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MODULATION OF CA^{2+} ION FLUX THROUGH MITOCHONDRIAL MEMBRANE OF THE RAT BRAIN STEAM SYNAPTOSOMES BY 17β-ESTRADIOL

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Abstract

In the present study the modulation of Ca^{2+} ion flux in the synaptosomal mitochondria isolated from the ovariectyomized rat Brain Steam and the possible roll of membrane bound estradiol was examined. Physiological concentrations of 17β-estradiol binds specifically to isolated mitochondria (V_{max} 3.37± 0.25 pmol/mg protein, K_m 1.85± 0.06 nmol/l of free estradiol). Addition of 17β-estradiol (10 pmol/l - 1 nmol/ l) *in vitro* decreased mitochondrial calcium ion efflux significantly (25%) after 10 minutes. Modulation of calcium ion efflux and mitochondrial ion retention may be the way that 17β-estradiol (E2) exerts its role in the nerve cell homeostasis.

Introduction

The maintaining of Ca^{2+} ion homeostasis is of great importance for the normal functioning of neuronal cells. Significant contribution of mitochondria in shaping intracellular calcium ion concentration ($[Ca^{2+}]_i$) was documented in various neuronal preparations [1]. In mitochondria, Ca^{2+} is taken up via ruthenium-red sensitive uniporter driven by the electrochemical gradient, while Ca^{2+} ion efflux is mainly mediated by Na^+/Ca^{2+} exchanger activity [1]. Steroid hormones can modulate various processes in mitochondria from nervous tissues [2]. Some of these effects seem to be mediated via the mitochondrial membrane. In our previous work specific binding of E2 to mitochondria isolated from nerve cell endings *fom whole rat brain* were found [3]. At the concentrations that specifically bind to mitochondrial membrane, estradiol was found to contribute to the modulation of mitochondrial Ca^{2+} transport. In that way estradiol regulate mitochondrial homeostasis and cytosolic Ca^{2+} concentrations by changing sequestration and release of those ions [3]. In order to examine relations between effects on mitochondrial Ca^{2+} flux and binding to mitochondrial membrane in specific brain area, Brain Stem, in the present study we quantified specific binding sites and concentration-dependent modulation of influx and efflux of Ca^{2+} in the presence of E2.

Experimental

Synaptosomal mitochondria used for E2 binding and Ca^{2+} transport measurements were isolated from the Brain Steam of chronically (3 weeks prior to use)

ovariectomized (OVX) female rats as described previously [4]. For E2 binding and Ca^{2+} transport monitoring mitochondria were preincubated at 22 °C for 10 min in medium containing (in mmol/l): 300 mannitol, 10 KCl, 1 maleate, 5 glutamate, 10 Tris-HCl, pH 7.4. In the cease of binding assay, after preincubation without hormone, mitochondria (0.5 mg/ml) were incubated with (^{3}H)estradiol (0.1-100 nmol/l) an additional 10 min, for total hormone binding. Nonspecifically bound estradiol was determined incubating mitochondria with labeled and 100-fold excess of unlabelled estradiol. Specific hormone binding was calculated by subtracting nonspecific bound from total bound estradiol. The influx of Ca^{2+} to synaptosomal mitochondria was initiated by adding 0.2 mmol/l $CaCl_2$ (0.6 μCi $^{45}CaCl_2$), lasted 5 min and stopped by ruthenium red (17.5 μg/mg protein), a specific inhibitor for Ca^{2+} uniporter. For Ca^{2+} efflux monitoring, mitochondria were loaded with calcium in the same way and after adding ruthenium red the efflux of Ca^{2+} was initiated by adding NaCl (20 mmol/l) and 0.2 mmol/l EDTA and lasted 5 min. The effect of E2 on Na-dependent Ca^{2+} efflux was measured by incubating Ca^{2+}-preloaded mitochondria with 0.5 pmol/l - 50 nmol/l of E2 for 10 minutes.

Results and Discussion

As presented in Fig. 1, E2 specifically binds to synaptosomal mitochondria from BS and this binding reaches plateau in the presence 3.25 nmol/l and higher of E2. Michaelis-Menten plot of specific estradiol binding to mitochondria indicates one binding site with estimated V_{max} of 3.37± 0.25 pmol/mg protein and K_m of 1.85± 0.06 nmol/l free estradiol. Scatchard plot (fig 1 inset) concave upward indicates the existence of positive cooperativity. When compared with our previous results [5], on synaptic plasma membranes from rat BS (capacity of 0.3 pmol/mg and affinity 26 nmol/l free estradiol for high capacity/low affinity site and capacity 0.06 pmol/mg and affinity of 4 nmol/l free estradiol for low capacity/high affinity site) we found on mitochondria one binding site with different binding properies indicating that diferent proteins (receptors) are responsible for specific estradiol binding to synaptosomal plasma membrane and mitochondria of BS.

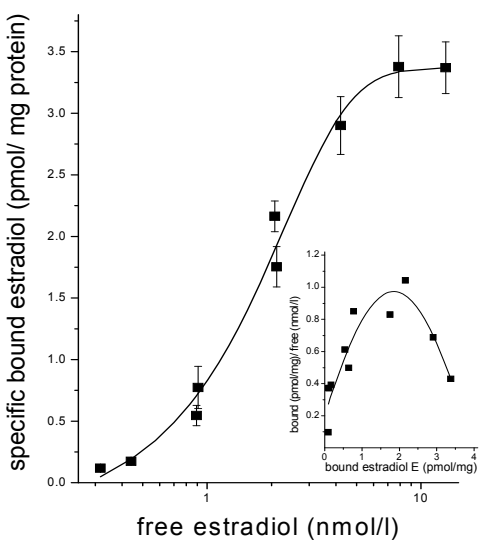

Fig. 1. Specific (^{3}H)estradiol binding to synaptosomal mitochondria isolated from Brain Stem

Ca^{2+} influx through the

Table 1. Dose-dependent effect of estradiol *in vitro* on mitochondrial Ca^{2+} flux (% of inhibition or stimulation of control value)

E2 conc.	Ca^{2+} influx	Ca^{2+} efflux
0.5 pmol/l		- 14.9
1 pmol/l	+ 13	- 13.9
5 pmol/l	0	- 14.9
10 pmol/l	+ 1	**- 22.4**
50 pmol/l	+ 4	**- 27.8**
0.1 nmol/l	- 12.5	**- 24.1**
0.5 nmol/l	- 6	**- 26.6**
1 nmol/l	+ 12	**- 25.9**
5 nmol/l	- 5	- 11.2
10 nmol/l	+ 10	+ 5.6
50 nmol/l	- 2	+ 11.1
0.1 µmol/l	- 11.5	+ 14.8
1 µmol/l	+ 10	+ 13.2
10 µmol/l	- 7	+ 15.1

ruthenium red sensitive uniporter and Na-dependent Ca^{2+} efflux was measured in $^{45}Ca^{2+}$ preloaded synaptosomal mitochondria isolated from the BS, in the presence and absence of E2 *in vitro*. The uniporter, compared to the control values (0.484 nmol Ca^{2+}/mg protein) was unaffected by E2. In the case of Ca^{2+} efflux (control value in the absence of estradiol 0.54 nmol Ca^{2+}/ mg protein), E2 exerts a dose-dependent effect (Table 1.). Estradiol at concentrations up to 5 pmol/l inhibited Ca^{2+} efflux about 15%, while the concentrations between 10 pmol/l and 1 nmol/l decreased Ca^{2+} efflux in the BS mitochondria about 25%. This result is in accordance with our earlier findings on synaptosomal mitochondria isolated from rat brain nucleus caudatus and hippocampus [6]. The estradiol concentrations that affect the BS Ca^{2+} efflux are between E2 concentrations exerting effects on two structures mentioned above. The exsistence of multiple populations of mitochondrial binding sites may reflect the diversity of neuronal cell types and their physiological properties within the brain structures.

Conclusion

Ca^{2+} transport in Brain Steam mitochondria can be modulated by estradiol. While influx of Ca^{2+} was unchanged, inhibition of Ca^{2+} efflux was detected. The estradiol decrease mitochondrial Na^{+}/Ca^{2+} exchanger activity, at the same concentrations at which it bound specifically to mitochondria, possibly acting *via* mitochondrial membrane binding sites for estradiol.

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