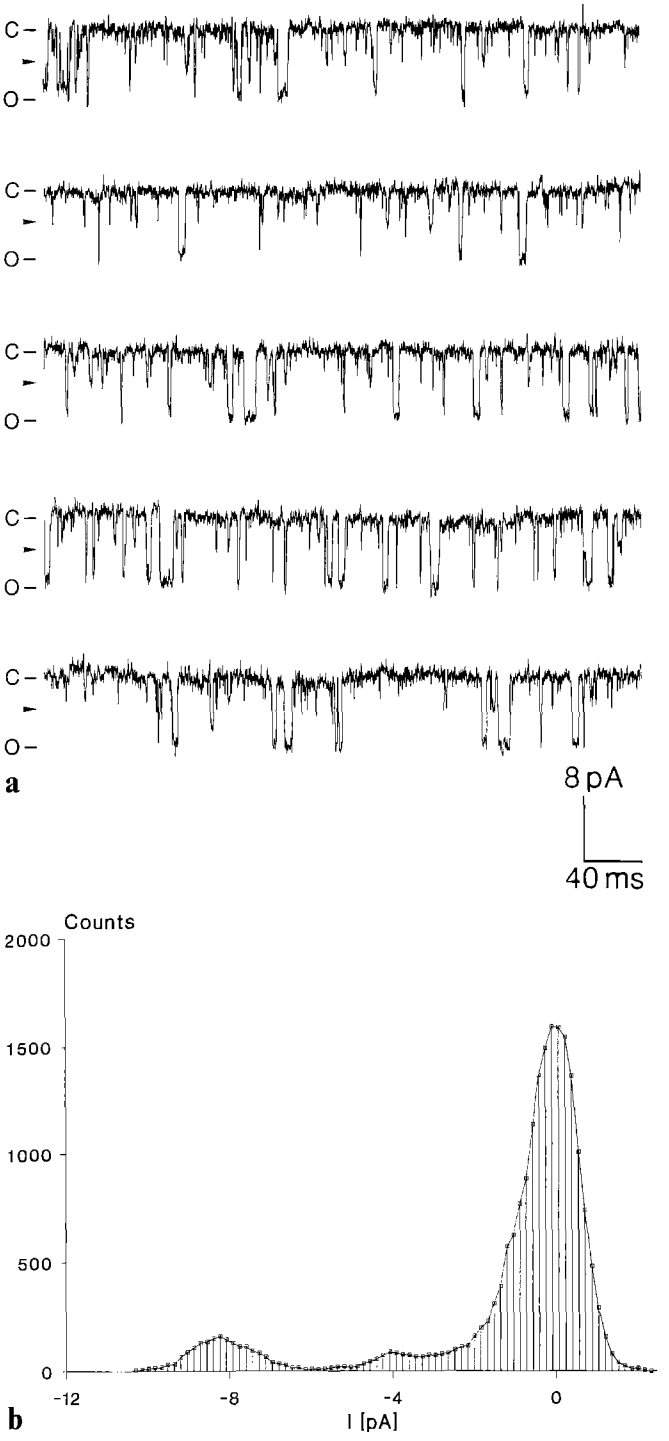

Fig. 1. a Recordings of unitary currents from synaptosomal membranes reconstituted into giant liposomes using the dehydration–rehydration technique (Keller et al. 1988). Ionic currents were measured in the excised patch configuration under voltage clamp conditions. Steplike currents between two discrete current levels correspond to the open state (o) and closed state (c) of the channel. The trace demonstrates the relative homogeneity of the channel fluctuation pattern; besides the main conductance of 150 pS, a substate of about 70 pS can be detected. The patch holding potential was -60 mV (recording time, $t = 2$ s; 3 kHz filtering). **b** Amplitude histogram of current fluctuations shown in Fig. 1 a; sampling rate was 10 kHz and the applied voltage was -60 mV. The histogram is mainly bimodal with a well defined closed state, $I = 0$ pA, and a well-defined open state, $I = -9$ pA; in addition the substate ($I = -4$ pA) can be seen. The mean probability of the channel being in the open state is $P_o = 0.1$

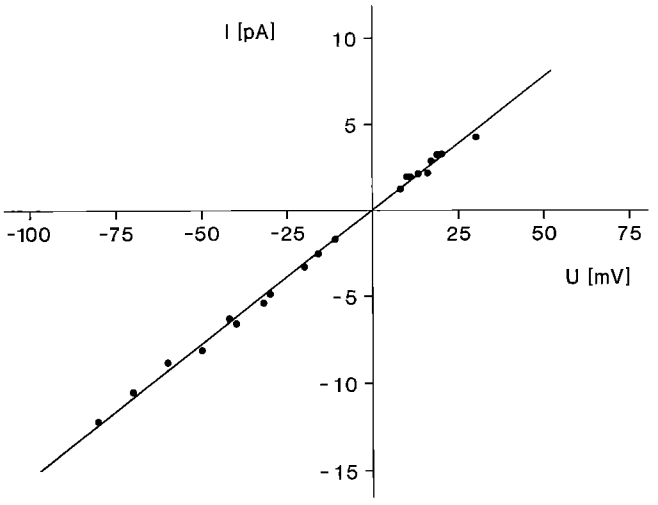

Fig. 2. Single channel current as a function of membrane potential. The $I-V$ characteristic is linear over the whole voltage range investigated, giving a single channel conductance of 150 pS for the given example

Table 1. Conductance of an ion channel in synaptosomal membranes reconstituted in giant liposomes by different techniques. Giant proteoliposomes were prepared from synaptosomal membranes and asolectin-liposomes using either the dehydration/rehydration method (Keller et al. 1988) or the detergent-free procedure as described by Schindler (1980)

Reconstitution procedure	Solution [150 mM]	Conductance [pS]	Experiments [n]
Dehydration/rehydration	NaCl	140 ± 37	9
Dehydration/rehydration	NaSCN	139 ± 23	22
Detergent-free	NaSCN	140 ± 26	4
Dehydration/rehydration	NaGlu	127 ± 19	8
Detergent-free	KCl	128 ± 10	3

150 pS a short-lived subconductance state (70 pS) can be distinguished. An analysis of this recording is given in Fig. 1 b, showing the amplitude histogram of closed (0 pA) and open states (−9 pA); the substate can also be identified in this histogram (−4 pA). The Gaussian fit routines revealed a probability of the channel being in the open state (P_0) of 0.1. The lifetime distribution of the closed state of the channel is presented in Fig. 3 a as a linear (top) and half-logarithmic plot (bottom); for the closed state a double-exponential decay is observed, giving mean lifetime constants $\tau_{c1} = 0.26$ and $\tau_{c2} = 3.2$ ms. The lifetime distribution of the open state of the channel is shown in Fig. 3 b. The lifetimes are clearly single-exponentially distributed, giving a mean open state lifetime of $\tau_0 = 1.1$ ms.

Ion selectivity

The ion selectivity of the channel was determined by the conductance ratios ($Na^+ = 1$) and the reversal potentials for asymetric salt conditions. As shown in Fig. 4 the channel is almost equally well permeable for the different alkali cations (measured with 150 mM monovalent salt) and also highly conductive for Ca^{2+}-ions (estimated for 75 mM Ca^{2+}); however it was only slightly permeable for anions; the permeability was 0.3 ± 0.1 for Cl^- and < 0.1 for glutamate, as derived from reversal potentials (see also Table 1). Thus, the channel may be described as a general cation channel.

Voltage dependence of the channel kinetics

To demonstrate the voltage dependence of the channel kinetics, four traces of single channel fluctuations at different voltages are presented in Fig. 5. Traces of single channel fluctuations at different membrane potentials are displayed; the applied potential and the closed state are marked for each trace. The probability of the channel being in the open state increases as the membrane is depolarized. This result is even more clearly demonstrated by the integral histograms for the fluctuations at different membrane potentials. At positive potentials the open probability of the channel was close to one.

A more quantitative analysis of the channel activity at different membrane potentials is given in Fig. 6 a. The probability of the channel being in the open state (P_0) is plotted as a function of the applied membrane potential. The channel is mainly closed at potentials ≤ -80 mV and most sensitive to voltage between −60 mV and −20 mV. At more positive membrane potentials the open probability approaches unity. The P_0 versus voltage relationship can be fit with a curve derived from the Boltzmann equation assuming an 'equivalent gating charge' of 2.4. The resulting voltage dependent macroscopic current is shown in Fig. 6 b; it represents the product of the open probability multiplied by the single channel current for different voltages. As can be seen, the channel mediates a depolarizing current in the voltage range between −40 mV and 0 mV.

Calcium dependence of the channel kinetics

All measurements studying the voltage dependence of the channel activity were performed in the presence of 1 mM Ca^{2+}. During the course of this study it became evident that changing the levels of $[Ca^{2+}]_i$ in inside-out experiments induced a modified behaviour of the channel. Elevated concentrations of Ca^{2+} caused a change in the open probability at a given voltage. Figure 7 illustrates the proportion of open channels as a function of membrane potential at three different concentrations of $[Ca^{2+}]_i$. At a given potential the probability of the channel being in the open state increases with an elevated Ca^{2+} concentration. The applied voltage at which P_0 equals 0.5 (the midpoint voltage of the P_0- versus V-relation) changes with increasing Ca^{2+} concentrations to more negative values.

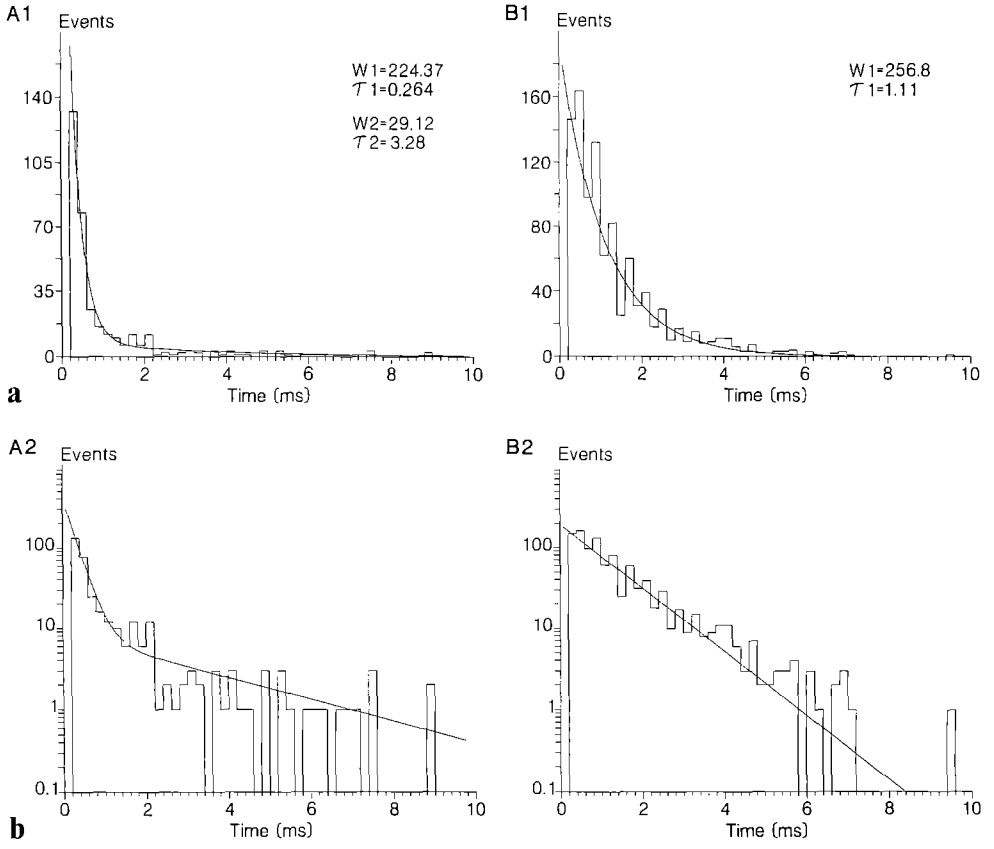

Fig. 3. **a** The lifetime distribution of the closed state of the channel is depicted in a linear (*top*) and half-logarithmic plot (bottom). The lifetimes are double-exponentially distributed giving mean lifetime constants of $\tau_{c1}=0.26$ and $\tau_{c2}=3.2$ ms. **b** The lifetime distribution of the open state of the channel in a linear (*top*) and a half-logarithmic plot (*bottom*). The lifetimes are single-exponentially distributed, giving a mean open state lifetime of $\tau_0=1.1$ ms

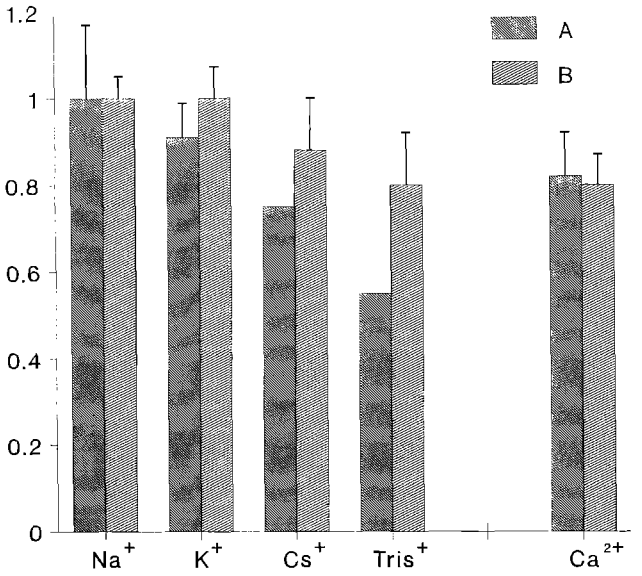

Fig. 4. Ion selectivity of the channel as determined from the conductance ratios $[Na^+=1]$ (A) and from the reversal potentials (B) for asymmetric salt conditions. Data were obtained from excised patches by perfusion of the bath with different salt solutions and are the mean of 3–10 experiments ± S.D. The ion selectivity was calculated from the reversal potentials using the Goldman-Hodgkin-Katz-equation

Blocking pharmacology of the channel

A number of drugs, including TTX, verapamil, BAY-K 8644 and apamine have been employed in order to pharmacologically characterize the cationic channel of synaptosomal membranes; none of them showed any effect in micromolar concentrations. However, the channel activity was totally blocked by 1 m*M* Co^{2+}. In a subsequent set of experiments the effect of ryanodine, previously shown to block the Ca^{2+}-channel of the sarcoplasmic reticulum, as well as the effect of ruthenium red, which was previously identified as an effective blocker of the voltage-dependent increase in synaptosomal calcium, have been analysed. The results are demonstrated as amplitude histograms showing three channels in an isolated patch (Fig. 8). In Fig. 8 a (control) the amplitude histogram of a fluctuation trace with three active channels, recorded at +50 mV in the presence of 0.5 μ*M* Ca^{2+}, is depicted. Under these conditions, the activity of the different channels resulted in three different conductance states of 6, 12 and 18 pA. As can be seen, treatment with ryanodine (10 μ*M*) did not affect the channel activity (Fig. 8 b). However, application of 100 μ*M* ruthenium red significantly changed the channel activity: Two unitary conductances are completely blocked, only one channel remains active (Fig. 8 c).

In order to minimize the possibility that reconstitution artifacts may affect the results, a number of control experiments were performed: *1.* Liposomes prepared without addition of synaptosomal membranes never showed any channel fluctuations. *2.* Dehydration–rehydration experiments using only synaptosomal membranes without exogenous lipids gave only a very limited amount of vesicular structures. Attempts to analyse those vesicles usually resulted in rather unstable seals, however, recordings demonstrated a relatively high channel density. The channel recorded showed the same voltage

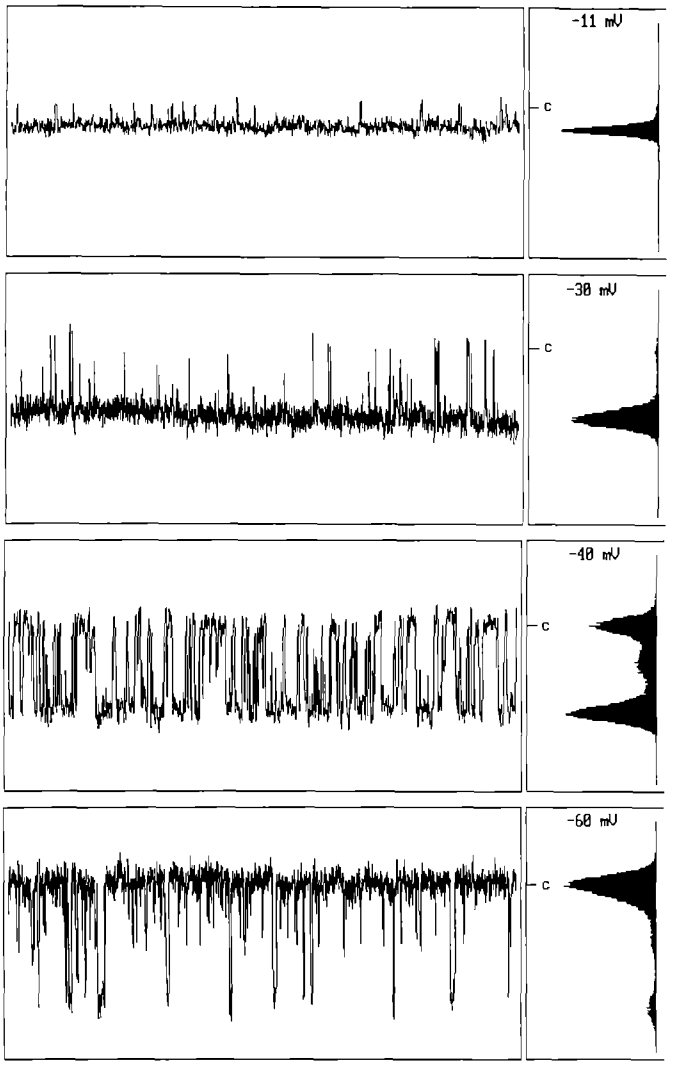

Fig. 5. Voltage dependence of the channel kinetics. Traces of fluctuations of the same channel at different membrane potentials are shown. The applied potential and the open and closed states are marked for each trace. Integral histograms for the current fluctuations at the different membrane potentials are depicted

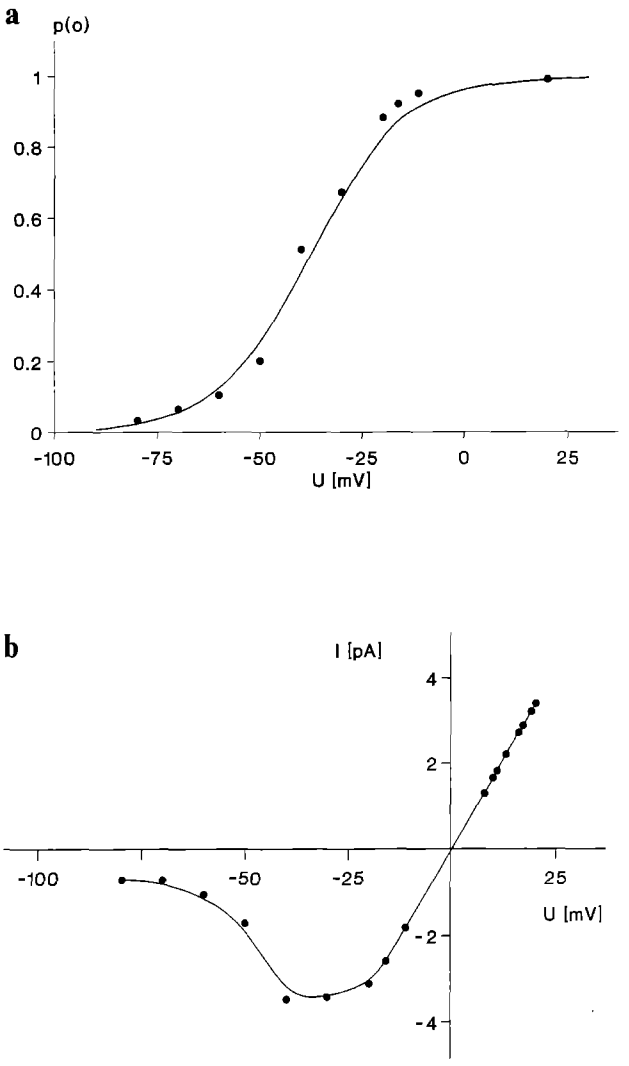

Fig. 6. **a** Open state probability (P_0) is plotted as a function of the applied membrane potential. All data derived from the same preparation. **b** Voltage dependent time averaged current deduced from the P_0-values multiplicated with the single channel current (from Fig. 2) for each voltage

dependence and kinetic behaviour as channels in proteoliposomes (see above). *3.* Analysis of proteoliposomes formed with mitochondrial membranes (P_H-fraction, see Material and methods) never showed channel activity with similar properties. *4.* When synaptosomal proteoliposomes, which were formed without detergents using the procedure of Schindler and Quast (1980) in combination with freeze-thaw-cycles, were analysed, similar channel properties were evaluated.

Discussion

The small size of presynaptic nerve terminals and their inaccessibility in situ precludes the use of conventional electrophysiological techniques for directly exploring the ion channels in the presynaptic membrane. To bypass this problem, in the present study, synaptosomal membranes from insect ganglionic neuropile were reconstituted into giant liposomes (Keller et al. 1988), which can be approached by patch pipettes and yield stable high resistance seals. An ion channel with a large unitary conductance, selectively permeable for cations, was detected. This cationic channel type was characterized on the basis of its conductance and gating properties, its sensitivity to voltage and calcium, and to some degree its pharmacology. Due to the preparation of the membranes it is not possible to say for certain from which region of the neuropile and from which region of neurons this channel was obtained. However, it is highly likely that the channel was from presynaptic nerve terminals since the subcellular fractions used have thoroughly been characterized (Breer 1981; Breer and Knipper 1985) and channels with similar properties have not been identified in other preparations. It is not immediately obvious from its properties what role this channel may play in synaptic function, however, a number of possibilities can be taken into account. Calcium channels are considered as key elements in synaptic

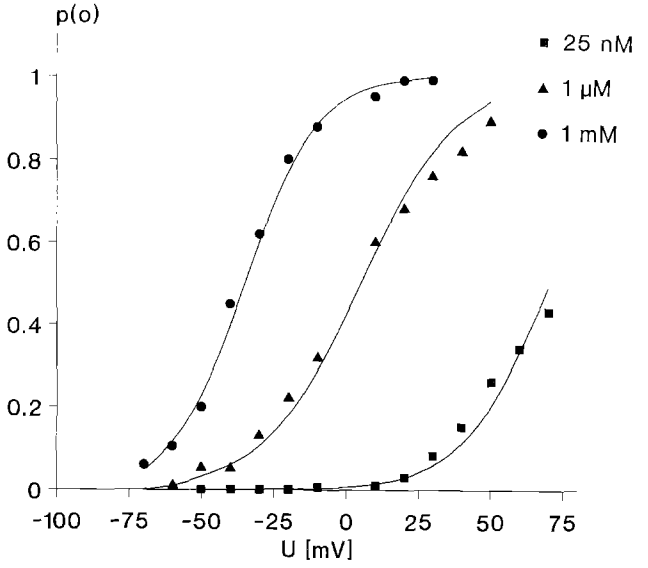

Fig. 7. Effect of calcium ions and voltage on the open state probability of the channel. P_0 is depicted as a function of membrane potential at three different Ca^{2+} concentrations. The fitted curves are derived from the Boltzmann equation

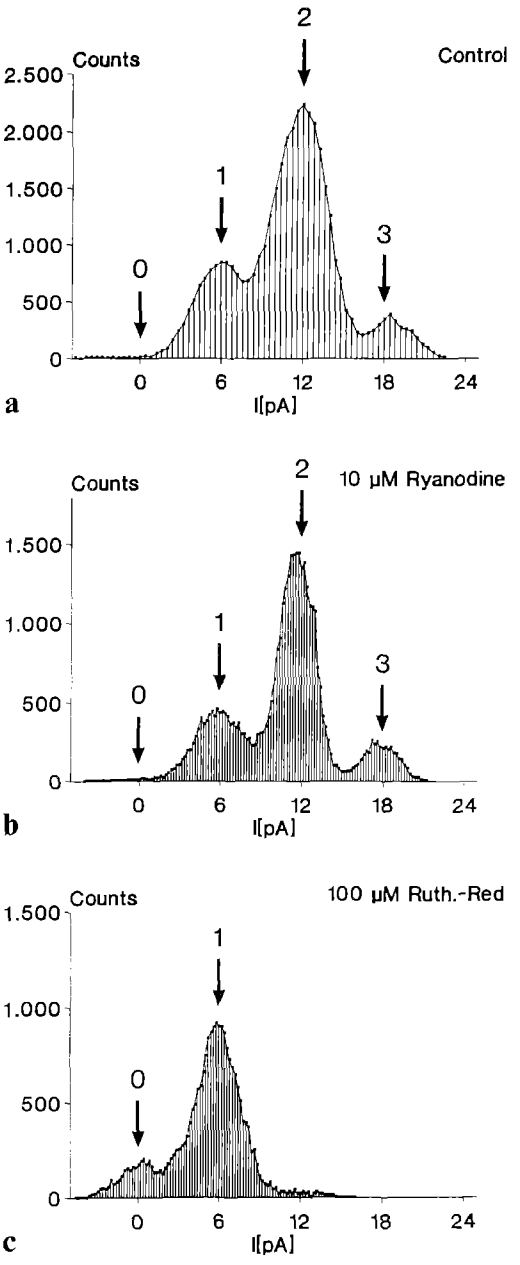

Fig. 8a–c. Amplitude histogram of a current fluctuation trace with three active channels. Recordings were performed at +50 mV in the presence of 0.5 μ*M* Ca^{2+}. **a** Control; **b** in the presence of ryanodine (10 μ*M*); **c** in the presence of ruthenium red (100 μ*M*)

functions, transducing changes in membrane potential into an influx of Ca^{2+} ions down their electrochemical gradient. A variety of ion flux studies (Blaustein et al. 1980, Suszkiw et al. 1989) as well as electrophysiological measurements (Umbach et al. 1984; Nelson et al. 1984) have established voltage gated Ca^{2+} currents through synaptosomal membranes, however the type of channel has not unequivocally been established (Augustine 1987). It is unclear if the channel characterized in this study may be involved in voltage-dependent calcium influx, but its sensitivity to ruthenium red represents an interesting feature in view of a current observation by Taipale et al. (1989). These authors noticed that micromolar concentrations of this drug prevents the evoked release of transmitter from presynaptic terminals due to a reduction of the voltage-dependent increase of cytosolic free calcium in nerve terminals. The results obtained were consistent with the notion that ruthenium red blocks a cation channel in synaptosomal membranes which is permeable for calcium as well as for sodium ions. It is interesting to note that a calcium activated cation channel, with a conductance of 213 pS in 310 m*M* KCl, has recently been detected in peptidergic nerve terminals from a crustacean neurohaemal organ (Lemos et al. 1986). This channel has been termed the 'maxi' Ca^{2+}-activated cation channel in analogy to the Ca^{2+}-activated K^+ channels.

An interesting role for an unspecific channel in exocytotic active membranes has recently been proposed by Breckenridge and Almers (1987a, b). The authors found a so-called 'fusion pore' with a mean conductance about 200 pS, which is active during early events of exocytosis in mast cells. Similar studies have not yet been possible to perform during exocytosis from presynaptic terminals, but a voltage- and calcium-gated cation channel in presynaptic membranes as described in this study may be considered as an putative functional element involved in initiation or maintenance of exocytotic release processes.

The molecular mechanisms of transmitter release from nerve terminals are still not unequivocally established (Augustine 1987). An interesting pathway, alternative to an exocytotic process, has recently emerged from molecular studies, identifying the vesicular protein p38-synaptophysin. This characteristic membrane protein of synaptic vesicles exhibits a hydrophobicity profile similar to the protein that forms the gap junction channel (Leube et al. 1987). Biophysical studies of synaptophysin, reconstituted into lipid bilayers, have shown that this protein forms voltage-dependent cation channels with a conductance of about 150 pS (Thomas et al. 1988). Based on these observations it has been proposed that the vesicular 'synaptophysin-pore' together with an equivalent element in the

presynaptic membrane, may produce a gap-junction-like channel, which in turn would mediate the release of transmitters. It will be of particular interest to evaluate the possibility that the cationic channel in the synaptosomal membrane may represent an equivalent of synaptophysin.

The observation that synaptosomes release their cytoplasmic acetylcholine in response to an appropriate stimulus, and a calcium-dependent release of transmitter can also be observed using synaptosomal ghosts, refilled with soluble acetylcholine (Israel et al. 1981), led to the identification of a specific presynaptic membrane protein, called mediatophore, which mediates the release of acetylcholine from liposomes in response to calcium influx (Birman et al. 1986). Single channel studies are in progress trying to explore the possibility that the Ca^{2+}-dependent cation channel identified in insect synaptosomal membranes, which may preferentially derive from cholinergic synapses (Breer 1981), may be permeable for acetylcholine, and thus represent an alternative pathway for releasing transmitter from the nerve terminal.

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References

Augustine GJ, Charlton MP, Smith SJ (1987) Calcium action in synaptic transmitter release. Ann Rev Neurosci 10:633–693

Bangham AD, Hill MW, Miller NGA (1974) Preparation and use of liposome as models of biological membranes. Methods Membr Biol 1:1–68

Berrier C, Coulombe A, Houssin C, Ghazi A (1989) A patch-clamp study of ion channels of inner and outer membranes and the contact zones of *E. coli*, fused into giant liposomes. FEBS Lett 259:27–32

Birman S, Israel M, Lesbats B, Morel N (1986) Solubilization and partial purification of a presynaptic membrane protein ensuring calcium-dependent acetylcholine release from proteoliposomes. J Neurochem 47:433–444

Blaustein MP, Goldring JM (1975) Membrane potentials in pinched-off presynaptic nerve terminals, monitored with a fluorescent probe: evidence that synaptosomes have potassium diffusion potentials. J Physiol (London) 247:589–615

Blaustein MP, Ratzlaff RW, Schweitzer ES (1980) Control of intracellular calcium in presynaptic nerve terminals. Fed Proc 39:2790–2795

Breckenridge LJ, Almers W (1987a) Currents through the fusion pore that forms during exocytosis of a secretory vesicle. Nature 328:814–817

Breckenridge LJ, Almers W (1987b) Final steps in exocytosis observed in a cell with giant secretory granules. Proc Natl Acad Sci USA 84:1945–1949

Breer H (1981) Isolation and characterization of synaptosomes from the central nervous system of insects. Neurochem Int 3:155–163

Breer H, Knipper M (1985) Synaptosomes and neuronal membranes from insects. In: Breer H, Miller TA (eds) Neurochemical techniques in insect research. Springer, New York Berlin Heidelberg, pp 125–154

Breer H, Lueken W (1983) Transport of choline by membrane vesicles prepared from synaptosomes of insect nervous tissue. Neurochem Int 5:713–720

Cohen FS, Zimmerberg J, Finkelstein A (1979) Fusion of phospholipid vesicles with planar phospholipid bilayer membranes. J Gen Physiol 75:251–270

Cook NJ, Zeilinger C, Koch KW, Kaupp UB (1986) Solubilization and functional reconstitution of the cGMP-dependent cation channel from bovine rod outer segments. J Biol Chem 261: 17033–17039

Criado M, Keller BU (1987) A membrane fusion strategy for single-channel recordings of membranes usually non-accessible to patch-clamp pipette electrodes. FEBS Lett 224:172–176

Gray EG, Whittaker VP (1962) The isolation of nerve endings from brain: an electronmicroscopic study of cell fragments derived by homogenisation and centrifugation. J Anat 96:79–88

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Hirashima N, Kirino Y (1988) Potassium channels in synaptosomal membrane examined using patch-clamp techniques and reconstituted giant proteoliposomes. Biochim Biophys Acta 946:209–214

Israel M, Lesbats B, Manaranche R (1981) ACh release from osmotically shocked synaptosomes refilled with transmitter. Nature 294:474–475

Keller BU, Hedrich R, Vaz WL, Criado M (1988) Single channel recordings of reconstituted ion channel proteins: an improved technique. Pflügers Arch 411:94–100

Krueger BK, Worley JF, French RJ (1983) Single sodium channels from rat brain incorporated into planar lipid bilayer membranes. Nature 303:172–175

Lemos JR, Nordmann JJ, Cooke IM, Stuenkel EL (1986) Single channels and ionic currents in peptidergic nerve terminals. Nature 319:410–412

Leube RE, Kaiser P, Seiter A, Zimbelmann R, Franke WW, Rehm H, Knaus P, Prior P, Betz H, Reinke H, Beyreuther K, Wiedenmann B (1987) Synaptophysin: molecular organization and mRNA expression as determined from cDNA. EMBO J 6:3261–3268

Nachshen DA, Blaustein MP (1980) Some properties of potassium-stimulated calcium influx in presynaptic nerve endings. J Gen Physiol 76:709–728

Nelson MT (1986) Interactions of divalent cations with single calcium channels from rat brain synaptosomes. J Gen Physiol 87:201–222

Reichardt LF, Kelly RB (1983) A molecular description of nerve terminal function. Ann Rev Biochem 52:871–926

Schindler H (1980) Formation of planar bilayers from artificial or native membrane vesicles. FEBS Lett 122:77–79

Schindler H, Quast U (1980) Functional acetylcholine receptors from *Torpedo marmorata* in planar membranes. Proc Natl Acad Sci USA 77:3052–3056

Suszkiw JB, Murawsky MM, Shi M (1989) Further characterization of phasic calcium influx in rat cerebrocortical synaptosomes: inferences regarding calcium channel type(s) in nerve endings. J Neurochem 52:1260–1269

Taipale HT, Kauppinen RA, Komulainen H (1989) Ruthenium red inhibits the voltage-dependent increase in cytosolic free calcium in cortical synaptosomes from guinea-pig. J Biochem Pharmacol 38:1109–1113

Tank DW, Miller C, Webb WW (1982) Isolated-patch recording from liposomes containing functionally reconstituted chloride channels from *Torpedo electroplax*. Proc Natl Acad Sci USA 79:7749–7753

Thomas L, Hartung K, Langosch D, Rehm H, Bamberg E, Franke WW, Betz H (1988) Identification of synaptophysin as a hexameric channel protein of the synaptic vesicle membrane. Science 242:1050–1053

Umbach JA, Gunderson CB, Baker PF (1984) Giant synaptosomes. Nature 311:474–477

Whittaker VP (1984) The synaptosome. In: Lajtha A (ed) Handbook of neurochemistry, 2nd edn, vol 7. Plenum Press, New York, pp 41–69