Some Peculiarities of the Na^+-Ca^{2+} Exchange Reaction in Choline, Tetramethylenammonium and Tris Media

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Introduction

Na^+-Ca^{2+} exchange has been well defined in the membranes of excitable tissues. Its main role is suggested to be the extrusion of calcium from the cells at the expense of transmembrane electrochemical sodium gradient. The system has been studied in detail *in vitro* in membrane vesicles loaded with sodium ions and transferred into media containing ions other than sodium (e.g. Philipson 1985; DiPolo and Beaugé 1988; Reeves 1985; Sanchez-Armass and Blaustein 1987). The highest rates of the Na^+-Ca^{2+} exchange were observed in media containing potassium ions. With a high probability, this is due to the fact that in potassium medium two independent systems participate in calcium accumulation: voltage dependent calcium channels and Na^+-Ca^{2+} exchange (Coutinho et al. 1983; Duarte et al. 1991; Tagliatella et al. 1990). On the contrary, in our experiments with Na^+-Ca^{2+} exchanger from crayfish striated muscle reconstituted into asolectin phospholipids we observed higher $^{45}Ca^{2+}$ accumulation in vesicles incubated in choline medium as compared with potassium medium (unpublished results). The experiments with the rat brain nerve endings and microsomes presented herein as well as those with the crayfish striated muscle membranes have shown that not only choline, but also tetramethylammonium and Tris ions in the external medium exhibited a higher $^{45}Ca^{2+}$ accumulation *via* Na^+-$^{45}Ca^{2+}$ exchange as compared with the external potassium medium.

Abbreviations: TMA – tetramethylammonium, Tris – tris(hydroxymethyl)aminomethane

Materials and Methods

Reagents: Choline chloride (Serva), tetramethylammoniumchloride (Sigma), Tris base (Loba), sucrose (Merck), asolectin (Fluka), $^{45}CaCl_2$ (Amersham), bovine serum albumin (Serva); all other chemicals of analytical grade were purchased from Lachema (Brno).

Preparation procedures and Na^+-Ca^{2+} exchange measurements: Rat brain synaptosomal and microsomal fractions were prepared by common differential centrifugation of sucrose homogenates (Žúbor et al. 1980), and crayfish muscle membrane preparations were prepared as described in Ruščák et al. (1987b). The fractions of synaptosomes, brain microsomes and crayfish muscle membranes were stirred in 200 mmol.l^{-1} NaCl buffered to pH 8 with Tris-HCl in a ratio 30–40 μg protein per 10 μl medium, left overnight; the Na^+-Ca^{2+} exchange in these preparations was estimated as follows: 20 μl of specimens containing 60–80 μg of protein were transferred into 1 ml of the medium containing $^{45}CaCl_2$ (actual concentrations are given in Table and Figures) and the isotonic (420–425 mosmol) NaCl, KCl, TMA and Tris solutions. After 15 s of incubation at room temperature the specimens were rapidly filtered through GF/C glass fibre filters and the $^{45}Ca^{2+}$ retained on the filters was measured in a Rackbeta scintillation counter in SLT-41 (Spolana) scintillation cocktail.

Pure asolectin liposomes or proteoliposomes containing crayfish muscle Na^+-Ca^{2+} exchanger were prepared according to the description by Ruščák et al.(1989). The specimens loaded in 200 mmol.l^{-1} NaCl were transferred into a medium containing NaCl, KCl or choline, and the radioactivity retained in the liposomes and proteoliposomes was measured as in Ruščák et al. (1987a) either by filtration through CM Sephadex C-25 column or through GF/C glass fibre filters.

The Na^+-Ca^{2+} exchange is expressed as the difference in $^{45}Ca^{2+}$ accumulation between the external Na^+ media and external media of other compositions (nmol.mg^{-1}prot. .min^{-1}). For protein determination the method of Lowry et al. (1951) was used with bovine serum albumin as the standard.

Table 1. Na^+-Ca^{2+} exchange in crayfish striated muscle microsomal fraction (*A*), in rat brain synaptosomes (*B*), and in rat brain microsomes (*C*) in nmol.mg^{-1} prot.min^{-1} as arithmetical mean ± S.E.M. Numbers in the parentheses indicate numbers of independent experiments. Index 1 indicates the presence of 30 mmol.l^{-1} sodium in the external medium. 20 μmol.l^{-1} $^{45}Ca^{2+}$ in the medium. Sub (*D*), Ca^{2+} uptake in rat brain microsomes with 200 mmol.l^{-1} internal KCl medium in nmol.mg^{-1} prot.min^{-1}. For details see Materials and Methods.

External medium	*A* (5)	*B* (3)	*C* (3)	*D* (2)
K	8 ± 2.3	3.4 ± 0.2	5.4 ± 0.4	0
K_1	1.9 ± 0.8	1.0 ± 0.08	1.1 ± 0.06	0
Ch	45 ± 12.0	15.3 ± 2.3	16.7 ± 2.5	3.2
Ch_1	6.5 ± 2.0	3.2 ± 0.9	5.1 ± 1.1	1.0
TMA	42 ± 10.3	12.5 ± 2.1	12.1 ± 1.8	8.6
TMA_1	5 ± 1.8	4.4 ± 0.4	4.8 ± 0.6	1.1
Tris	17 ± 3.1	11.1 ± 1.6	12.8 ± 2.0	2.3
$Tris_1$	6.3 ± 2.0	5.6 ± 1.3	6.9 ± 0.9	0.7

Results and Discussion

Our experiments have shown that in the nerve endings, brain microsomes and crayfish muscle membranes significantly higher $^{45}Ca^{2+}$ accumulation *via* Na^+-Ca^{2+} exchange occurs in choline, TMA and Tris containing isotonic media as compared with potassium medium. Addition of 30 mmol.l^{-1} NaCl, by preserving the isotonicity of the external media, inhibited $^{45}Ca^{2+}$ uptake by 60–80%; 100 mmol.l^{-1} sodium concentration in the uptake media completely abolished the $^{45}Ca^{2+}$ accumulation. It is noteworthy, however, that limited $^{45}Ca^{2+}$ accumulation was observed in brain microsomes when the internal ion was potassium and the external solutions contained monovalent organic cations. Under these experimental conditions sodium ions in the external medium also inhibited calcium uptake (Table 1). Higher values of $^{45}Ca^{2+}$ uptake were found also in reconstituted proteoliposomes containing crayfish muscle exchanger in choline medium than as compared with the potassium medium: while in the external potassium medium Ca^{2+} uptake was 123 ± 14 nmol.mg^{-1} prot.min^{-1}, in choline medium it reached the value 380 ± 38 nmol.mg^{-1} prot.min^{-1} ($n = 6$). The differences in $^{45}Ca^{2+}$ uptake rates between native vesicles and proteoliposomes reconstituted from crayfish striated muscle were also preserved when $^{45}Ca^{2+}$ accumulation was estimated in preparations containing 1 mmol.l^{-1} unlabeled intravesicular Ca^{2+}. The presented results are contradictory to the find-

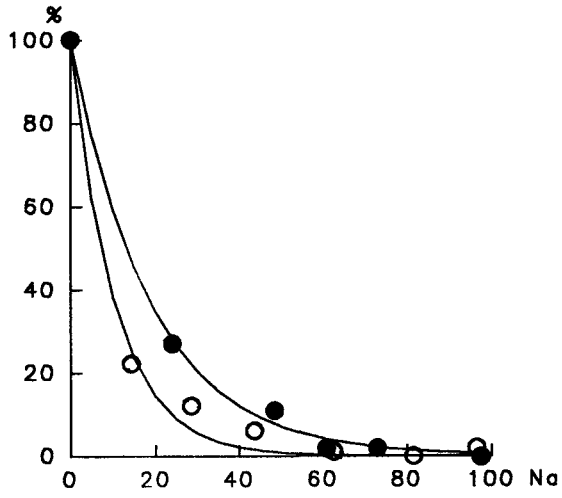

Figure 1. Relative inhibition of increasing external sodium concentrations on the Na^+-Ca^{2+} exchange as percentage of control values in crayfish striated muscle microsomal membranes. Δ – potassium in the external medium, o – choline in the external medium. External $^{45}Ca^{2+}$ concentration, 20 μmol.l^{-1}. Each point represents the mean value of 4 measurements. For details see Materials and Methods.

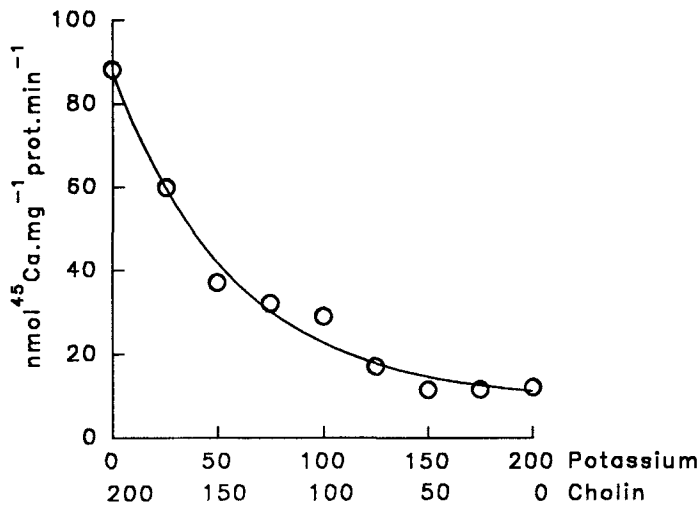

Figure 2. Inhibition of the Na^+-Ca^{2+} exchange in crayfish striated muscle microsomal membranes by increasing potassium and decreasing choline concentrations in the medium. External $^{45}Ca^{2+}$ concentration, 20 μmol.l^{-1} pH of the medium, 8. Each point represents the mean value of 4 measurements.

ings of Duarte et al. (1991) and Tagliatela et al. (1990) who found higher Ca^{2+} uptake rates in synaptosomes in potassium as compared to choline media; Hale and Keller (1990) did not observe any difference in Ca^{2+} uptake in the heart sarcolemma between potassium and choline media.

$^{45}Ca^{2+}$ uptake was inhibited, relatively to the same extent, by extravesicular sodium in a concentration-dependent manner, both in potassium and choline external medium (Fig. 1). A partial inhibition of $^{45}Ca^{2+}$ uptake, however, was also observed in the choline medium with the increasing potassium concentrations in the external medium (Fig. 2). Our results confirm the well established inhibitory effect of sodium ions on the Na^+-Ca^{2+} exchange (Gilbert and Meissner 1982; Murphy et al. 1986; Philipson 1985). Inhibition of $^{45}Ca^{2+}$ uptake by potassium ions in combination with choline in the external medium indicate that, under the experimental condition mentioned, another mechanism of the inhibition of calcium accumulation during Na^+-Ca^{2+} exchange reaction, should also be present. Probably, potassium ions diminish the membrane permeability for Ca^{2+} ions in the presence of choline as the inhibitory effect was observed not only in native membranes but also in the proteoliposomes reconstituted from crayfish microsomes (data not shown). This assumption is supported by the results summarized in Fig. 3: potassium ions at 100 mmol.l^{-1} concentration in the external medium inhibited, but not completely abolished, $^{45}Ca^{2+}$ accumulation at increasing $^{45}Ca^{2+}$ concentrations in the exter-

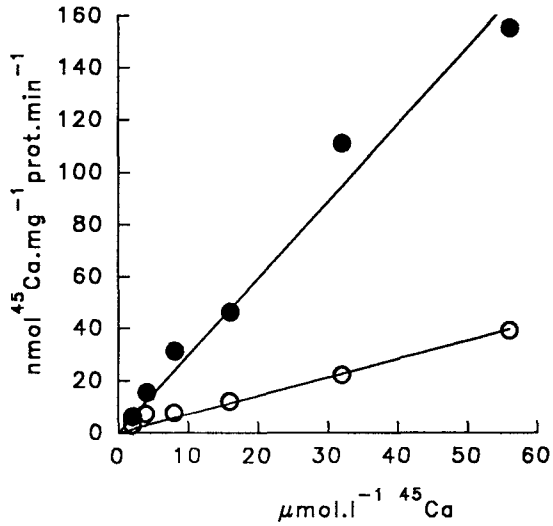

Figure 3. Inhibition of the Na^+-Ca^{2+} exchange in crayfish striated muscle microsomal membranes in the choline (●) and the choline:potassium medium (1 : 1) (○) by increasing concentrations of $^{45}Ca^{2+}$ in the medium. Each point represents the mean value of 4 measurements.

nal medium. The inhibition of $^{45}Ca^{2+}$ uptake by potassium ions did not depend on the pH of the external media, (data not shown).

Our results indicate that, in addition to Na^+-Ca^{2+} exchange, another sodium dependent unsaturable system is involved in calcium translocation across the membrane in the rat brain and the crayfish striated muscle.

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