Estradiol Affect Na-dependent Ca^{2+} Efflux From Synaptosomal Mitochondria

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Abstract. The effects of gonadal steroid hormone, 17β-estradiol (E_2), *in vitro* on rat brain mitochondria Ca^{2+} movement were investigated. Intrasynaptosomal mitochondria Ca^{2+} uptake *via* an energy-driven Ca^{2+} uniporter have K_m = 112.73 ± 7.3 μmol.l^{-1} and V_{max} =21.97 ± 1.7 nmol $^{45}Ca^{2+}$ mg^{-1}. Ca^{2+} release trough a Na^+/Ca^{2+} antiporter was measured with a K_m for Na^+ of 43.7 ± 2.6 mmol.l^{-1}, and V_{max} of 1.5 ± 0.3 nmol $^{45}Ca^{2+}$ mg^{-1}. Addition of estradiol in preincubation mixture did not affect the uptake of Ca^{2+} mediated by the ruthenium red-sensitive uniporter, while it produced biphasic effect on Na-dependent Ca^{2+} efflux. Estradiol at concentrations up to 1 nmol.l^{-1} decreased the efflux significantly (63% inhibition with respect to the control), and at concentrations above 10 nmol.l^{-1} increased it exponentially. The maximum inhibiting concentration of estradiol (0.5 nmol.l^{-1}) increased the affinity of the uniporter (K_m reduced by about 30%), without affecting significantly the capacity (V_{max}) for Na^+. The results presented suggest that estradiol inhibits Na-dependent Ca^{2+} efflux from mitochondria and acts on mitochondrial retention of Ca^{2+}, which may modulate mitochondrial and consequently synaptosomal content of Ca^{2+}, and in this way exerts its role in the homeostasis of calcium in nerve terminals.

Key words: Mitochondria — Ca^{2+} transport — Estradiol — Synaptosomes

Introduction

Maintaining Ca^{2+} homeostasis is of great importance for the normal functioning of cells especially the excitable ones. In neurons, neurotransmitter release and other specialized cell functions are associated with changes in free cytosolic Ca^{2+} concentration (Smith and Augustine 1988; Nicholls 1989; Miller 1991). Besides two membrane mechanisms for Ca^{2+} extrusion, Na^+/Ca^{2+} exchange (Blaustein and Ector 1976; Rahamimoff and Spanier 1979; Metlaš et al. 1988) and ATP-driven Ca^{2+} ef-

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flux (Gill et al. 1984; Horvat and Metlaš 1984; Peković et al. 1986), cytosolic Ca^{2+} concentration can be maintained by sequestration to the endoplasmatic reticulum (Hankart et al. 1978; McGraw et al. 1980). Additionally, the role of mitochondria in Ca^{2+} buffering has been suggested from experiments on neurons (Duchen et al. 1990; Thayer and Miller 1990; Werth and Thayer 1994; White and Reynolds 1995). Influx of Ca^{2+} to mitochondria, which occurs through ruthenium-red sensitive channels, is an electrogenic process driven by the large electrical gradient across the inner mitochondrial membrane, set up through the proton extrusion by the electron-transport chain (Rottenberg and Marbach 1990). An increase in extramitochondrial Ca^{2+} in the range of 0.05–1 μmol.l^{-1} should bring about a proportional increase in matrix calcium (Garcia et al. 1996). The efflux of calcium from brain mitochondria is an Na-dependent, electroneutral process mediated by antiporter (Na^+/Ca^{2+} exchanger); to a lesser extent calcium may exit mitochondria by an Na-independent mechanism (Gunter and Pfeiffer 1990). It is established that the Na^+/Ca^{2+} exchange through the inner mitochondrial membrane has a role in maintaining a low intramitochondrial Ca^{2+} concentration (Li et al. 1992; Cox and Matlib 1993).

It is well known that steroid hormones regulate Ca^{2+} transport in various tissues and cell types (Batra 1986; Nikezić et al. 1988, 1996, 1997; Blackmore et al. 1990; Horvat et al. 1991; Foresta et al. 1993). Thus, we have recently demonstrated that estradiol 17β-benzoate and progesterone *in vivo* (Nikezić et al. 1988; Horvat et al. 1991) and estradiol *in vitro* (Nikezić et al. 1996, 1997) modulate both, the voltage dependent and the Na-dependent Ca^{2+} transport in rat brain synaptosomes. The described effects of the steroids, or at least some of them, appear to be non-genomic and mediated *via* plasma membrane binding sites (Duval et al. 1983; Horvat et al. 1995). The aim of the present work was to examine if estradiol exerts its effect on synaptosomal mitochondria Ca^{2+} transport, and whether it transfers in this way its message to the mitochondrial compartment. Thus, the Ca^{2+} influx and the Na-dependent Ca^{2+} efflux, were investigated in mitochondria isolated from synaptosomes of female rat brain in the absence and presence of estradiol *in vitro*.

Materials and Methods

Mature, cycling and chronically ovariectomized (3 weeks prior to use) female Wistar rats were used in experiments. The animals were kept in constant conditions (light on: 5–17 h, and temperature 24 ℃) and had free access to food and water.

$^{45}CaCl_2$ (specific activity 68 mCi/nmol) was purchased from New England Nuclear Chemical Co. 17β-estradiol and other chemicals were purchased from Sigma Chemical Co., St. Louis, USA or Calbiochem-Boehringer, Mannheim, Germany. Cellulose nitrate filters (pore size 0.45 μm) were obtained from Whatman Limited, England.

Preparation of synaptosomal mitochondria

The mitochondria used for Ca^{2+} transport measurements were prepared from puri-

fied female rat brain nerve endings (synaptosomes). The animals were sacrificed by cervical dislocation. The whole brains were rapidly removed and homogenized in ice-cold buffered sucrose (0.32 mol.l^{-1} sucrose, 5 mmol.l^{-1} Tris-HCl, pH 7.4). The synaptosomes were isolated and purified from pools of brain tissue (6 brain/pool) according to the method of Cotman and Matheus (1971). Brain homogenate was centrifuged at 1,000×g for 10 min, the pellet was resuspended in the same volume of buffered sucrose and centrifuged at the same conditions. Resulting supernatants from both centrifugations were pelleted at 12,500×g for 20 min. The pellet was suspended in buffered sucrose and layered on two-step discontinuous Ficoll gradient, consisting of 13% and 7.5% (w/v) Ficoll in sucrose-EDTA (0.32 mmol.l^{-1} sucrose, 10 mmol.l^{-1} Tris-HCl, pH 7.4 and 50 mmol.l^{-1} K-EDTA) buffer. After centrifugation at 65,000×g for 55 min the interphase between 7.5% and 13% Ficoll was collected and suspended in sucrose-EDTA buffer. The obtained purified synaptosomes in the pellet after centrifugation at 12,000×g for 30 min were lysed in hypotonic buffer containing 5 mmol.l^{-1} Tris-HCl, pH 7.6 and freezed at −20 °C. The synaptosomal lysate was used for mitochondria preparation, according to the procedure of Lai and Clark (1970). After thawing and homogenizing, the synaptosomal lysate was centrifuged at 11,800×g for 10 min. The pellet was resuspended in 5 mmol.l^{-1} Tris-HCl, pH 8 and centrifuged at 8,300×g for 10 min, the pellet was resuspended to a final volume of 10 ml in 3% Ficoll medium (3% Ficoll, 0.12 mol.l^{-1} mannitol, 0.03 mol.l^{-1} sucrose, 25 mmol.l^{-1} K-EDTA, pH 7.4). This suspension was carefully layered onto a discontinuous gradient consisting of 5 ml 4.5% and 10 ml 6% Ficoll in 0.24 mol.l^{-1}, 0.06 mol.l^{-1} sucrose, 50 mmol.l^{-1} K-EDTA pH 7.4, and centrifuged for 30 min at 11,500×g. The pellet was saved on ice and layers of 4.5% and 6% Ficoll were collected, suspended in buffered sucrose and centrifuged at 12,000×g for 10 min. The obtained pellet and the pellet from former centrifugation were pooled and suspended in 10 ml buffered sucrose and centrifuged for 10 min at 9,800×g. The pellet containing purified synaptosomal mitochondria (termed by authors SM and SM_2), was suspended in 0.3 mol.l^{-1} mannitol. Aliquots of the suspension were rapidly frozen and stored at −70 °C until use. According to the authors mitochondria obtained in this way have no requirement for BSA to show respiratory activity and control. Protein concentration was determined by the method of Lowry et al. (1951).

Purity of mitochondrial preparation

The purity of the mitochondrial preparation was estimated by measuring F1/F0-ATPase activity, (specific marker of mitochondrial membranes) in the absence and presence of different ATPase inhibitors. The assay was performed according to Martinez et al. (1995). Enzyme activity was determined by colorimetric determination of inorganic phosphate (Pi) liberated from ATP hydrolysis. A typical incubation mixture for F1/F0-ATPase activity measurement contained 10 mmol.l^{-1} Tris-HCl, pH 8.0, 2 mmol.l^{-1} $MgCl_2$, 2 mmol.l^{-1} ATP and 130 μg of mitochondrial proteins in the final volume of 0.5 ml. The mixture was preincubated 10 min at 30 °C with or without ATPase inhibitors. Addition of 2 mmol.l^{-1} ATP started the enzyme

assay and the reaction was stopped after 10 min by adding 55 μl of 3 mol.l^{-1} perchloroacetic acid and immediate cooling in ice-water. Concentrations of Pi were measured according to the method of Pennial (1966). The inhibitors used were 1 mmol.l^{-1} ouabain (specific inhibitor of membrane Na^+/K^+-ATPase), 1 mmol.l^{-1} NaF/theophylline (inhibitor of nonspecific alkaline phosphatase), and oligomycine (75 μg/mg protein) as specific inhibitor of F1/F0-ATPase.

Mitochondrial Ca^{2+} transport

Ca^{2+} uptake: Synaptosomal mitochondria were preincubated at 22 ℃ for 10 min in uptake medium (MUM) which contained (in mmol.l^{-1}): 300 mannitol, 10 KCl, 1 maleate, 5 glutamate, 10 Tris-HCl, pH 7.4, in a final volume of 200 μl. After preincubation, uptake of Ca^{2+} was initiated by adding 0.6 μCi $^{45}CaCl_2$ containing $CaCl_2$ (25–300 μmol.l^{-1}); it was let to run for 1–60 min. The reaction was stopped by adding 10–15 μmol.l^{-1} (17.5 μg/mg protein) ruthenium red and 2 ml of 0.2 mol.l^{-1} sucrose. Aliquots of 1 ml were vacuum – filtered through 0.45 μm pore size cellulose-nitrate filters and washed two times with 3 ml of 0.2 mol.l^{-1} sucrose. Ca^{2+} retained in mitochondria was calculated from radioactivity counting corrected for radioactivity in the filter blank (without mitochondria) and nonspecific $^{45}Ca^{2+}$ binding (measured immediately after addition of Ca^{2+}) and calculated as pmol/mg protein. Effects of estradiol on mitochondrial Ca^{2+} uptake were examined by incubating mitochondria in the presence of 1×10^{-11}–1×10^{-6} mol.l^{-1} estradiol dissolved in 0.1% ethanol. The corresponding control was run in the presence of 0.1% ethanol alone.

Ca^{2+} efflux: Loading mitochondria with Ca^{2+} was performed for 5 min in the presence of 200μmol.l^{-1} $CaCl_2$ (0.6 μCi $^{45}CaCl_2$), uptake of Ca^{2+} was stopped by adding 10–15 μmol.l^{-1} (17.5 μg/mg protein) ruthenium red. Ca^{2+} efflux was initiated by adding NaCl (0–250 mmol.l^{-1}) and 0.2 mmol.l^{-1} EDTA for 1–30 min. Ca^{2+} retained by mitochondria was calculated from radioactivity counting (corrected for blank without mitochondria and nonspecific binding). Na-dependent Ca^{2+} efflux was calculated by subtracting Ca^{2+} concentration retained in mitochondria after addition of Na/EDTA from Ca^{2+} concentration in mitochondria after addition of ruthenium red (no Na/EDTA). The effect of estradiol on Na-dependent Ca^{2+} efflux was measured by incubating Ca^{2+}-preloaded mitochondria with E_2 (5×10^{-12}–1×10^{-6} mol.l^{-1}) for 10 min and initiating efflux by NaCl and EDTA for 5 min.

Results

Lai and Clark (1970) showed in their procedure for mitochondrial isolation little contamination by synaptosomes. The purity of our isolated synaptosomal mitochondria preparation (contamination by synaptic membranes) was estimated by measuring the activity of F1/F0-ATPase in the presence of specific ATPase inhibitors (Table 1). In the presence of oligomycin less than 30% residual activity was detected, while 1 mmol.l^{-1} ouabain decreased the enzyme activity up to 10%, probably due to some membrane contamination. Finally, theophylline and sodium

Table 1. F1F0-ATPase activity in the presence of inhibitors

Inhibitors	Inhibition (%)
Control	0
Ouabain	7.36±3.19
NaF/theophylline	3.76±1.25
Oligomycine	71.25±2.37

ATPase activity was assayed in the incubation mixture containing 130 μg of mitochondrial proteins for 10 min. The mixture was preincubated for 10 min at 30°C without or with ouabain (0,2 mmol.l^{-1}), NaF/theophylline(1 mmol.l^{-1}/1 mmol.l^{-1}) and oligomycine (75 μg/mg of mitochondrial protein) as indicated in Materials and Methods. The results are presented as % of inhibition with respect to control values (means of three determinations ± S. D.).

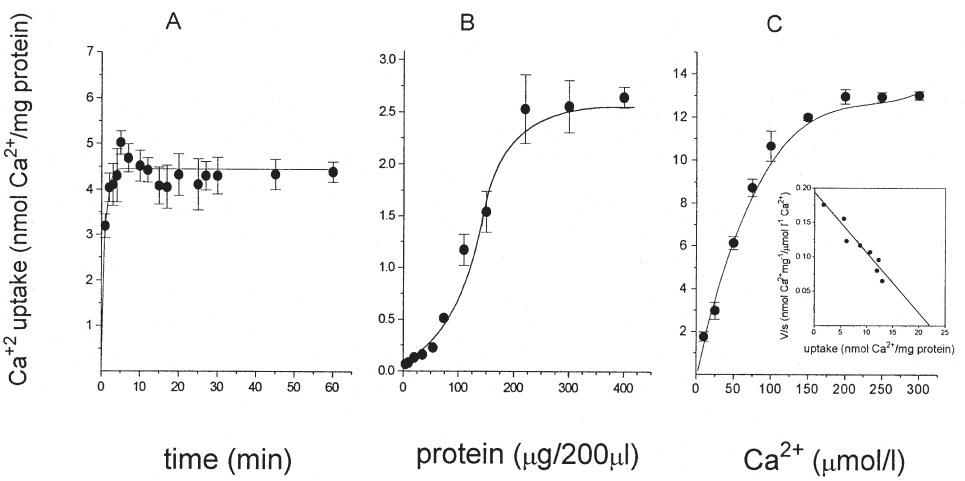

Figure 1. Uptake of Ca^{2+} by synaptosomal mitochondria. Mitochondria were incubated at 22°C in the presence of $^{45}CaCl_2$ (0.6 μCi) in a final volume of 200 μl for: *A*) various times in the presence of 0.1 mg/ml of mitochondria and 100 μmol.l^{-1} $CaCl_2$; *B*) 5 min in the presence of 100 μmol.l^{-1} $CaCl_2$ and various concentrations of mitochondria; *C*) 5 min in the presence of 0.8 mg/ml of mitochondria and various concentrations of $CaCl_2$. The mitochondrial content of Ca^{2+} was measured and calculated as indicated in Materials and Methods. Results shown are mean ± S.E. from four to five separate experiments in triplicate determinations.

fluoride reduced F1/F0-ATPase activity less than 5%. The purity of the mitochondrial preparation, estimated on the basis of enzymatic markers, was deemed to be satisfactory for further work.

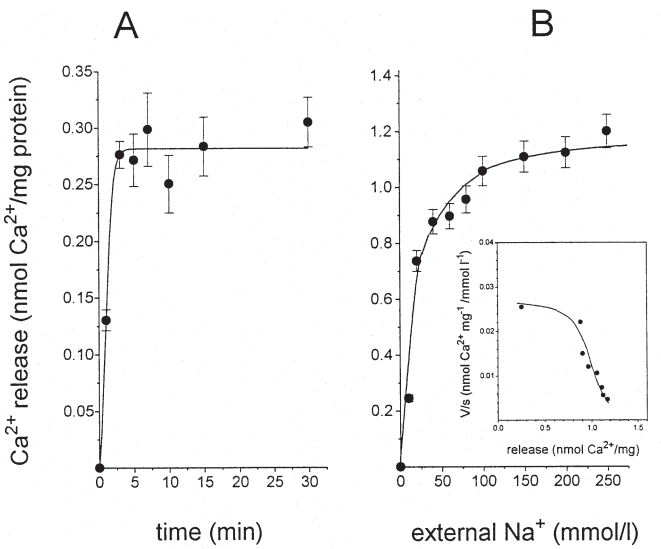

Figure 2. Na-dependent Ca^{2+} release from mitochondria. Synaptosomal mitochondria (0.8 mg/ml) were preloaded with Ca^{2+} for 5 min in the presence of 200 μmol.l^{-1} $CaCl_2$ (0.6 μCi $^{45}CaCl_2$), uptake of Ca^{2+} was stopped by adding 17.5 μg/mg protein of ruthenium red. After 2 min Na-dependent Ca^{2+} release was initiated by adding *A*) 20 mmol.l^{-1} NaCl and 0.2 mmol.l^{-1} EDTA, and was left to run for various periods of time; *B*) various concentrations of NaCl (0–250 mmol.l^{-1}) and 0.2 mmol.l^{-1} EDTA for 5 min (inset represents the Scatchard plot). Released Ca^{2+} by mitochondria was calculated from radioactivity counting as indicated in Materials and Methods. Results shown are mean ± S.E. from five separate experiments in triplicate determinations.

Mitochondrial Ca^{2+} transport

Ca^{2+} uptake. The dependence of mitochondrial Ca^{2+} influx with respect to the duration of incubation, mitochondrial content and extramitochondrial Ca^{2+} concentration was determined in order to establish the optimum conditions for uptake of Ca^{2+} by synaptosomal mitochondria, which occurs through ruthenium red-sensitive uniporter. As can be seen from Fig. 1*A*, 1 min after addition of Ca^{2+} (100 μmol.l^{-1}, 0.6 μCi $^{45}Ca^{2+}$) to the incubation medium, mitochondria filled about 60% of their capacity for Ca^{2+} whereas the ability of mitochondria to take up Ca^{2+} was saturated after 5 min. After 5 min of incubation, Ca^{2+} uptake was in direct relation to the mitochondrial content up to 1 mg/ml of protein concentration (Fig. 1*B*). The dependence of the uniporter on extramitochondrial Ca^{2+} concentration (Fig. 1*C*) has a hyperbolic shape with a saturation plateau in the presence of 200 μmol.l^{-1} $CaCl_2$ (40 nmol). Ca^{2+} uptake was non-cooperative with a K_m =112.73 ± 7.3 μmol.l^{-1} and V_{max}=21.97±1.7 nmol Ca^{2+} mg^{-1} protein (Fig. 1*C*, inset Scatchard plot).

Ca^{2+} efflux. Ca^{2+} efflux of preloaded mitochondria (1 mg ml^{-1}) 5 min. in the

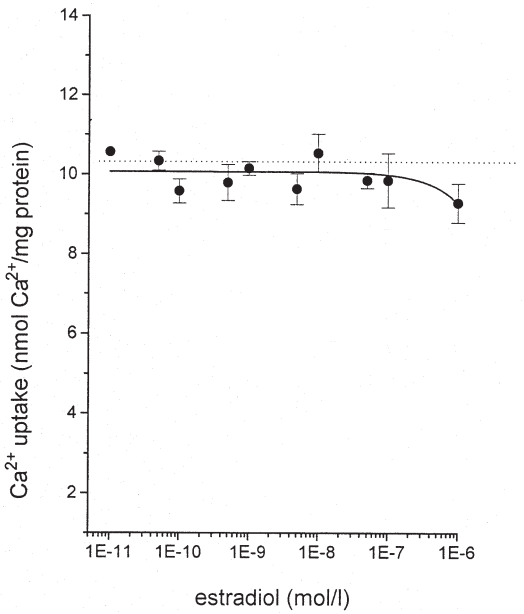

Figure 3. Effect of estradiol *in vitro* on mitochondrial Ca^{2+} uptake. Mitochondria (0.65 mg/ml) were preincubated in the presence of estradiol for 10 min at 22 ℃ in a final volume of 200 μl. Uptake of Ca^{2+} (200 μmol.l^{-1}, 0.6 μCi) was performed as indicated in Materials and Methods. Results shown are mean ± S.E. from five separate experiments in triplicate determinations (the dotted line represents control values).

presence of 200μmol.l^{-1} external Ca^{2+}, was examined with respect to duration of incubation and concentration of external Na^+, $[(Na^+)_o]$. The divalent cation chelator, EDTA, was included since Na-stimulated Ca^{2+} efflux was greatest in the absence of external Ca^{2+}/Mg^+ (Clark and Roman 1980). In the presence of 20 mmol.l^{-1} external NaCl, efflux of Ca^{2+} was very rapid and reached its maximum after 3 min (Fig. 2*A*). Ca^{2+} efflux was directly proportional to $[(Na^+)_o]$ concentration up to 100 mmol.l^{-1} (Fig. 2*B*). The hyperbolic shape plot with a plateau in the presence of 100 mmol.l^{-1} NaCl reached a V_{max} of 1.5 ± 0.3 nmol Ca^{2+} mg^{-1} mitochondrial protein while the calculated value of K_m for Na^+ was 43.7 ± 2.6 mmol.l^{-1}, as estimated from Scatchard plot (Fig. 2*B*, inset).

Effect of estradiol

Ca^{2+} uptake. Effect of various concentrations of estradiol *in vitro* on intrasynaptosomal mitochondria Ca^{2+} uptake is presented in Fig. 3. As can bee seen, after 10 min. of preincubation, E_2 at concentrations up to 1 μmol.l^{-1} was unable to modify mitochondrial Ca^{2+} uptake through ruthenium red-sensitive uniporter compared to the control values. The capacity and affinity of the uniporter for Ca^{2+} were

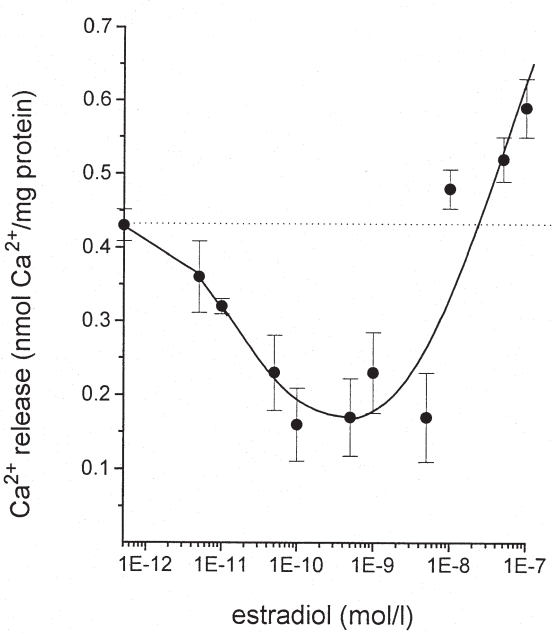

Figure 4. Effect of estradiol *in vitro* on mitochondrial Ca^{2+} release. Mitochondria (0.5 mg/ml) preloaded with 200 μmol.l^{-1} $CaCl_2$ (0.6 μCi) for 5 min, after addition of ruthenium red (0.01 mmol.l^{-1}), were incubated for 10 min. in the presence of various concentrations of estradiol. Na-dependent release of Ca^{2+} was initiated by 20 mmol.l^{-1} NaCl and 0.2 mmol.l^{-1} EDTA. Released Ca^{2+} was calculated as indicated in Materials and Methods. Results shown are means from six experiments (triplicate determination) $\pm$ S.E. (the dashed line represents control values).

similar in control conditions and under the influence of estradiol (data not shown). It may thus be concluded that Ca^{2+} uptake was not affected by E_2.

Ca^{2+} efflux. Na-dependent Ca^{2+} efflux in the presence of various concentrations of E_2 was measured in $^{45}Ca^{2+}$ preloaded synaptosomal mitochondria. E_2 had a biphasic effect on Na-dependent Ca^{2+} efflux (Fig. 4). Estradiol in near-physiological conditions (up to 1 nmol. l^{-1}) decreased Ca^{2+} efflux up to 63%. In the presence of higher E_2 concentrations Ca^{2+} efflux recovered to control levels and started rising exponentially at concentrations higher than 10 nmol.l^{-1}. Comparing the estimated capacity of the exchanger for Ca^{2+} efflux and the affinity for external Na^+ in the absence and presence of E_2 (0.5 nmol.l^{-1}), a slight increase in V_{max} (1.5 $\pm$ 0.3 and 1.78 $\pm$ 0.14 nmol Ca^{2+} mg^{-1} respectively) and a 25% decrease in K_m (43.7 $\pm$ 2.6 and 31.86 $\pm$ 0.42 mmol.l^{-1} respectively) for Na^+ were seen as compared to control values (Fig. 5). Also, nonlinear Scatchard plot (insets, Figs. 2 and 5) indicates the existence of cooperativity for Na^+.

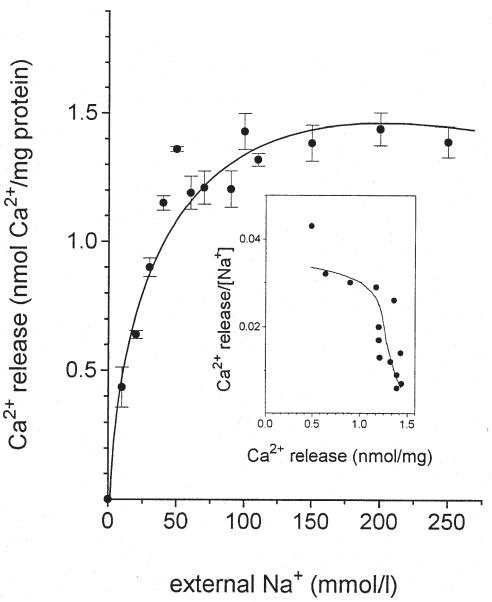

Figure 5. Na-dependence of mitochondrial Ca^{2+} release in the presence of estradiol. Ca^{2+} preloaded mitochondria (as indicated in legend to Fig. 4) were incubated in the presence of 0.5 $nmol.l^{-1}$ estradiol for 10 min. and the release was followed after addition of various concentrations of external NaCl and 0.2 $mmol.l^{-1}$ EDTA. Amounts of released Ca^{2+} were estimated as indicated in Materials and Methods. Results shown are means from five experiments (triplicate determinations) ± S.E. The insert shows the Scatchard plot.

Discussion

The transport of Ca^{2+} into brain mitochondria was examined in the absence and presence of the gonadal steroid hormone estradiol to evaluate if estradiol *in vitro* may modulate Ca^{2+} uptake and efflux to and from synaptosomal mitochondria. Results under control conditions, presented in this paper, were similar to those reported for mitochondria isolated from liver and adrenal medulla (Kraus-Friedman 1995; Palmiero et al. 1995) with respect to affinity for Ca^{2+} and hyperbolic dependence of the uniporter on external Ca^{2+} concentration. Some discrepancies concerning the affinity and capacity of the brain mitochondrial uniporter for Ca^{2+} (Rottenberg and Marbach 1990) probably reflect different sources of the mitochondria (Café et al. 1994); brain "free" mitochondria from male rats used by Café et al. (1994), differ in their properties from female brain synaptosomal mitochondria used in our experiments. Rapid influx of calcium into mitochondria seen in our experiments may have significant effects on cytoplasmic concentrations which are important in stabilizing cytoplasmic calcium during short periods of intense neu-

ronal activity, and may act as a reversible protective system preventing short-term injurious large rises in cytoplasmic free Ca^{2+} (Nicholls 1989). In brain mitochondria Na^+/Ca^{2+} exchange is the main Ca^{2+} operating system, similarly as in other excitable tissues and adrenal medulla (Carafoli 1987; Palmiero et al. 1995). The estimated Hill coefficient > 1 and the upward concavity of the Scatchard plot (this paper) clearly indicate the likelihood that two Na ions are exchanged for one Ca ion by electroneutral exchanger. Similar results have been reported for numerous other tissues (Deliconstantinos 1985; Carafoli 1987; Palmiero et al. 1995).

Preincubation of mitochondria in the presence and absence of E_2, did not affect the estimated affinities and the capacity of the uniporter for Ca^{2+} (K_m =125 and 112 μmol.l^{-1} and V_{max} = 27 and 21 nmol Ca^{2+} mg^{-1} protein, respectively). Since Ca^{2+} uptake occurs through ruthenium red-sensitive uniporter (Rottenberg and Marbach 1990), it may be concluded that E_2 does not affect this mitochondrial Ca^{2+} transport mechanism. In studying Na-dependent Ca^{2+} efflux from $^{45}Ca^{2+}$ preloaded synaptosomal mitochondria it was found that this efflux can be inhibited by nanomolar concentrations of E_2. These concentrations of estradiol augmented the affinity of the exchanger for Na^+ decreasing the value of K_m. The results suggest that E_2 modulates the mitochondrial Na^+/Ca^{2+} antiporter in a competitive manner. Consequently, it could be postulated that E_2, acting directly on the exchanger or indirectly *via* some other membrane constituent, changes the Na-dependent Ca^{2+} efflux. Concentrations of E_2 higher than 10 nmol.l $^{-1}$ potentiate Ca^{2+} release almost exponentially. The stimulatory effect of E_2 could be explained by non-specific effects of E_2 which may act on membrane fluidity similarly as detergents which solubilize membrane proteins (Delikonstantinos 1985, 1990; Schwartz et al. 1996; Nikezić et al. 1997). These findings are in concordance with our finding on synaptic plasma membranes and membrane vesicles (Horvat et al. 1995; Nikezić et al. 1996, 1997), where E_2 had a biphasic effect on Na^+/Ca^{2+} exchanger in potentiating nonspecifically Na/Ca exchange at concentrations higher than 100 nmol.l^{-1}. Specific effects on mitochondrial Ca^{2+} efflux were seen at lower E_2 concentrations than on synaptosomal membranes which is in agreement with the assumption that physiological concentrations of E_2 available to mitochondria are lower than those available to the synaptosomal membrane (Nikezić et al. 1996, 1997), and that mitochondria posses higher affinity for estradiol.

It was shown that an increase of the cytosolic Ca^{2+} concentration up to 1 nmol.l^{-1} might increase the matrix concentration of Ca^{2+} (Garcia et al. 1996). Increases in cytosolic calcium in neurons and synaptosomes produce an enhancement in cell respiration, in NAD-linked dehydrogenase, and respiratory chain activities and in mitochondrial membrane potential (Duchen et al. 1990). Even if estradiol *in vitro* does not influence mitochondrial Ca^{2+} uptake through ruthenium red-sensitive uniporter, as shown in this work, it could indirectly increase the matrix concentration of Ca^{2+}. Indeed, in our earlier work, we found that estradiol increased voltage-dependent uptake of Ca^{2+} (Nikezić et al. 1996) by synaptosomes isolated from rat brain. Estradiol, by indirectly increasing Ca^{2+} content in the matrix and Ca^{2+} retention, may increase the activity of Ca^{2+}-sensitive dehydroge-

nases in intrasynaptosomal mitochondria as has been reported for heart and other tissues (Denton and McCormack 1990; Hansford 1991; Garcia et al. 1996; Matsuda et al. 1997). As a consequence, a change occurs in citric acid cycle flux, resulting in stimulation of ATP synthesis (Aprille 1988; Garcia et al. 1996) which is the mode of action of some hormones (McCormack et al. 1990; Hoek et al. 1995). The inhibition of Na^+/Ca^{2+} exchange by nanomolar concentrations of estradiol can be expected to increase the ability of mitochondria to buffer changes in cellular Ca^{2+}. The results shown in this study, along with the finding that estradiol modulates synaptosomal Ca^{2+} transport (Nikezić et al. 1996, 1997) implicate estradiol as a modulator of Ca^{2+} transport mechanisms in the rat brain which may be the way how it exerts its role in nerve cell homeostasis.

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