Na^+—Ca^{2+} Exchanger in the Soluble Fraction of Crayfish Striated Muscle

M. RUŠČÁK, M. JUHÁSZOVÁ, J. ORLICKÝ and J. ZACHAR

Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 5, 833 06 Bratislava, Czechoslovakia

Abstract. Proteins with Na^+—Ca^{2+} exchange activity from the soluble fraction of crayfish striated muscle were inserted into asolectin proteoliposomes. A pH dependent calcium uptake with an optimum at the alkaline side and inhibition in the presence of sodium or strontium ions in the external medium was observed. When expressed per tissue wet weight the capacity for Na^+—Ca^{2+} exchange of proteoliposomes with inserted soluble proteins was by one half higher than that of the membrane fraction and more than twice higher in comparison with the reconstituted membrane bound exchanger. Using polyacrylamide gel electrophoresis two most prominent proteins with M_r over 200 and 43 kDa could be detected in proteoliposomes with the highest Na^+—Ca^{2+} exchange. It is assumed that protein(s) with M_r 43 kDa could represent the soluble Na^+—Ca^{2+} exchanger in crayfish striated muscle soluble fraction.

Key words: Crayfish striated muscle — Na^+—Ca^{2+} exchanger — Soluble muscle proteins

Introduction

Several reports have confirmed the existence of integral membrane proteins in the intracellular compartment. Soluble proteins with sodium channel properties have been found in the rat brain (Malysheva et al. 1984) and in the myocardium of rats and rabbits (Doyle et al. 1982; Lishko et al. 1985). A higher capacity for calcium antagonists was found in the soluble fraction of rabbit striated muscle than in the membrane fraction (Križanová et al. 1989). During the early stages of postnatal development in rats a large intracellular pool of α-subunits of the sodium channels was reported to prevail in the brain over the membrane bound subunit (Schmidt et al. 1987). Our preliminary experiments (Formelová et al. 1988) have shown a soluble protein to be present in the rat brain 8—10 days after birth, in the period of the maximal growth of membrane bound Na^+—Ca^{2+} exchange activity. Soluble brain proteins, when inserted into proteo-

liposomes, exhibited properties identical with those of the Na^+—Ca^{2+} exchanger, i.e. sodium gradient generated calcium uptake and its inhibition by extravesicular Na^+ or Sr^{2+}. After successful reconstitution of the Na^+—Ca^{2+} exchanger from crayfish muscle plasma membranes (Ruščák et al. 1987a), further experiments were designed to look for a soluble protein with Na^+—Ca^{2+} exchange properties in crayfish striated muscle in which calcium ions play a crucial role in membrane bioelectric phenomena.

Materials and Methods

Chemicals: bovine serum albumin, surcose (Merck), CM-Sephadex C-25, protein calibration kit (Pharmacia Fine Chemicals), $^{45}CaCl_2$, specific activity 111 (GBq/g Ca (Radioisotope of the Hungarian Academy of Sciences), 2-mercaptoethanol, EGTA, Tris-base, sodium dodecylsulphate (SDS), acrylamide, N,N'-bis-acrylamide, N,N,N',N'-tetramethylenediamine (TEMED) (Serva), asolectin (Fluka); all other reagents were of analytical grade and were purchased from Lachema.

Preparation and insertion of soluble proteins from crayfish muscles into proteoliposomes: muscles excised from claws and tails were homogenized by repeated strokes at 800 rpm in a Polytron PT 10—20 homogenizer in media of the following final composition (in mmol.l^{-1}): **1** — NaCl 200; Tris-HCl pH = 8, 20. **2** — sucrose 320, Tris-HCl pH = 8, 20; **3** — pure Tris-HCl pH = 8, 20. The tissue : medium ratio was 1 : 1 (w : v). The homogenate was centrifuged for 1 h in a Beckman L7-55 ultracentrifuge (swing-out rotor SW 40 Ti) at 140,000 or 200,000 × g_{max}. The sediment was rehomogenized and recentrifuged at the same conditions and the pooled supernatants were mixed with a solution of the following composition (in mmol.l^{-1}): sodium cholate 60, sodium chloride 1000, sodium oxalate 10, 2-mercaptoethanol 5, Tris-HCl pH = 8 20, asolectin 40 mg per 1 ml to obtain 1 mg soluble proteins in 1 ml of the solubilization medium. After 6 h solubilization under continuous stirring the specimens were centrifuged 1 h at 100,000 × g_{max}. The supernatant was transferred into dialyzing membranes (Serva) and dialyzed overnight against a 1000-fold the volume of the above medium without lipids and detergent. The dialyzate was then mixed with 6 volumes of the dialyzing medium, sonicated 3 × 15 s at 20 kHz (MSE-ultrasonic desintegrator MK2) and centrifuged for 3 h in a Beckman L7-55 ultracentrifuge (rotor SW 40 Ti) at 240,000 × g_{max}. The sedimented proteoliposomes were washed with 200 mmol.l^{-1} NaCl buffered with Tris-HCl to pH 8, recentrifuged at the same centrifugal force for 3 h, the final sediment was stirred in 200 mmol.l^{-1} NaCl pH 8, and left overnight to equilibrate with sodium ions. In another series experiments the following protease inhibitors were used throughout the preparation procedure: phenanthroline, phenylmethylsulfonylfluoride, iodacetamide and benzamidine, 1 mmol.l^{-1} each. In a further series of experiments the excised muscles were frozen in liquid N_2, powdered in frozen state under liquid nitrogen and the tissue powder was further extracted with 20 mmol.l^{-1} Tris-HCl pH 8 in the presence of protease inhibitors and treated for proteoliposome preparation in the same way as described above.

The same procedure as with the soluble proteins was also used for the reconstitution into proteoliposomes of proteins precipitated at 40% $(NH_4)_2SO_4$ saturation and the subsequent saturation with 80% ammonium sulphate. The precipitated proteins were sedimented for 1 h at 30,000 × g_{max} and the pooled sediments were further treated as described for reconstitution of the soluble fraction.

Na^+—Ca^{2+} exchange measurements: $^{45}CaCl_2$ accumulation was estimated in 50 μl of proteoliposomes loaded with NaCl containing cca 5 μg proteins which were transferred into 1 ml of the

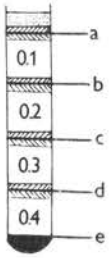

Fig. 1. Schematic representation of the separation of proteoliposomes loaded with $^{45}Ca^{2+}$ via Na^+—Ca^{2+} exchange reaction on a sucrose density gradient. Dotted layer — applied proteoliposomes, symbol *a—e* denote the subfractions at the limits of the gradients. Figures indicate sucrose concentration in mol.l^{-1}.

medium containing either 200 mmol.l^{-1} KCl, choline chloride, or NaCl, pH 8; after 1 min incubation at room temperature (20—22 °C) in the presence of (as a rule) 20 μmol.l^{-1} $^{45}CaCl_2$ the specimens were rapidly cooled and filtered through a column (1.5 cm in diameter × 1.5 cm in height) of CM-Sephadex C-25 by elution with KCl, choline chloride, or NaCl solutions. The eluates were collected in scintillation flasks (10 drops of eluate per one flask), 7 ml of the Bray scintillation cocktail (Spolana) were added per flask, and the radioactivity was measured in a Rackbeta (LKB) scintillation counter. In another series of experiments proteoliposomes loaded with Ca^{2+} at 50 μmol.l^{-1} $^{45}Ca^{2+}$ via Na^+—Ca^{2+} exchange reaction were applied on a discontinuous surcose density gradient (0.1—0.2—0.3—0.4 mol.l^{-1}) and centrifuged for 3h at 240,000 × g_{max}. The subfractions at the gradient limits (Fig. 1) were sucked off with a Pasteur pipette, and the calcium accumulation and protein content were quantitatively estimated and proteins in all subfractions were qualitatively analyzed.

The technique of the reconstitution and purification of the membrane bound exchanger was described elsewhere (Ruščák et al. 1987a, b).

Na^+—Ca^{2+} exchange was taken as the difference of $^{45}Ca^{2+}$ uptake in specimens transferred into potassium or choline medium versus values of ^{45}Ca found in vesicles incubated in sodium medium, and was expressed in nmoles of accumulated calcium per 1 mg proteoliposomal protein per minute.

The ATPase activities in specimens with soluble proteins or with ammonium sulphate precipitated proteins were estimated according to Ziegelhöffer et al. (1983).

Prior to both quantitative and qualitative protein determination using the methods of Lowry et al. (1951) and Laemli (1970) the proteoliposomes were mixed with excess distilled water; the hypoosmotic shock liberated the proteins in the intraliposomal space. Then the specimens were sonicated 3 × 15 s at 20 kHz and centrifuged for 3 h at 240,000 × g_{max}. The sediment was stirred in 12.5 % TCA solution, the mixture was centrifuged at 40,000 × g_{max} for 30 min and the obtained sediment was repeatedly (3 times) extracted with a warm ethanol-ether (1 : 1) mixture. The insoluble material was dissolved in 2 % SDS solution and used for further analyses. The electrophoretically separated proteins were stained according to Oakley et al. (1980) and the electrophoreograms were measured densitometrically (laser densitometer LKB Ultrascan 2202).

Except for Na^+—Ca^{2+} exchange measurements all operations were carried out at 2—4 °C.

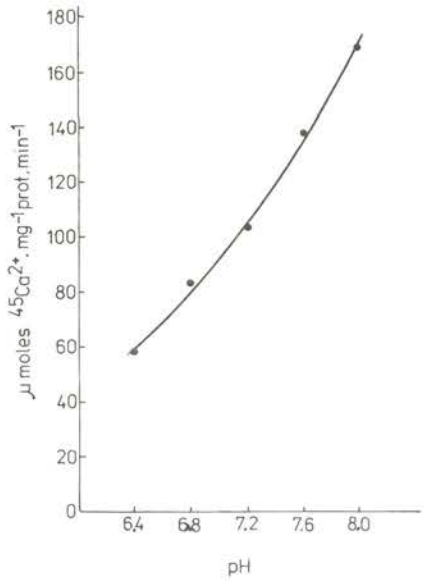

Fig. 2. pH dependence of the Na^+—Ca^{2+} exchange rate in proteoliposomes with inserted soluble proteins. Proteoliposomes loaded with sodium were transferred int 0.2 mol . l^{-1} choline chloride medium containing 20 μmol . l^{-1} $^{45}CaCl_2$. Each point represents the mean value of 4 measurements. For details see section Materials and Methods.

Results

When proteoliposomes loaded in 200 mmol . l^{-1} sodium or 200 mmol . l^{-1} potassium media were incubated for 1 min in the media of the same composition in the presence of 20 μmol . l^{-1} $^{45}Ca^{2+}$ and then filtered through CM-Sephadex C-25 column they eluted in the void volume; the amounts of ^{45}Ca bound to proteoliposomes did not differ, reaching values of 21 ± 2 nmol . mg^{-1} prot. min^{-1} ($n = 4$). It can therefore be stated that differences in $^{45}Ca^{2+}$ accumulation between proteoliposomes preloaded with Na^+ and incubated in potassium versus sodium media represent the real Na^+—Ca^{2+} exchange. Filtration of proteoliposomes through GF/C fiber filters was not successful as only 5 % of the proteoliposomal radioactivity remained on the filters. The soluble proteins of crayfish striated muscle inserted into asolectin proteoliposomes exhibited relatively high Na^+—Ca^{2+} exchange activities, with average values of 145 ± 19 nmol . mg^{-1} prot. min^{-1}; upon diminishing the sodium gradient by raising extravesicular sodium to 30 mmol . l^{-1}, the rate of Na^+—Ca^{2+} exchange dropped to 40.6 % of the control value. The addition of 0.5 mol . l^{-1} $SrCl_2$ into

Table 1.

Subfractions	I		II	
	S. A.	% T. A.	S. A.	% T. A.
a	1200	14	1140	20
b	1097	18.1	1100	38
c	460	26.8	580	13
d	170	7	230	8
e	17	0.4	7	80.7
Recovery %		66.3		80.7

Na^+—Ca^{2+} exchange in subfractions of proteoliposomes prepared on sucrose density gradient *a*—*e*: subfractions shown in Fig. 1. I — proteoliposomes with inserted membrane proteins, II — proteoliposomes with inserted soluble proteins. S. A. — specific activities of accumulated Ca^{2+} in nmol . mg^{-1} prot, % T. A. — percentage of the total amount of $^{45}Ca^{2+}$ accumulated in Na^+—Ca^{2+} exchange reaction applied to the gradient. In I the concentration of $^{45}Ca^{2+}$ in the medium was 25 μmol . l^{-1}, in II 50 μmol . l^{-1}. The total amount of calcium accumulated in proteoliposomes applied to the gradient in column I — 88 nmol, in column II — 36 nmol. For details, see section Materials and Methods.

the extravesicular medium reduced Na^+—Ca^{2+} exchange to 7.7 % of the control value. The final results were independent of the homogenization media used (see Materials and Methods), of the presence of protease inhibitors or of whether the muscles were freezed in liquid nitrogen; however, large seasonal variations were observed: substantially higher absolute values were measured during the summer as compared with the winter months.

Raising pH of the incubation media increased also the Na^+—Ca^{2+} exchange rate in proteoliposomes with inserted soluble proteins (Fig. 2).

In proteoliposomes loaded with Ca^{2+} via Na^+—Ca^{2+} exchange and separated on sucrose density gradient the highest Ca^{2+} accumulation was measured in the less dense media till 0.3 mol . l^{-1} sucrose, where about 70 % of the total activity applied to the gradient was recovered. The subfractions sedimented under 0.3 mol . l^{-1} sucrose showed much lower Na^+—Ca^{2+} exchange rates; the sediment at the limit between 0.3—0.4 mol . l^{-1} sucrose had a specific activity of only 20 % of that of the lightest subfractions and it represented about 8 % of the total activity applied to the gradient. The sediment at 0.4 mol . l^{-1} sucrose limit showed negligible Na^+—Ca^{2+} exchange. A similar pattern of Na^+—Ca^{2+} exchange was observed also in reconstituted proteoliposomes with membrane bound Na^+—Ca^{2+} exchanger (Table 1).

The total Na^+—Ca^{2+} exchange expressed per gram tissue wet weight was 14 ± 0.5 nmol Ca^{2+} . min^{-1} in the crude membrane fraction, ($n = 10$), 8.4 ± 0.6 nmol Ca^{2+} . min^{-1} in proteoliposomes prepared from the membrane

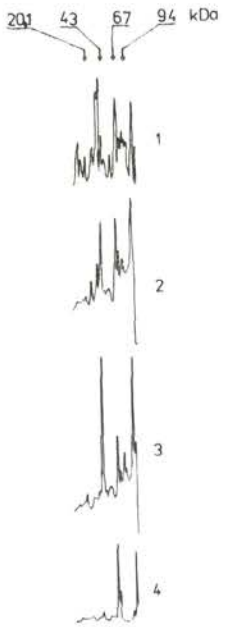

Fig. 3. Densitograms of SDS polyacrylamide gel electrophoresis: 1 — muscle soluble fraction; 2 — proteoliposomes with inserted soluble fraction proteins; 3 — proteoliposomes with inserted proteins precipitated 40 % ammonium sulphate concentration; 4 — proteoliposomes with inserted proteins precipitated at 80 % ammonium sulphate. Molecular weights are shown.

fraction, ($n = 6$), but 26.3 ± 1.4 nmol $Ca^{2+} \cdot min^{-1}$ in proteoliposomes prepared from the soluble fraction ($n = 8$).

The soluble proteins and those inserted into proteoliposomes either as a whole or after separation on the sucrose density gradient were further analyzed by SDS polyacrylamide gel electrophoresis. Only a few soluble proteins were firmly bound with asolectin proteoliposomes, the most distinct proteins being those with M_r above 200, of 74 and 43 kDa. When soluble proteins were stepwisely precipitated with 40 % and 80 % ammonium sulphate saturation and reconstituted into proteoliposomes the whole activity appeared in the first precipitate. The proteoliposomes prepared from those proteins exhibited 2—3 times higher specific activities as compared with the original soluble fraction proteoliposomes, while only traces (less than 5 %) of Na^+—Ca^{2+} exchange were found in proteoliposomes prepared from 80 % ammonium sulphate precipitate. SDS polyacrylamide gel electrophoresis revealed a substantial reduction of protein with M_r 74 kDa in proteoliposomes from 40 % ammonium sulphate

precipitate; this was precipitated with 80 % ammonium sulphate saturation. The most distinct proteins of the 40 % precipitate in proteoliposomes with high Na^+—Ca^{2+} exchange were those with M_r above 200 and 43 kDa (Fig. 3). There were no differences in protein profiles of proteoliposomes irrespective of whether electrophoresis was performed under reducing or non-reducing conditions. In reconstituted proteoliposomes prepared from the solubilized membrane proteins 3 distinct proteins with M_r 100, 55—72 and 43 kDa were observed (Ruščák et al. 1987).

It should be stressed that no membrane-specific ATPase activity was observed either in preparations containing the total soluble fraction or in the fraction precipitated at 40 % ammonium sulphate saturation.

Discussion

The Na^+—Ca^{2+} exchanger could be reconstituted and partially purified from membranes of crayfish striated muscle (Ruščák et al. 1987a). In the present work soluble proteins from crayfish striated muscle could be inserted into asolectin phospholipids, exhibiting Na^+—Ca^{2+} exchange activity, with specific activities reaching about one half of that of the reconstituted membrane-bound exchanger (Ruščák et al. 1987a). The presence of the Na^+—Ca^{2+} exchanger in the soluble fraction of crayfish striated muscle is confirmed by a number of results presented herein. It is well established that Na^+—Ca^{2+} exchange diminishes upon reducing sodium gradient between the intra- and extravesicular space (Gilbert and Meissner 1982; Philipson 1985; Reeves and Sutko 1983; Ruščák et al. 1987b), half-maximal inhibition being observed at 20—25 mmol . l^{-1} extravesicular sodium. This was also observed in our experiments: in the presence of 30 mmol . l^{-1} extravesicular sodium Na^+—Ca^{2+} exchange was reduced by more than 50 %. Sr^{2+} is a competitive inhibitor of Na^+—Ca^{2+} exchange (Grower and Kwan 1987); 0.5 mmol . l^{-1} $SrCl_2$ nearly completely inhibited Na^+—Ca^{2+} exchange in rat brain membranes (Orlický et al. 1985). The same results were obtained with the soluble Na^+—Ca^{2+} exchanger from crayfish striated muscle inserted into asolectin proteoliposomes. The alkaline pH optimum of Na^+—Ca^{2+} exchange in proteoliposomes with inserted soluble proteins was similar to that obtained for the membrane bound exchanger in the crayfish muscle membranes (Ruščák et al. 1987b), rabbit muscle membrane (Gilbert and Meissner 1982) or cardiac sarcolemma (Philipson 1985). These results indicate that the soluble fraction of the crayfish striated muscle contains protein(s) with Na^+—Ca^{2+} exchanger properties.

The finding of a soluble protein with Na^+—Ca^{2+} exchange activity which

should be an integral membrane component, is unique for crayfish muscle. No clearly detectable Na^+—Ca^{2+} exchange activity was observed in the soluble proteins inserted into asolectin proteoliposomes from the crayfish, frog and rat hearts or from frog and rat striated muscles (Formelová et al. 1988 and unpublished results). A number of membrane bound proteins, however, are known to be present in tissues in soluble form. A soluble protein with the sodium channel properties has been described in the rat brain (Malysheva et al. 1984); Lishko and Zhukareva 1987). The saxitoxin binding protein in the frog heart is by one half present in not membrane-bound form (Doyle et al. 1982), a tetrodotoxin binding protein was found in the heart of adult rats (Lishko et al. 1985), calcium antagonists binding protein prevails in the soluble fraction of rabbit striated muscle (Križanová et al. 1989). Considering the above findings the existence of a soluble Na^+—Ca^{2+}exchanger in crayfish muscle is very plausible. It is noteworthy that the total capacity of the soluble Na^+—Ca^{2+} exchanger in crayfish muscle is higher than that of the membrane bound exchanger. Whether some regulatory inhibitory proteins suggested to be present in native membranes (Hale et al. 1984) were lacking in our preparations cannot be answered from the present experiments. Also, the question remains unanswered whether all soluble exchanger or only its part was inserted into asolectin membranes.

The question concerning the origin of the soluble Na^+—Ca^{2+} exchanger remains open. The exchanger is unlikely to originate from membrane fragments as no membrane-specific ATPase activity was found either in the soluble fraction or in 40 % $(NH_4)_2SO_4$ protein precipitate specimens and no differences in Na^+—Ca^{2+} exchange rates were observed between proteoliposomes from supernatants obtained at 140,000 × g_{max} or 200,000 × g_{max} and among preparations from the various homogenization media. The liberation of soluble proteins with Na^+—Ca^{2+} exchange properties which could be loosely bound to the membranes and would be split off during the preparation procedure cannot be excluded. Unlikely is the suggestion that the protein with Na^+—Ca^{2+} exchange properties originates from the membrane bound exchanger as a result of its proteolysis during the preparation procedure as no differences were observed in Na^+- -Ca^{2+} exchange rates between specimens prepared in the presence or in the absence of protease inhibitors and/or those frozen in liquid nitrogen. If the protein with Na^+—Ca^{2+} exchanger properties is split off the membrane during the short period of the muscle excision then a specific effect must be suggested on the exchanger, as the ratio of the Na^+—Ca^{2+} exchange rates of the preparations reconstituted from membrane to soluble fraction was 1 : 2 on average. Therefore the assumption seems to be acceptable that there exists in crayfish striated muscle a non membrane bound protein with Na^+—Ca^{2+} exchanger

properties which under suitable conditions can be inserted into phospholipid membranes and acquire the properties of the membrane bound exchanger.

A close relationship was observed between Na^+—Ca^{2+} exchange and the soluble protein(s) with Mr over 200, of 74 and 43 kDa inserted into asolectin proteoliposomes from the soluble fraction of crayfish muscle. The 74 and/or 43 kDa molecular mass is close to that reported for the Na^+—Ca^{2+} exchanger identified in crayfish muscle plasma membranes (Ruščák et al. 1987a), heart sarcolemma (Hale et al. 1984; Longoni and Carafoli 1987) or synaptic plasma membranes (Barzilai et al. 1984, 1987). Further experiments with precipitated soluble proteins at different ammonium sulphate saturations revealed a relationship between the Na^+—Ca^{2+} exchange and the occurrence of 43 kDa protein in proteoliposomes (Fig. 3). M_r of this protein is close to that reported for partially digested brain microsomal membranes with still preserved Na^+—Ca^{2+} exchange (Ruščák et al. 1985).

Nevertheless, two questions remain still open: the exact identification of the soluble protein with Na^+—Ca^{2+} exchange properties and its significance for muscle physiology.

Acknowledgement. We are indebted to Dr. A. Džurba for his performing analyses of ATPase activity.

References

Barzilai A., Spanier R., Rahamimoff H. (1984): Isolation, purification, and reconstitution of the Na^+ gradient-dependent Ca^{2+} transporter (Na^+—Ca^{2+} exchanger) from brain synaptic plasma membranes. Proc. Nat. Acad. Sci. USA **81**, 6521—6525

Barzilai A., Spanier R., Rahamimoff H. (1987): Immunological identification of the synaptic plasma membrane Na^+—Ca^{2+} exchanger. J. Biol. Chem. **262**, 10315—10320

Doyle D. D., Wong M., Tanaka J., Barr L. (1982): Saxitoxin binding site in frog myocardial cytosol. Science **215**, 1117—1119

Formelová J., Juhászová M., Orlický J., Ruščák M., Travěncová I., Zachar J. (1988): Na^+—Ca^{2+} exchange in the soluble fraction of rat brain proteins. Physiol. Bohemoslov. **37**, 362

Gilbert J. R., Meissner G. (1982): Sodium-calcium ion exchange in skeletal muscle sarcolemmal vesicles. J. Membrane Biol. **69**, 77—84

Grower A. K., Kwan Ch. Y. (1987): Sodium-calcium exchange in rat myometrium. Biochem. Arch. **3**, 353—362

Hale C. C., Slaughter R. S., Ahrens D. C., Reeves J. P. (1984]: Identification and partial purification of the cardiac sodium-calcium exchange protein. Proc. Nat. Acad. Sci. USA **81**, 6569—6573

Križanová O., Hudecová S., Orlický J., Zachar J. (1989): Is Ca^{2+} antagonists binding protein from cytosolic fraction the precursor of α_1 subunit of Ca^{2+} channel? Gen. Physiol. Biophys. **8**, 99—111

Laemli U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature **227**, 680—685

Lishko V. K., Storchak L. G., Himmelreich N. H. (1985): Transmembrane localization of cytoplasmic proteins from the cardiac muscle in proteoliposomes. Ukr. Biokh. Zh. **57**, 3—7 (in Russian)

Lishko V. K., Zhukareva V. A. (1987): Purification and properties of the TTX-sensitive protein from bovine brain soluble fraction. In: Receptors and Ion Channels. (Ed. by Y. A. Ovchinnikov and F. Hucho, W. de Gruyter) pp. 1987—192, Berlin—New York 1987

Longoni S., Carafoli E. (1987): Identification of the Na^+/Ca^{2+} exchanger of calf heart sarcolemma with the help of specific antibodies. Biochem. Biophys. Res. Commun. **145**, 1059—1063

Lowry O. O., Rosebrough N. J., Farr A. L., Randall R. J. (1951): Protein measurements with the Folin phenol reagent. J. Biol. Chem. **193**, 266—275

Malysheva M. K., Lishko V. K., Zhukareva V. A., Lysenko V. V. (1984): Integration of soluble tetrodotoxin-sensitive brain protein with liposomal membranes. Neurophysiology **16**, 550—558

Oakley B. R., Kirsch D. R., Morris N. R. (1980): A simplified ultrasensitive silver stain for detecting proteins in polyacralamide gels. Anal. Biochem. **105**, 361—363

Orlický J., Ruščák M., Juhász O. (1985): Effects of calcium channel antagonists on the Na^+—Ca^{2+} exchange in microsomal membranes of bovine cerebellar cortex. Brat. Lekár. Listy **83**, 272—280 (in Slovak)

Philipson K. D. (1985): Sodium-calcium-exchange in plasma membrane vesicles. Annu. Rev. Physiol. **47**, 567—571

Reeves J. P., Sutko J. L. (1983): Competitive interactions of sodium and calcium with the sodium-calcium exchange system of cardiac sarcolemmal vesicles. J. Biol. Chem. **258**, 3178—3182

Ruščák M., Orlický J., Juhász O., Zachar J. (1985): Na^+—Ca^{2+} exchange in rat brain microsomal membranes pretreated with pronase and/or SDS. Gen. Physiol. Biophys. **4**, 249—256

Ruščák M., Juhászová M., Orlický J., Zachar J. (1987a): Reconstitution and partial purification of Na^+—Ca^{2+} exchanger from crayfish striated muscle plasma membranes. Gen. Physiol. Biophys. **6**, 523—528

Ruščák M., Orlický J., Juhászová M., Zachar J. (1987b): Na^+—Ca^{2+} exchange in plasma membranes of crayfish striated muscle. Gen. Physiol. Biophys. **6**, 469—478

Schmidt J., Rossie S., Catterall W. A. (1987): A large intracellular pool of inactive Na channel α-subunit in developing rat brain. Proc. Nat. Acad. Sci. USA **82**, 4847—4851

Ziegelhöffer A., Breier A., Džurba A., Vrbjar N. (1983): Selective and reversible inhibition of heart sarcolemmal ($Na^+ + K^+$)ATPase by bromphenyl isothiocyanate. Evidence for a sulfhydryl group in the ATPase binding site of the enzyme. Gen. Physiol. Biophys. **2**, 447—456

Final version accepted December 16, 1988