Na^+—Ca^{2+} Exchange in the Rat Brain During Ontogeny

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Abstract. A rise of Na^+—Ca^{2+} exchange during ontogenic development was found in the rat brain which parallels brain maturation. Nerve endings are the main structure which contributes to the rise of the exchange activity.

Key words: Na^+—Ca^{2+} exchange — Brain maturation

Introduction

Na^+—Ca^{2+} exchange has been studied in detail in nerve tissue synaptosomal and microsomal preparations (Blaustein and Oborn 1975; Coutinho et al. 1983; Schellenberg and Swanson 1981), the exchanger from the nerve tissue was reconstituted into arteficial phospholipid vesicles (Schellenberg and Swanson 1982), partially purified (Barzilai et al. 1984) and identified immunologically using antibodies against the proteins suggested to be the components of the exchanger (Barzilai et al. 1987). No data are available concerning the Na^+—Ca^{2+} exchange activity during brain maturation. Since the rat brain undergoes morphological, metabolic and functional maturation after the birth only (Davison and Dobbing 1968), we decided to follow the Na^+—Ca^{2+} exchange activity during ontogenic development in rat brain homogenates as well as in brain subcellular fractions.

Materials and Methods

Chemicals: $^{45}CaCl^2$ (Institute of Radioisotopes of the Hungarian Academy of Sciences), Tris-base, 2-mercaptoethanol, bovine serum albumin, choline chloride (Serva), SDS, sucrose (Merck), scintillation cocktail (Spolana); all other chemicals of analytical grade were from Lachema Brno.

Preparation procedure: Rat brain hemispheres pooled from 3—10 animals of various ages were homogenized by 20 strokes in a glass homogenizer with a 0.5 mm slit between the wall and the pestle, in isolation medium of the following final composition (in $mmol.l^{-1}$): NaCl 200, Tris-HCl 20, pH 8,

2-mercaptoethanol 5. The tissue to medium ratio was 1:10 (w:v). The homogenates were used for Na^+—Ca^{2+} exchange measurements. In another series of experiments the hemispheres of the same litters were homogenized in the same way in a medium containing (in mmol.l^{-1}): sucrose 300, EDTA 0.1, EGTA 0.1, 2-mercaptoethanol 5, TrisHCl 20, pH 7.4. The homogenates were centrifuged at $1400 \times g$ for 20 min in a K24D centrifuge. The sediments were used for measurements of Na^+—Ca^{2+} exchange, and the supernatant was further centrifuged for 30 min at $10,000 \times g$. The sediment, denoted as the crude mitochondrial fraction (CM), was rehomogenized in 0.3 mol.l^{-1} sucrose, pH 8, and layered on the top of a discontinuous sucrose density gradient (0.8—1.2 mol.l^{-1}, 12.5 ml of each concentration) and centrifuged for 1 hr at 100,000xg in a Beckman L8—80 ultracentrifuge, rotor SW 28. The layers (myelin, synaptosomes and mitochondria; Orlický et al. 1981) obtained at the limits of the gradients were sucked off into Pasteur pipettes, diluted with an equal volume of 5 mmol.l^{-1} Tris-HCl pH 8, and centrifuged at $100,000 \times g$ for 30 min. The obtained pellets were stirred in 200 mmol.l^{-1} NaCl pH 8, and were used for Na^+—Ca^{2+} exchange measurements.

Microsomal membrane fraction was prepared by centrifugation of postmitochondrial supernatant for 1 hr at $100,000 \times g$. The obtained pellet was stirred in 200 mmol.l^{-1} NaCl pH 8 and tested for Na^+—Ca^{2+} exchange.

Na^+—Ca^{2+} exchange measurements: $^{45}CaCl_2$ accumulation was estimated in 50 μl of homogenate or subcellular fraction suspensions preloaded with NaCl by overnight incubation in NaCl media at 4 °C, containing cca 50—100 μg protein. The specimens were transferred into 2 ml of the medium containing either 200 mmol.l^{-1} choline chloride or NaCl, pH 8; after 15 s incubation at room temperature in the presence of 20 μmol.l^{-1} $^{45}CaCl_2$, the mixtures were rapidly filtered through GF/C glass fibre filters (Whatman) on a Millipore (Aminco) equipment; the filters were washed twice with 3.0 ml of 200 mmol.l^{-1} cold choline chloride or NaCl solution. Then, they were dried in hot air, solubilized in the SLT-41 scintillation cocktail medium and counted in a Rackbeta LKB scintillation counter.

Na^+—Ca^{2+} exchange was taken as the difference of $^{45}Ca^{2+}$ accumulation in specimens transferred into choline medium versus vesicles incubated in sodium medium. Total activities were expressed in nmol of accumulated calcium per gram tissue wet weight per minute, and specific activities in nmol of accumulated calcium per mg protein per minute.

The protein content in the preparations was determined by the method of Lowry et al. (1951) after previous solubilization of the undissolved material with 2 % SDS; bovine serum albumin was used as a standard.

Results

As can be seen from the data shown in Table 1, Na^+—Ca^{2+} exchange is already present in the brain homogenates of 2 days old rats. A sharp increase was then observed in the period between days 5—15 after birth, and a nearly steady state was reached 20 days after birth. Relatively large amounts of the total activity remained in the sediment obtained at $1400 \times g$. This activity in the sediment increased with the increasing age of the animals, whereas the activity in the postnuclear supernant decreased. If one suggests identical subcellular distribution of the Na^+—Ca^{2+} exchange in undisrupted cells of the nuclear fraction as

Table 1. Na^+—Ca^{2+} exchange in the developing rat brain: homogenates (H), nmol.g^{-1} tissue w w.min^{-1} (T.A.); percentages of the T. A. in the nuclear fraction (N) and in the postunclear supernatant (PS). For details see Materials and Methods. All values are means of 2 independent determinations.

Age (days)	H	N	PS	Recovery (%)
2	162	33	71	104
5	227	47	60	107
10	321	56	45	101
15	435	55	42	97
20	480	58	40	98
Adult	445	59	40	99

Table 2. Recalculated values of the Na^+—Ca^{2+} exchange from the data of Table 1 in crude mitochondrial (CM) and microsomal (Mic) fractions of the rat brain during ontogenic development (nmol.g^{-1} tissue w w.min^{-1}). The values are means of 2 independent determinations.

Age (days)	CM	Mic
2	76	97
5	135	105
10	190	151
15	289	109
20	340	70
Adult	366	80

well as in the postnuclear supernatant, then the Na^+—Ca^{2+} exchange activity can be calculated for the tissue as a whole. The calculated values (Table 2) indicate that the increase in Na^+—Ca^{2+} exchange during rat brain maturation is due to the rise of the exchange activity in the crude mitochondrial fraction.

Postnuclear fractions were further separated into crude mitochondrial fraction, its subfractions: myelin, nerve endings and purified mitochondria, as well as the microsomal fraction. As can be seen from the results summarized in Table 3, a total increase of the Na^+—Ca^+ exchange was observed in the crude mitochondrial fraction which parallels the increase of the activity found in the brain homogenates. When the crude mitochondrial fraction was fractionated into myelin, nerve endings and mitochondrial subfractions, an increase of the Na^+—Ca^{2+} exchange was observed in the myelin and nerve endings subfractions. On the other hand, only low activities were found in purified mitochondria, and no relationship was noticed between mitochondrial Na^+—Ca^{2+} exchange and the brain maturation.

Table 3. Na^+—Ca^{2+} exchange in the developing rat brain: crude mitochondrial fraction (CM), subfractions of myelin (My), nerve endings (Ne), purified mitochondrie (M) and microsomal fraction (Mic). T. A. — Na^+—Ca^{2+} exchange in $nmol.g^{-1}$ tissue w $w.min^{-1}$, S. A. — Na^+—Ca^{2+} exchange in $nmol.mg^{-1}$ prot. min^{-1}. The values are means of 2 independent determinations. For details see Materials and Methods.

Age (days)	CM		My		Ne		M		Mic	
	T.A.	S.A.	T.A.	S.A.	T.A.	S.A.	T.A.	S.A.	T.A.	S.A.
2	51	4.4	3.3	2.2	20	4.9	3.5	0.7	65	13.4
5	65	4.6	7.9	3.9	36	7.7	3.6	0.9	70	13.8
10	82	5.1	12.4	4.7	52	8.1	3.1	0.7	65	15.4
15	133	7.2	12.6	8.1	78	8.1	2.7	0.7	50	16.4
20	140	7.7	13.3	8.4	78	8.9	2.0	0.7	30	16.6
Adult	148	7.8	12.5	8.9	80	10.6	2.9	0.7	33	17.1

Discusion

The results presented show that Na^+—Ca^{2+} exchange in the rat brain hemispheres increases during the ontogenic development. This rise in the exchange activity parallels the period of morphological, metabolic and functional maturation of the brain (Davison and Dobbing 1968). A similar pattern exhibited another transport system, ($Na^+ + K^+$)ATPase in the rat brain (Křivánek et al. 1989).

Na^+—Ca^{2+} exchange was found to be present in the nerve tissue cell membranes and in the synaptosomes (Coutinho et al. 1983; Schellenberg and Swanson 1981). From our results it can be concluded that during brain maturation significant differences in the total amount of Na^+—Ca^{2+} exchange occur between the nerve endings and microsomal fraction. Na^+—Ca^{2+} exchange increases in the crude mitochondrial fraction (as expressed per wet weight), whereas simultaneously the total exchange activity in the microsomal fraction decreases as compared with the early postnatal age (Table 2). When the crude mitochondrial fraction was separated into myelin, nerve endings and purified mitochondria subractions, a 3-fold rise of the total activity was found in the myelin and the nerve edings subfraction during ontogenic development, but no differences were noticed in the purified mitochondria subfraction. We assume that the increase in the Na^+—Ca^{2+} exchange activity in the myelin subfraction is not connected with myelin itself, but is probably related to the existence of axolemmal fragments of myelinated axons as well as microsomal membranes and light nerve endings present in the myelin subfractions (Reiss et al. 1981; Zetusky et al. 1979).

The main growth of the Na^+—Ca^{2+} exchange during the early postnatal period is related to the multiplication of the nerve endings; the Na^+—Ca^{2+} exchange in these preparations increased both when expressed per tissue wet weight and per mg protein, indicating that not only the volume of nerve endings but also the abundance of the exchanger in them increased. The Na^+—Ca^{2+} exchange remained constant in the purified mitochondria subfraction under our experimental conditions, i.e. the forward mode of Na^+—Ca^{2+} exchange. This finding is in agreement with the general view that sodium ions rather extrude calcium from mitochondria (Brand 1985). A small accumulation of ^{45}Ca in purified mitochondria seems to be related to the presence of small amounts of nerve endings in this subfraction as can be deduced from our earlier experiments using the same preparation procedure for the preparation of purified brain mitochondria (Orlický et al. 1981).

A relative decrease of Na^+—Ca^{2+} exchange in the brain microsomal fraction at a simultaneous increase of its specific activity can be explained by the fact that during brain maturation the volume of the nerve endings in the tissue

increases and thus the relative amount of cell body membranes, the second carrier of Na^+—Ca^{2+} exchange in the nerve tissue, decreases.

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