# $Na^+ - Ca^{2+}$ Exchange in Locust Striated Muscles

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**Abstract**. High $Na^+ + Ca^{2+}$ exchange rates comparable with those reported for crayfish striated muscle, rat heart and rat brain, were observed in locust striated muscle homogenates and membrane preparations. The $Na^+ - Ca^{2+}$ exchange followed the 1st order kinetics with a $K_m$ value of 18 $\mu$mol . $l^{-1}$ for Ca, the pH optimum was at 8, the temperature optimum at 30° C, and the exchange was inhibited in the presence of sodium in the incubation medium, with a $K_{i\,Na}$ of approx. 25 mmol . $l^{-1}$. The present results suggest a high $Na^+ - Ca^{2+}$ exchange in locust striated muscles which operate on the calcium electrogenesis principle.

**Key words**: Locust muscle — $Na^+ - Ca^{2+}$ exchange

## Introduction

Membrane preparations of various tissues show great differences in $Na^+ - Ca^{2+}$ exchange. This diversity can be explained either by different abundance of the exchanger in the membrane preparations or by different amounts of superficial membranes in the examined preparations (Matlib 1988; Philipson 1985; Ruščák and Juhászová 1988; Smith et al. 1987). Significant differences have been reported between the membranes of striated muscles with calcium (Ruščák et al. 1987b) and sodium (Gilbert and Meissner 1982) electrogenesis, the $Na^+ - Ca^{2+}$ exchange being one order of magnitude higher in the membranes of muscles with calcium electrogenesis. The present work was aimed at the study of $Na^+ - Ca^{2+}$ exchange in another type of muscles with calcium electrogenesis, the locust muscles (Mandelshtam 1983).

## Materials and Methods

*Reagents used*: $^{45}CaCl_2$, specific activity 189 GBq/g Ca (Institute of Isotopes, Hungarian Academy of Sciences), Tris-base, 2-mercaptoethanol, choline chloride, bovine serum albumin (Serva), sucrose (Merck), asolectin (Fluka), scintillation cocktail (Spolana); all other chemicals of analytical grade were purchased from Lachema.

*Tissue preparation*: Muscle tissue excised from 10—15 locust legs and chests, crayfish tails, frog thigh, rat iliopsoas muscle and from rat heart ventricles was homogenized by 3 × 30 strokes in a Polytron PT 10—20 homogenizer (Luzern) at 800 r.p.m. in 200 mmol . $l^{-1}$ NaCl, buffered to pH 8.0 with 20 mmol . $l^{-1}$ Tris HCl. The tissue to medium ratio was 1:10 (w:v). Rat cerebral hemispheres were homogenized in the same way in a glass homogenizer, the slit between the wall and the pestle of the homogenizer being 100 $\mu$m, by 30 strokes. The homogenates were used for $Na^+ - Ca^{2+}$ exchange measurements.

Locust muscle homogenates were further centrifuged at $1400 \times g_{max}$ for 20 min in a K24D centrifuge, and the supernatant obtained was further centrifuged for 30 min at $10{,}000 \times g_{max}$. The sedimented pellet was suspended in 200 mmol . $l^{-1}$ NaCl solution, pH 8 and used for $Na^+ - Ca^{2+}$ exchange measurements and for electron microscopic analyses. The $10{,}000 \times g_{max}$ supernatant was further centrifuged for 60 min at $100{,}000 \times g_{max}$ in a Beckman L7-55 ultracentrifuge (rotor SW 40 Ti). The sediment was stirred in 200 mmol . $l^{-1}$ NaCl solution buffered to pH 8 with 20 mmol . $l^{-1}$ Tris-HCl and used for $Na^+ - Ca^{2+}$ exchange measurements and electron microscopy analyses. In another serie of experiments, $1400 \times g_{max}$ supernatant was centrifuged for 1 h at $100{,}000 \times g_{max}$, the obtained sediment was rehomogenized in 0.3 mol . $l^{-1}$ sucrose pH 8, layered on the top of a discontinuous sucrose density gradient consisting of 0.6—0.8—1.0 mol . $l^{-1}$ sucrose (9 ml of each concentration) and centrifuged for 1 h at $100{,}000 \times g_{max}$, the sediment in 1.2 mol . $l^{-1}$ sucrose was then taken for $Na^+ - Ca^{2+}$ exchange estimations and for electron microscopic analyses. Purified rat brain mitochondria were prepared according to Orlický et al. (1981). Postnuclear sediments were reconstituted into asolectin proteoliposomes as described in detail for crayfish muscle (Ruščák et al. 1988).

*$Na^+ - Ca^{2+}$ exchange measurements*: $Na^+ - Ca^{2+}$ exchange in the specimens was estimated by the rapid filtration technique through GF/C glass fibre filters (Whatman) on a Millipore (Aminco) equipment. $^{45}CaCl_2$ accumulation was estimated by diluting 50 $\mu$l of the respective homogenate, of the $10{,}000 \times g_{max}$, and $100{,}000 \times g_{max}$ sediments in 1.2 mol . $l^{-1}$ sucrose preloaded with 200 mmol . $l^{-1}$ NaCl pH 8, into 2 ml of $Ca^{2+}$ uptake medium containing either 200 mmol . $l^{-1}$ choline chloride or NaCl, pH 8; after 15 s incubation at room temperature (except for the determination of the temperature dependence) in the presence of 20 $\mu$mol . $l^{-1}$ $^{45}CaCl_2$ (except for the determination of the calcium concentration dependence), the specimens were rapidly filtered, the filters were washed twice with 3.0 ml of 200 mmol . $l^{-1}$ cold choline chloride or NaCl solution, dried on air, and after addition of 7 ml SLT-41 scintillation cocktail, the radioactivity of the filters was estimated in a Rackbeta LKB scintillation counter.

pH dependence was measured in the incubation media containing 200 mmol . $l^{-1}$ choline chloride or NaCl and 20 mmol . $l^{-1}$ imidazol -HCl buffer of desired pH values.

Temperature dependence was estimated in media held at desired temperatures in water bath.

$Na^+ - Ca^{2+}$ exchange was taken as the difference of $^{45}Ca^{2+}$ accumulation in specimens transferred into choline chloride medium versus values of Ca measured in sodium medium, and was expressed in nmoles of accumulated calcium per minute, either per gram tissue wet weight or per mg protein.

Proteins were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

**Table 1.**

| Locust muscle | | Crayfish muscle | | Rat heart | | Rat brain | |
|---|---|---|---|---|---|---|---|
| T.A. | S.A. | T.A. | S.A. | T.A. | S.A. | T.A. | S.A. |
| 640 | 4.6 | 322 | 1.9 | 430 | 1.3 | 514 | 4.9 |
| ± | ± | ± | ± | ± | ± | ± | ± |
| 207 | 1.3 | 22 | 0.12 | 116 | 0.23 | 131 | 0.5 |

$Na^+$–$Ca^{2+}$ exchange rates in nmol . $min^{-1}$ as arithmetical mean ± S.E.M. T.A. — activity per gram tissue wet weight, S.A. — activity per miligram of proteins. Number of experiments: rat heart and rat brain $n = 4$, crayfish and locust muscles $n = 8$.

*Electron microscopy*: The $10{,}000 \times g_{max}$, $100{,}000 \times g_{max}$ and sucrose gradient centrifugation sediments were each mixed with 4 % glutaraldehyde to give final glutaraldehyde concentration of 2 %, and after 30 min fixation, the specimens were centrifuged for 30 min at $10{,}000 \times g_{max}$. The fixed sediments were washed with cacodylate buffer and postfixed with 1 % $OsO_4$ for 30 min. The centrifuged samples were washed with distilled water and contrasted with 2 % uranyl acetate in distilled water for 24 h. After dehydration in ethanol, the samples were transferred through propylene oxide to Durcupan. Sections were cut with glass knives on a Reichert ultracut E. After staining with lead citrate, they were examined in a JEM 1200 EX electron microscope at 80 kV.

## Results

Comparison of the $Na^+ - Ca^{2+}$ exchange rates in different tissues is difficult because of unknown amounts of superficial membranes in the preparations used; therefore, we decided to investigate the total $Na^+ - Ca^{2+}$ exchange in locust muscle homogenates and to compare the findings in the locust muscle with values measured for some other excitable tissues. As can be seen from the results summarized in Table 1, high $Na^+ - Ca^{2+}$ exchange rates were measured in locust muscle, crayfish muscle, rat heart and rat brain. One order of magnitude lower $Na^+ - Ca^{2+}$ exchange was found in homogenates of frog muscles, but no detectable $Na^+ - Ca^{2+}$ exchange was found in homogenates of rat iliopsoas muscles. The relatively large spread of the values for locust muscle is due to the fact that 2—3 times higher $Na^+ - Ca^{2+}$ exchange rates were found in 3—4 days starving animals in comparison with those having free access to food and water.

Quantitative analysis of the $Na^+ - Ca^{2+}$ exchange in subcellular fractions failed as even after repeated homogenization of the pellet sedimented at $1400 \times g_{max}$ nearly 70 % of the activity remained in the pellet. The specific

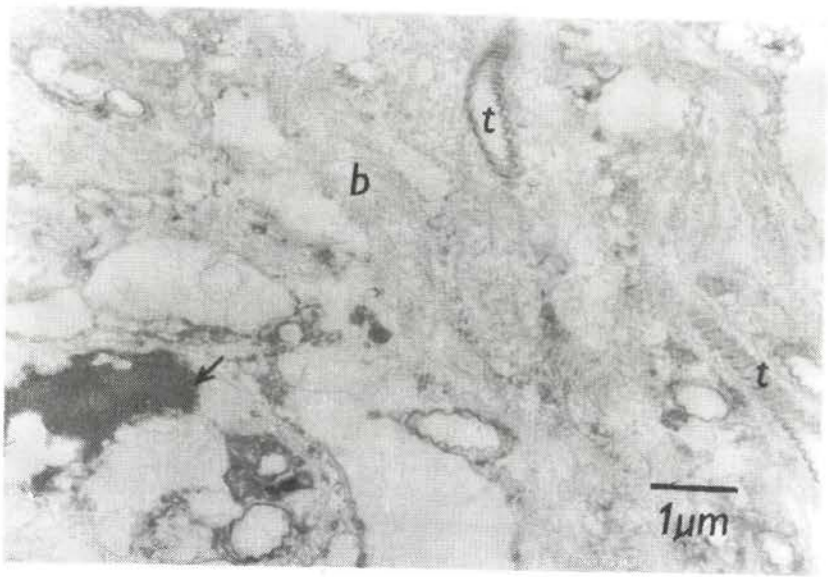


**Fig. 1.** Electron micrograph of the $10,000 \times g_{max}$ sediment. Tracheolar fragments (*t*), basal membrane (*b*) and membrane vesicles were mainly observed. The arrow points the nuclear fragment. Mitochondria in this fraction were very seldom.

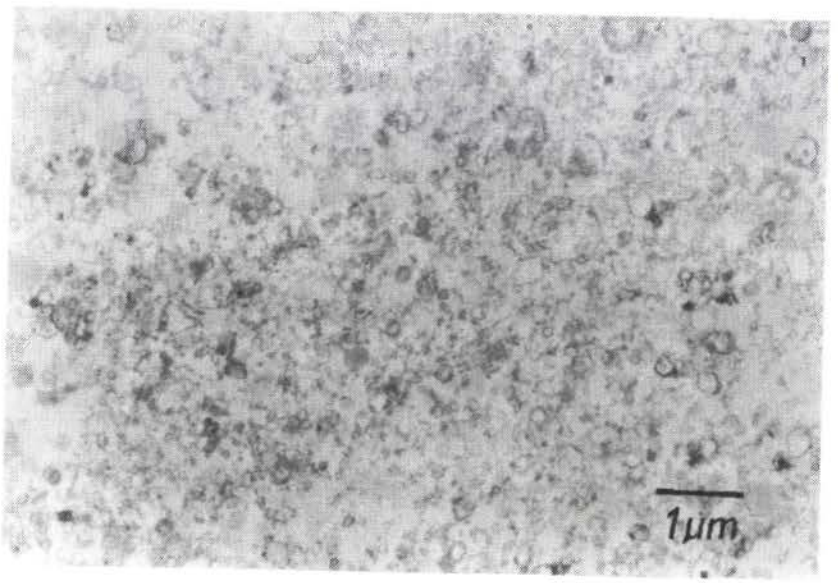


**Fig. 2.** Fraction of the $100.000 \times g_{max}$ sediment exhibiting typical sarcotubular structure.

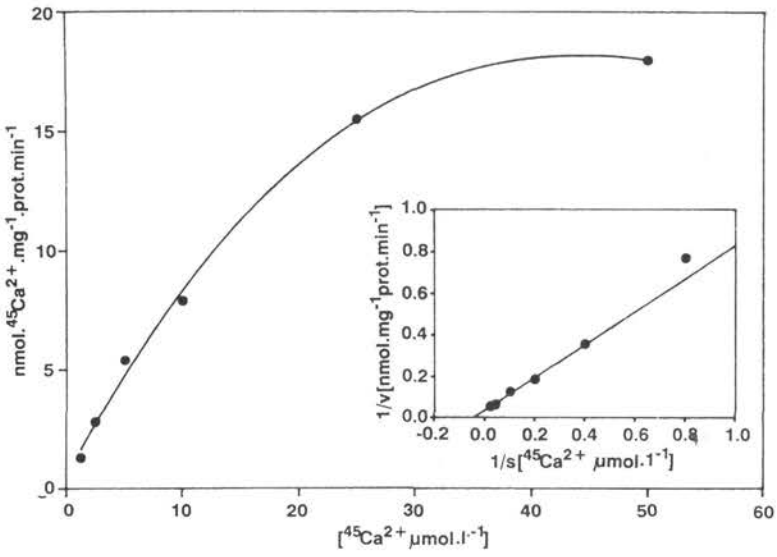


**Fig. 3.** $Ca^{2+}$ uptake in locust muscle homogenates via $Na^+ - Ca^{2+}$ exchange in relation to $^{45}Ca^{2+}$ concentration in the medium. Insert: Lineweaver–Burk's plot. Each point represents mean value of 4 measurements. Results are expressed in nmol . mg$^{-1}$ prot . min$^{-1}$. Incubation time: 15 s.

activities in the sediments obtained at 10,000 and 100,000 × $g_{max}$ did not differ significantly; 10—14 nmol . mg$^{-1}$ prot . min$^{-1}$ were measured for the 10,000 × $g_{max}$ sediment, and 6—12 nmol . mg$^{-1}$ prot. min$^{-1}$ for the 100,000 × $g_{max}$ sediment. Electron microscopy revealed mainly tracheolar fragments, membrane vesicles and rare mitochondrial fragments (Fig. 1), in the 10,000 × $g_{max}$ fraction whereas the 100,000 × $g_{max}$ fraction exhibited typical vesicular microsomal formations (Fig. 2). A similar $Na^+ - Ca^{2+}$ exchange rate and a similar electron microscopical picture were also obtained for the 1.2 mol . l$^{-1}$ sucrose pellet.

As we failed to prepare the mitochondrial fraction from locust muscle, $Na^+ - Ca^{2+}$ exchange was estimated in purified rat brain mitochondria. Only 2.5 % of the activity of the postnuclear brain supernatant were found in purified mitochondria: the activity of the postnuclear supernatant was 260 nmol . g$^{-1}$ tissue wet weight . min$^{-1}$, whereas the $Na^+ - Ca^{2+}$ exchange in purified mitochondria was only 6.3 nmol . g$^{-1}$ tissue wet weight . min$^{-1}$.

After reconstitution of both the 10,000 × $g_{max}$ and the 100,000 × $g_{max}$ sediments into asolectin proteoliposomes, the specific activity of the $Na^+ - Ca^{2+}$ exchange rose to reach 100—140 nmol . mg$^{-1}$ prot . min$^{-1}$. SDS polyacrylamide gel electrophoresis showed many proteins in proteoliposomes, and no relation

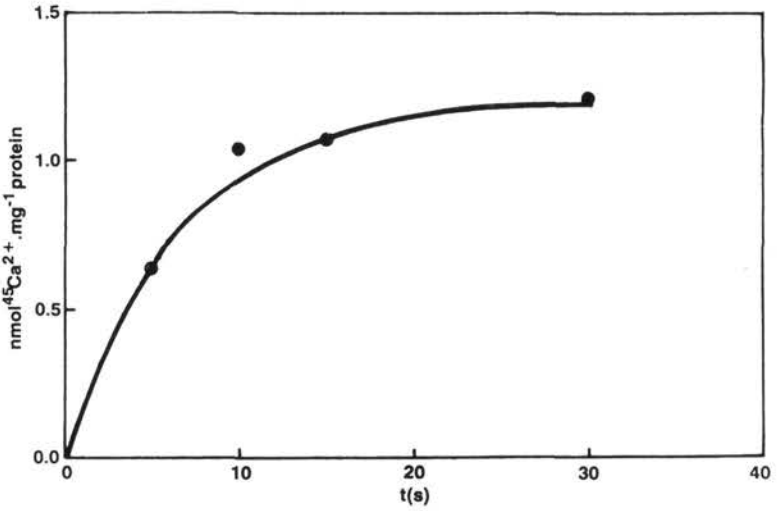


**Fig. 4.** The time dependence of $Ca^{2+}$ uptake in locust muscle homogenates via $Na^{+} - Ca^{2+}$ exchange (nmol.mg$^{-1}$ prot). Each point represents mean value of 4 measurements. $^{45}Ca^{2+}$ in the medium: 20 $\mu$mol.l$^{-1}$.

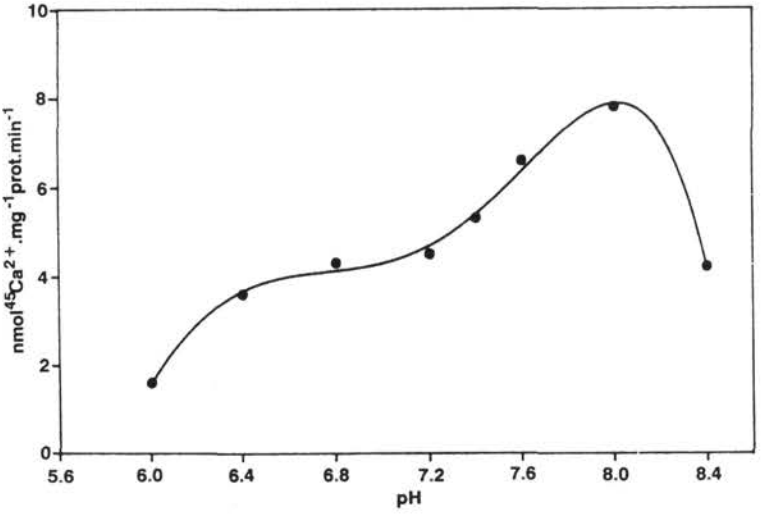


**Fig. 5.** $Na^{+} - Ca^{2+}$ exchange rates (nmol.mg$^{-1}$ prot.min$^{-1}$) in locust muscle homogenates in relation to pH of the incubation medium. Each point represents mean value of 4 measurements. $^{45}Ca^{2+}$ in the medium: 20 $\mu$mol.l$^{-1}$. Incubation time: 15 s.

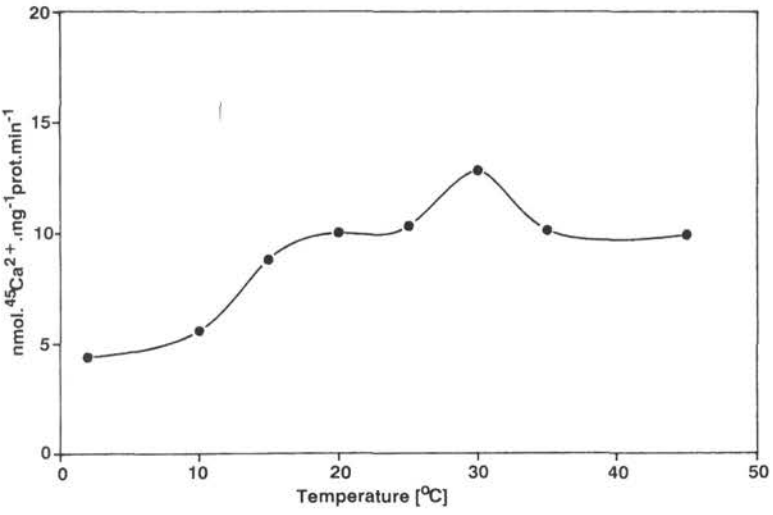


**Fig. 6.** $Na^+ - Ca^{2+}$ exchange in locust muscle homogenates (nmol . mg$^{-1}$ prot . min$^{-1}$) in relation to the temperature of the incubation media. Each point represents mean value of 4 measurements. $^{45}Ca^{2+}$ in the medium: 20 $\mu$mol . l$^{-1}$. Incubation time: 15 s.

could be derived with respect to the identification of the exchanger protein (data not shown). When proteins not sedimented at 100,000 × $g_{max}$ ("soluble fraction") were inserted into asolectin phospholipids, the proteoliposomes did not exhibit any $Na^+ - Ca^{2+}$ exchange.

The concentration dependence of $Ca^{2+}$ uptake in $Na^+ - Ca^{2+}$ exchange reaction in locust muscle homogenates is illustrated in Fig. 3. Half-maximal velocity of calcium uptake was reached at about 18 $\mu$mol . l$^{-1}$ $^{45}Ca^{2+}$ in the medium. The $Na^+ - Ca^{2+}$ exchange in locust muscle homogenates was time dependent. Maximal $Ca^{2+}$ uptake was observed during first 10 s of incubation, and a steady state was reached at 30 s of incubation, with approx. 1 mg homogenate protein present in the incubation medium and at 20 $\mu$mol . l$^{-1}$ $^{45}Ca^{2+}$ in the medium (Fig. 4). $Ca^{2+}$ accumulation was dependent on the pH of the incubation medium, with a maximum rate at approx. pH 8. There was a sharp decline in the exchange rate below and over this value (Fig. 5). The $Na^+ - Ca^{2+}$ exchange in locust muscles was strongly temperature dependent, with an optimum at 30 °C (Fig. 6). $Na^+ - Ca^{2+}$ exchange decreased with the increasing $Na^+$ concentration in the extravesicular incubation media (Fig. 7), half maximal inhibition occurring at approx. 25 mmol . l$^{-1}$ extravesicular sodium concentration.

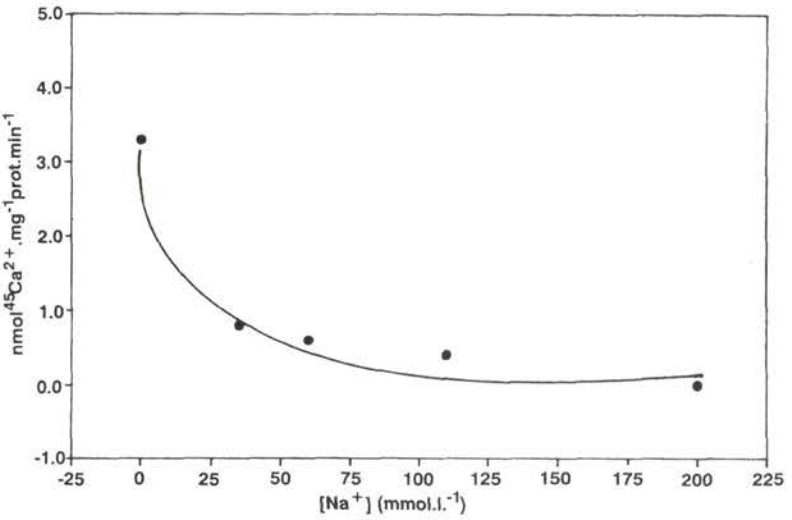


**Fig. 7.** Inhibition of the $Na^+ - Ca^{2+}$ exchange in muscle homogenates in relation to external $Na^+$ concentration. Results are expressed as the mean of 4 measurements (nmol . mg$^{-1}$ prot . min$^{-1}$). $^{45}Ca^{2+}$ in the medium: 20 $\mu$mol . l$^{-1}$. Incubation time: 15 s.

## Discussion

The presented results confirm the existence of high $Na^+ - Ca^{2+}$ exchange in locust muscle homogenates, comparable with those reported for crayfish striated muscle, rat heart and rat brain (Table 1). High $Na^+ - Ca^{2+}$ exchange was also found in membrane preparations of the tissues mentioned (Philipson 1985; Ruščák et al. 1986, 1987b), as contrasting with very low exchange rates in membranes of rabbit (Gilbert and Meissner 1982) and rat striated muscle (unpublished results). It is unlikely that the high $Na^+ - Ca^{2+}$ exchange in homogenates is related to the mitochondrial $Na^+ - Ca^{2+}$ exchange system. Only approx. 3 % of the total activity was measured in brain mitochondrial fractions. A low contribution of mitochondrial $Na^+ - Ca^{2+}$ exchange to total tissue activity has been also suggested by the reports showing that sodium rather liberates calcium from mitochondria (Brand 1985; Crompton 1985; Matlib et al. 1985).

The membrane bound exchanger could be reconstituted into asolectin proteoliposomes, with the specific activities of proteoliposomes being one order of magnitude higher than those of the membrane fraction. These results are comparable with those reported for the reconstituted crayfish muscle

$Na^+ - Ca^{2+}$ exchanger (Ruščák et al. 1987a); contrary to the crayfish muscle (Ruščák et al. 1989), no activity could be detected in the "soluble" fraction of locust muscle.

$Na^+$ driven $Ca^{2+}$ accumulation was dependent on the $Ca^{2+}$ concentration in the medium, half maximal velocity of the uptake being observed at 18 $\mu$mol . $.l^{-1}$ $Ca^{2+}$ in the incubation medium. This value is close to those reported for rabbit striated muscle (Gilbert and Meissner 1982), heart sarcolemma (Philipson 1985), nerve tissue membranes (Schellenberg and Swanson 1981), and smooth muscle membranes (Matlib 1988). The $Na^+ - Ca^{2+}$ exchange was also time dependent; maximal rates of $Ca^{2+}$ uptake were observed in the first seconds of incubation, similarly as it has been the case with heart (Philipson 1985), brain (Schellenberg and Swanson 1981) and smooth muscle membranes (Matlib 1988).

The $Na^+ - Ca^{2+}$ exchange was strongly dependent on the pH of the incubation medium, the optimal pH (Fig. 4) being at slightly alkaline side (approx. pH 8), similarly as with other tissues (for review see Ruščák and Juhászová 1988).

The $Na^+ - Ca^{2+}$ exchange depended on the temperature of the incubation media, and the optimum was close to the optimal body temperature. For mammalian membranes the optimal temperature ranges within 37—40 °C (Orlický et al. 1984; Reuter 1982; Schellenberg and Swanson 1981), whereas it is 20 °C for crayfish muscle membranes (Ruščák et al. 1987b). The optimal temperature for the locust growth is 28—32 °C (Hunter-Jones 1966). This was also the range of the temperature optimum for the $Na^+ - Ca^{2+}$exchange in locust muscle preparations (Fig. 5).

It is well established that sodium ions present in the incubation medium inhibit the $Na^+ - Ca^{2+}$ exchange in various tissues, the half-maximal inhibition being observed at 20—25 mmol . $l^{-1}$ sodium in the external medium (Gilbert and Meissner 1982; Murphy et al. 1986; Ruščák et al. 1987b). Similar sodium concentrations in the external medium were necessary to reach half maximal $Na^+ - Ca^{2+}$ exchange rates in locust muscle preparations (Fig. 6).

$Na^+ - Ca^{2+}$ exchange plays a dominant role in the contraction — relaxation cycle of the heart and smooth muscle (Reuter 1982; Mullins 1984; Matlib 1988). The question of its physiological significance in striated muscle remains open. The low $Na^+ - Ca^{2+}$ exchange in rabbit plasmalemma (Gilbert and Meissner 1982), but high exchange rates in crayfish (Ruščák et al. 1989), barnacle (Rasgado-Flores et al. 1989) and locust muscles with calcium electrogenesis indicate that in invertebrate muscles $Na^+ - Ca^{2+}$ exchange may play a dominant role in the regulation of intracellular calcium levels during contraction-relaxation cycle.

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