Calcium transport in synaptosomes and subcellular membrane fractions of brain tissue in spontaneously hypertensive rats

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Summary

1. The uptake of Na\(^+\) and Ca\(^{2+}\) by synaptosomes and uptake of Ca\(^{2+}\) by the mitochondria and microsomes of brain tissue of rats with spontaneous hypertension (SH rats) and normotensive Kyoto-Wistar rats (WKY rats) were studied with an isotope-exchange method.

2. By means of inhibitor analysis it has been shown that calcium influx into the synaptosomes during depolarization of their plasma membrane takes place only through the potential-dependent channels in both groups of animals.

3. Basal Ca\(^{2+}\) uptake by the synaptosomes of hypertensive rats was increased, apparently by partial depolarization of the synaptosome membrane caused by the increased membrane permeability to Na\(^+\) (basal Na\(^+\) uptake by synaptosomes was found to be increased in hypertensive rats).

4. Ca\(^{2+}\) uptake by mitochondria of hypertensive rats was increased, and the Ca\(^{2+}\) uptake by microsomes was decreased in these rats compared with controls.

5. The increment of the maximal Ca\(^{2+}\) transport rate in microsomes after the addition of calmodulin was decreased in spontaneously hypertensive rats compared with normotensive animals. Thus alterations in the interaction of calmodulin with the Ca\(^{2+}\)-transporting systems of the plasma membrane are an important part of the widespread membrane defect observed in spontaneous hypertension.

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6. The changes in the Ca\(^{2+}\)-transporting and Ca\(^{2+}\)-regulating systems of the synaptosomes of brain tissue in spontaneously hypertensive rats may be the basis for the increase of the intrasynaptosomal Ca\(^{2+}\) concentration and, in turn, for the alteration in the rate of neurotransmitter release.

Key words: brain, calcium transport, membranes, spontaneous hypertension, synaptosomes.

Abbreviations: TTX, tetrodotoxin.

Introduction

Recent studies have demonstrated abnormalities in the plasma membrane of several types of cells in primary hypertension (both in spontaneous hypertension of rats and in essential hypertension of humans). Altered membrane control over intracellular calcium distribution and increased membrane permeability for univalent cations were observed, particularly, in myocardiocytes and vascular smooth muscle cells of spontaneously hypertensive Kyoto-Wistar rats [1-4] and in adipocytes and erythrocytes in both forms of primary hypertension [5-8]. Thus it has become evident that membrane alterations are widespread in the tissues and that primary hypertension might be considered as a special kind of membrane pathology. Recent findings of decreased Ca\(^{2+}\)-binding ability in plasma membranes of synaptosomes in spontaneously hypertensive (SH) rats [9] supports this point of view.

It is known that the free calcium concentration in nerve endings plays the key role in regulation...
of neurotransmitter release [10]. Ca²⁺ concentration in cytoplasm is determined both by the rate of Ca²⁺ influx during membrane depolarization and by the activity of Ca²⁺-transporting systems. At present there is little information concerning the mechanism of Ca²⁺ influx into nerve endings; in particular, the relative role of Ca²⁺- and Na⁺-channels in the total Ca²⁺ uptake has not been established. Among the systems responsible for Ca²⁺ transport against the electrochemical gradient the leading role of Na⁺-Ca²⁺ exchange has been considered [11]. However, it has been shown that the significance of this system in the maintenance of free Ca²⁺ concentration of the nerve terminal during its depolarization is rather limited [12]. ATP-dependent Ca²⁺-pumps in the plasma membrane and in intrasynaptosomal vesicles, as well as the Ca²⁺-transport system of mitochondria, were considered to be more likely Ca²⁺-buffering systems of nerve terminals [13-15]. The relative roles of these systems in the handling of low concentrations of Ca²⁺ have not been established. In the present work the features of Ca²⁺ uptake by synaptosomes during their depolarization, as well as Ca²⁺ accumulation by subcellular membrane fractions of the brain, have been studied in spontaneously hypertensive rats.

Material and methods

Rats

Sixteen-week-old male Okamoto-Aoki spontaneously hypertensive (SH) rats weighing 230-260 g (blood pressure 170-200 mmHg) and normotensive Kyoto-Wistar rats (WKY rats) of the same age and sex (blood pressure 120-130 mmHg) were used.

Preparation of membrane fractions

Synaptosomes were prepared by a modification of Cotman's method [16]. Rat brain (without cerebellum) was homogenized in 20% (w/v) medium A (0.32 mol/l sucrose, 5 mmol/l HEPES, pH 7.4) at 0-2°C, with Teflon/glass (clearance 0.1-0.08 mm), once or twice at 400 rev./min, and diluted twice with the same medium. The homogenate was centrifuged at 3000 g for 10 min. The supernatant was centrifuged (20 000 g, 15 min) and the pellet was washed with medium A under the same conditions. The sediment was resuspended in 30 ml of medium A and transferred into a discontinuous Ficoll-sucrose density gradient, consisting of 10 ml each of 7.5%, 12% and 17% Ficoll (w/v in medium A). The fractions were separated by centrifugation (25 000 rev./min for 60 min; rotor type SW-27, Beckman, U.S.A.). The fractions between sucrose (0.32 mol/l)-7.5% Ficoll, -7.5-12% Ficoll and -12-17% Ficoll, and the fraction at the bottom (the fractions F₁, F₂, F₃ and F₄ respectively), were washed in 80 ml of 0.32 mol/l sucrose (57 000 g for 30 min). The supernatant obtained after 20 000 g centrifugation was sedimanted (30 000 g for 30 min), yielding a pellet (P₁) and supernatant (S₁). The P₁ pellet was resuspended and washed in 0.32 mol/l sucrose (57 000 g for 40 min): fraction F₅. The S₁ supernatant was centrifuged at 120 000 g for 90 min and washed in 0.32 mol/l sucrose under the same conditions: fraction F₆.

Identification of membrane fractions

Among the fractions F₁-F₆ of WKY rat brain the highest activity of plasma membrane markers (acetylcholinesterase [17], 5'-nucleotidase [18]) was observed in fraction F₂. The activity of mito-

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Protein (% of total)</th>
<th>Relative activities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetylcholinesterase</td>
<td>5'-Nucleotidase</td>
</tr>
<tr>
<td>F₁</td>
<td>4.59</td>
<td>2.7</td>
</tr>
<tr>
<td>F₂</td>
<td>2.1</td>
<td>8.9</td>
</tr>
<tr>
<td>F₃</td>
<td>2.3</td>
<td>6.3</td>
</tr>
<tr>
<td>F₄</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>F₅</td>
<td>1.5</td>
<td>11.5</td>
</tr>
<tr>
<td>F₆</td>
<td>2.9</td>
<td>67.2</td>
</tr>
</tbody>
</table>
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Chromatidial enzyme markers (succinate dehydrogenase [19]) was negligible in fraction F2 and the highest activity was in fraction F4 (Table 1). Data obtained in this study for identification of fractions F2 and F4 are in accordance with the results of other authors [16]. It should be noted that the maximum glucose 6-phosphatase activity [20] was observed in fraction F6, together with high activity of both 5'-nucleotidase and acetylcholinesterase (Table 1). Taking into consideration the data on the distribution of glucose 6-phosphatase activity in membrane fractions of liver and kidney, for which this enzyme was found to be a marker of endoplasmic reticulum [20], it may be suggested that our fraction F6 consisted mostly of endoplasmic reticulum, which contains the enzymes commonly acting as markers for plasma membranes, or non-synaptosomal plasma membranes enriched by glucose 6-phosphatase.

There were no significant differences in the activities of membrane marker enzymes between WKY and SH rats.

On the basis of the data mentioned above, membrane fractions F2 (synaptosomes), F4 (mitochondria) and F6 (microsomes) were used for study of Ca²⁺ transport.

Calcium uptake by synaptosomes

To study the kinetics of Ca²⁺ uptake, synaptosomes were preincubated for 10 min (37°C) in the following medium (mmol/l): HEPES-Tris, 20 (pH 7.4); MgCl₂, 135; K₂HPO₄, 0.5; glucose, 10. Protein concentration was 1-2 mg/ml. After preincubation, aliquots of synaptosomes were put in medium B [composition (mmol/l): HEPES-Tris, 20 (pH 7.4); MgCl₂, 1; KCl, 5; NaCl, 135; K₂HPO₄, 0.5; CaCl₂, 1; ⁴⁵CaCl₂, 5 μCi/ml; glucose, 10] or in medium C (the same as medium B, but with NaCl at 50 mmol/l and KCl at 80 mmol/l). In a number of experiments the Na⁺/K⁺ concentration ratio was varied.

Except for synaptosomal membrane depolarization due to the elimination of K⁺-diffusion potential (C media), synaptosomal depolarization was caused either by the addition of veratrine to medium B, leading to the opening of Na⁺-channels, or by ouabain, which depolarizes the membrane by Na⁺,K⁺-dependent ATPase inhibition. Specific Na⁺-channel (tetrodotoxin, TTX) and Ca²⁺-channel (verapamil) blockers were used in the experiments. Inhibitors of electron transport and ATPase of mitochondria (rotenone and oligomycin) were added 2 min before the end of preincubation. Inhibitor concentrations are presented in the Tables and Figures.

After the incubation, 100-150 µg of synaptosome protein was transferred to Millipore HA filters, which had been stored in KCl solution (1 mol/l) and washed just before the experiment with 5 ml of the following medium (mmol/l): NaCl, 150; EGTA, 1; Tris-HCl, 10 (pH 7.1; 0-2°C). The samples on the filters were washed three times with 5 ml of the same medium.

Sodium uptake by synaptosomes

To determine Na⁺ uptake synaptosomes were put in medium B containing 0.3 mmol of ouabain/l, where ⁴⁵CaCl₂ was substituted for ²²NaCl (20 µCi/ml). After 0.5, 10 and 30 min of incubation an aliquot of synaptosome suspension containing 2 mg of protein was placed on filters (Whatman, GF/c, U.K.) and washed four times with 5 ml of the same wash medium.

Ca²⁺ accumulation by mitochondria and microsomes

Membrane fraction protein (30-40 µg) was added to 50 ml of medium and incubated at 37°C with periodic shaking. The composition of the incubation medium was (mmol/l): imidazole-HCl, 40 (pH 6.9); H₄, 120; NaCl, 6.5; MgCl₂, 3; K₂HPO₄, 3; ATP-Tris, 4; EGTA, 0.1; CaCl₂, 0.01-0.3; ⁴⁵CaCl₂, 1.5 µCi/ml. Accumulation of Ca²⁺ by the mitochondria was determined in the presence of 4 mmol of Tris-succinate/l. Free calcium concentration in the incubation medium was calculated from the value $K_s [Ca-EGTA]^{2-} = 1.3568 \times 10^4$ mol/l [21]. After the incubation, the suspension (200 µl) was filtered through Millipore filters (HA) and the filters were washed three times with 5 ml of wash medium. Radioactivity of the filters and in the incubation medium was measured in Bray solution [22]. The kinetics of Ca²⁺ accumulation by the membrane fraction was linear up to 10 min. To determine the rate of Ca²⁺ accumulation the incubation time was limited to 5 min. The dependence of calcium accumulation rate on the Ca²⁺ concentrations was expressed in a Lineweaver-Burk plot. A HP-9815A computer was used to calculate the affinity ($K_m$) and the maximal rate ($V_{max}$) of Ca²⁺ accumulation.

Calmodulin concentration

Concentration of calmodulin in the nerve tissue cytosol (supernatant obtained after the microsome sedimentation) was determined by the increase of ATP-dependent Ca²⁺ accumulation by inside-out vesicles of erythrocyte membranes. Both the
method of preparation of rat erythrocyte ghosts enriched by inside-out vesicles and the method of determination of Ca²⁺ accumulation by these vesicles have been described before [23].

Reagents

TTX, rotenone and oligomycin (Bohringer, F.R.G.), veratrine (active principle of veratridine) (Sigma, U.S.A.) and verapamil (LEX, Yugoslavia) were used in this study. Calmodulin was kindly provided by Dr. V. A. Tkachuk (Biochemistry Department of Moscow University).

**Results**

**Effect of depolarizing agents on Ca²⁺ accumulation by synaptosomes**

Addition of veratrine (70 µg/ml) to medium B or changes in the K⁺/Na⁺ concentration ratio (medium C) increased Ca²⁺ uptake by synaptosomes 2–2.5-fold (Fig. 1). The maximum effect of potassium depolarization was observed at 3–5 min and, for veratrine depolarization, at 15 min of incubation (Fig. 1). Ouabain, a Na⁺,K⁺-ATPase inhibitor, caused an increase in Ca²⁺ uptake. The addition of TTX to medium B para-

![Graph](image)

**TABLE 2. Effect of blocking agents on calcium uptake by synaptosomes**

Means ± SE are given. The time of incubation was limited to 5 and 15 min in media B and C respectively. TTX, Tetrodotoxin.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Blocking agent</th>
<th>Ca²⁺ (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WKY rats</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 5)</td>
</tr>
<tr>
<td>B (low potassium)</td>
<td>TTX (500 nmol/l)</td>
<td>1.72 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Verapamil (100 µmol/l)</td>
<td>1.68 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Oligomycin (2 µg/ml) + rotenone (2 µg/ml)</td>
<td>1.55 ± 0.10</td>
</tr>
<tr>
<td>B + ouabain (0.3 mmol/l)</td>
<td>TTX (500 nmol/l)</td>
<td>2.68 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Verapamil (100 µmol/l)</td>
<td>2.03 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td>B + veratrine (70 µg/ml)</td>
<td>TTX (500 nmol/l)</td>
<td>4.26 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Verapamil (100 µmol/l)</td>
<td>1.68 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>1.58 ± 0.09</td>
</tr>
<tr>
<td>C (high potassium)</td>
<td>TTX (500 nmol/l)</td>
<td>4.81 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Verapamil (100 µmol/l)</td>
<td>4.70 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>1.53 ± 0.10</td>
</tr>
</tbody>
</table>

* Difference between SH and WKY rats with P < 0.01.
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tially decreased the Ca²⁺ uptake by synaptosomes caused by ouabain and completely blocked veratrine-induced Ca²⁺ uptake. There was no effect of TTX on K⁺-induced Ca²⁺ uptake (Table 2). Verapamil not only abolished the effect of all depolarizing agents on Ca²⁺ uptake by synaptosomes, but also decreased basal Ca²⁺ uptake in medium B by 10–15%. Oligomycin and rotenone, depolarizing the mitochondrial membrane, led to a drastic decrease both in basal and in veratrine- or K⁺-induced Ca²⁺ uptake (Fig. 1b).

Basal Ca²⁺ uptake by synaptosomes of hypertensive rats was 70% higher than that of normotensive control rats (Table 2). The differences disappeared only after verapamil treatment.

The dependence of Ca²⁺ uptake by synaptosomes on the K⁺/Na⁺ concentration ratio is shown in Fig. 2. Differences of Ca²⁺ uptake between synaptosomes from hypertensive and normotensive rats were revealed at moderate (up to 60 mmol/l) potassium concentrations, where Ca²⁺ uptake by synaptosomes of SH rats was considerably higher.

The effect of ouabain on Ca²⁺ uptake by the SH rat synaptosomes was greater than that observed in synaptosomes of normotensive rats (Table 2). In SH rats the magnitudes of ouabain-, veratrine- and potassium-induced Ca²⁺ uptake were practically the same. The differences in the ouabain effect on synaptosomes of both groups of animals remained after addition of TTX, whereas they were not observed after treatment with verapamil.

![Graph](image1)

**Fig. 2.** Dependence of calcium uptake by synaptosomes on the potassium/sodium concentration ratio in the incubation medium. The number of observations is 5 for each group. The brain tissue from eight rats was used for each observation.

![Graph](image2)

**Fig. 3.** Dependence of the rate of calcium accumulation by mitochondria on the free calcium concentration in the incubation medium. The number of observations is 4 for each group. The brain tissue from eight rats was used for each observation.

Ca²⁺ accumulation by mitochondria and microsomes

The affinity of the Ca²⁺-transporting system of mitochondria for Ca²⁺ in hypertensive rats was approximately twice that in normotensive rats (1.03 ± 0.08 μmol/l and 0.58 ± 0.60 μmol/l respectively; Fig. 3). The maximal rate of Ca²⁺ accumulation by mitochondria of both groups did not differ (Table 3). The affinity of the Ca²⁺-transporting system of microsomes for Ca²⁺ was thrice that of mitochondria (Fig. 4, Table 4). On the other hand, the maximal rate of Ca²⁺ accumulation by microsomes was about one-twentieth of that of mitochondria. The addition of calmodulin led to an increase both in the maximal rate of Ca²⁺ accumulation by microsomes of normotensive rats and in the affinity for Ca²⁺. The effect of calmodulin on the Ca²⁺ pump of SH rat microsomes was negligible (Fig. 4, Table 4).

Calmodulin content

The content of calmodulin in cytosol of brain tissue (supernatant obtained after sedimentation of microsomes) did not differ in the two groups of
FIG. 4. Dependence of the rate of calcium accumulation by microsomes on the free calcium concentration in the incubation medium: (1) without calmodulin; (2) with calmodulin (0.8 μmol/l). The number of observations is 4 for each group. The brain tissue from eight rats was used for each observation.

TABLE 3. Affinity for Ca\(^{2+}\) (K\(_{m}\)) and the maximal rate (V\(_{max}\)) of calcium accumulation by rat brain mitochondria

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>K(_{m}) (μmol/l)</th>
<th>V(_{max}) (nmol min(^{-1}) mg(^{-1}) of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 WKY rats</td>
<td>4</td>
<td>1.03 ± 0.08</td>
<td>9.93 ± 1.20</td>
</tr>
<tr>
<td>2 SH rats</td>
<td>4</td>
<td>0.58 ± 0.06</td>
<td>10.73 ± 1.41</td>
</tr>
<tr>
<td>P (1 vs 2)</td>
<td></td>
<td>&lt; 0.001</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

n is the number of observations. The brain tissue from eight rats was used for each observation. Means ± SE are given. N.S., Not significant.

animals. In both the addition of 3 μl of cytosol to the 500 μl of the incubation medium led to a 5.0-5.2-fold increase in the rate of Ca\(^{2+}\) accumulation by the inside-out vesicles of the erythrocyte membranes. The same increase in the rate of Ca\(^{2+}\) accumulation could be produced by the addition of 1.5 nmol of bovine brain calmodulin (Fig. 5).

Discussion

Fraction F\(_2\), which has been characterized as synaptosomes, was enriched by enzyme markers of plasma membranes and contained the minimal admixtures of mitochondria and endoplasmic reticulum (Table 1). This conclusion is in agreement with the practical absence of effect of ATP (1 mmol/l) on \(^{45}\)Ca uptake by fraction F\(_2\) (data are not presented). Fraction F\(_1\), which was similar to fraction F\(_2\) in 5'-nucleotidase activity, consists mostly of myelin [16].

The increase in Ca\(^{2+}\) uptake caused by potassium depolarization was completely inhibited by verapamil (Table 2). The Na\(^+\)-channel inhibitor TTX did not affect potassium-stimulated Ca\(^{2+}\) uptake; this agrees with previously published results [24]. Ouabain, the Na\(^+\),K\(^+\)-ATPase inhibitor, is capable of lowering the membrane potential (by 10-20 mV) by abolishing its electrogenic component dependent on the Na\(^+\) pump [25]. Such a decrease of potential apparently leads to the opening of a part of the potential-dependent Na\(^+\)-channels and to still greater membrane depolarization, which in turn causes the opening of Ca\(^{2+}\)-channels in the plasmalemma and leads to Ca\(^{2+}\) influx into the nerve endings (synaptosomes). The partial decrease of Ca\(^{2+}\) accumulation by synaptosomes under the effect of TTX, as well as its complete disappearance due to verapamil, support this explanation (Table 2).

Increases in Na\(^+\) permeability, induced by veratrine, give rise to synaptosome depolarization, which in its turn leads to the opening of potential-dependent Ca\(^{2+}\)-channels in the membrane and, as a result, to an increase in Ca\(^{2+}\) uptake. The alternative pathway is Ca\(^{2+}\) entry into synaptosomes through potential-dependent Na\(^+\) channels. TTX prevented the effect of veratrine (Table 2). However, verapamil also completely abolished the veratrine-induced Ca\(^{2+}\) uptake (Table 2). If Ca\(^{2+}\) influx occurred (if only in part) along the TTX-sensitive Na\(^+\)-channels, verapamil would not be able to abolish the veratrine effect completely.

Thus analysis with several inhibitors has shown that Ca\(^{2+}\) influx into synaptosomes in all types of membrane depolarization used in the study occurs only through potential-dependent Ca\(^{2+}\)-channels.

The differences in Ca\(^{2+}\) uptake by synaptosomes in SH rats and control rats were observed in the absence of depolarizing agents or during...
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TABLE 4. Effect of calmodulin on the affinity for Ca\textsuperscript{2+} (K_m) and the maximal rate (V_{max}) of calcium accumulation by rat brain microsomes

n is the number of observations. The brain tissue from eight rats was used for each observation. Means ± SE are given. N.S., Not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Without calmodulin</th>
<th>With calmodulin (0.8 \textmu mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_m (\textmu mol/l)</td>
<td>V_{max, \text{K}_m} (nmol min\textsuperscript{-1} mg\textsuperscript{-1} of protein)</td>
</tr>
<tr>
<td>1 WKY rats</td>
<td>0.334 ± 0.034</td>
<td>0.582 ± 0.036</td>
</tr>
<tr>
<td>2 SH rats</td>
<td>0.289 ± 0.061</td>
<td>0.397 ± 0.05</td>
</tr>
<tr>
<td>P (1 vs 2)</td>
<td>N.S.</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

FIG. 5. Dependence of the rate of Ca\textsuperscript{2+} uptake by inside-out vesicles of the rat erythrocyte membrane on calmodulin (1) or supernatant of WKY (2) and SH (3) rat brain tissue content in 0.5 ml of the incubation medium. The incubation medium also contained imidazole-HCl (50 mmol/l) (pH 6.9), KCl (100 mmol/l), MgCl\textsubscript{2} (5 mmol/l), EGTA (0.1 mmol/l), ATP (disodium; 4 mmol/l) and \textsuperscript{45}CaCl\textsubscript{2} (0.04 mmol/l) (for details see [23]). The number of observations is 4 for each group. The brain tissue from eight rats was used for each observation.

moderate plasma membrane depolarization (by means of Na\textsuperscript{+},K\textsuperscript{+}-ATPase inhibition and by increases in K\textsuperscript{+} concentration in the incubation media up to 50–60 mmol/l). Since these differences disappeared after verapamil action, it could be suggested that they were determined by the increase in SH rat synaptosomes of Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+}-channels owing to partial membrane depolarization.

Partial synaptolemma depolarization might result from the decrease of K\textsuperscript{+} diffusion potential (due to the decreased intrasynaptosomal K\textsuperscript{+} concentration) or from the increased permeability of the synaptolemma to Na\textsuperscript{+}. To examine the latter suggestion, the kinetics of \textsuperscript{22}Na influx into synaptosomes were studied. The results given in Fig. 6 show that Na\textsuperscript{+} uptake rate by synaptosomes in the absence of depolarizing agents (medium B) is considerably higher in hypertensive rats than in control animals.

Thus there are grounds to believe that altered permeability of the synaptolemma for Na\textsuperscript{+} in SH rats leads to its depolarization and, as a result, to an increased Ca\textsuperscript{2+} influx into nerve endings.

The high affinity constant for Ca\textsuperscript{2+} (K_m = 1.03 \textmu mol/l) and the high maximal Ca\textsuperscript{2+} accumulation rate (V_{max} = 9.2 nmol min\textsuperscript{-1} mg\textsuperscript{-1} of protein) enable mitochondria to compete effectively with Mg\textsuperscript{2+},Ca\textsuperscript{2+}-ATPase of the plasma membrane for cytosolic ionized calcium, during the early stages of depolarization. Additional data concerning the leading contribution of mitochondria to the regulation of intrasynaptosomal Ca\textsuperscript{2+} concentration during depolarization were obtained in
experiments with rotenone and oligomycin. The addition of these electron-transport inhibitors of mitochondria reduced both basal Ca\(^{2+}\) accumulation by mitochondria and the effect of all depolarizing agents used in the study (Fig. 1). These results are in good agreement with the data of Akerman & Nickolls [13].

The other membrane fraction of nerve tissue cells in our study possessing Ca\(^{2+}\) accumulating ability was the microsome fraction. Besides the high activity of marker enzymes of the plasma membrane (5'-nucleotidase and acetylcholinesterase), high activity of the endoplasmic reticulum marker (i.e. glucose 6-phosphatase) was observed in this fraction (Table 2). A membrane fraction similar in enzyme marker composition was obtained by Schellenberg & Swanson [26]. It should be noted that the affinity of the Ca\(^{2+}\)-transporting system of microsomes for Ca\(^{2+}\) (\(K_m = 0.33 \mu\text{mol/l}\)) (Fig. 4, Table 4) is similar to that found by Blaustein et al. for the fraction of lysed synaptosomes [27]. We suggest that the latter fraction may contain a microsome admixture. It should also be noted that the increase of Ca\(^{2+}\) accumulation by the microsome fraction was observed during K\(^+\)-induced depolarization and the existence of the Na\(^+\)-Ca\(^{2+}\) exchange system was shown to be identical with that described for synaptosomes [26]. In our study there was increased Ca\(^{2+}\) uptake by microsomes after the addition of calmodulin (Table 4). Previously the calmodulin effect on Ca\(^{2+}\) uptake has been demonstrated in inside-out vesicles of the presynaptic membranes [15]. Hence we assume that the properties of the Ca\(^{2+}\)-transporting system of both the synaptoplemma and microsomes are similar (if not identical). Therefore we can evaluate the contribution of plasma membrane and of mitochondria in Ca\(^{2+}\)-transport regulation in depolarized synaptosomes. The values of \(K_m\) and \(V_{\text{max}}\) for Ca\(^{2+}\)-transporting systems of mitochondria and microsomes suggest that mitochondria play the leading role in the maintenance of low intrasynaptosomal Ca\(^{2+}\) concentration.

Together with increased calcium influx in synaptosomes, defects of both mitochondrial and microsomal Ca\(^{2+}\)-transporting systems were observed in the brain tissue of spontaneously hypertensive rats. Whereas Ca\(^{2+}\) uptake by mitochondria of hypertensive rats was higher than that of normotensive animals, in microsomes of SH rats it was, on the contrary, reduced. Because of this the part played by changes in subcellular calcium handling in determining the rate of the neurotransmitter release by nerve terminals of spontaneously hypertensive rats may be obtained only by direct investigations. In hypertension the increment of the maximal Ca\(^{2+}\)-transport rate after calmodulin action was lowered both in microsomes of brain tissue (Table 4) and in erythrocytes [28]. This suggests that the altered interaction between calmodulin and membrane Ca\(^{2+}\)-transporting systems is an important part of a widespread membrane defect in spontaneous hypertension. The molecular mechanism of this defect, which also reveals itself by the increase of plasma membrane permeability for univalent cations [7, 8, 14, 18], the decrease of Ca\(^{2+}\)-binding ability [6, 9], has been investigated. The results of the present study, as well as the investigations carried out on the adipose tissue of SH rats [5], indicate, however, that the membrane defect in primary hypertension is not limited to plasma membranes but appears also in mitochondria.

References


