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FLUX OF Ca^{2+} IONS IN THE SYNAPTOSOMAL MITOCHONDRIAL MEMBRANE

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Abstract

In the present study, the flux of Ca^{2+} ions in the synaptosomal mitochondrial membrane isolated from the whole brain and hippocampus of chronically ovariectomized female rats was examined. Under basal conditions no significant difference was found. Addition of estradiol (0.5 nmol/l) in the preincubation mixture decreased significantly (25%) Na-dependent Ca^{2+} efflux in mitochondria from both sources which may be the way that it exerts its role in nerve cell homeostasis.

Introduction

The 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 [1]. Beside two membrane mechanisms for Ca^{2+} extrusion, Na^{+}/Ca^{2+} exchange and ATP-driven Ca^{2+} efflux, the role of mitochondria in Ca^{2+} buffering has been suggested [2]. The influx of Ca^{2+} in 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 [3].The efflux of calcium from brain mitochondria is an Na-dependent, electroneutral process mediated by antiporter, Na^{+}/Ca^{2+} exchanger [4]. In order to compare the influx and efflux of Ca^{2+} in mitochondria isolated from synaptosomes of the whole female rat brain and hippocampus were investigated. The effect of estradiol *in vitro* was found.

Experimental

Synaptosomal mitochondria used for Ca^{2+} transport measurements were isolated from the whole brain and hippocampus of chronically (3 weeks prior to use) ovariectomized (OVX) female rats as described previously [5]. Isolated synaptosomal mitochondrial pellets were suspended in 0.3 mannitol and kept at –20 °C until use. For Ca^{2+} transport monitoring mitochondria were preincubated at 22 °C for 10 min in medium containing (in mM): 300 mannitol, 10 KCl, 1 maleate, 5 glutamate, 10 Tris-HCl, pH 7.4. The influx of Ca^{2+} to synaptosomal mitochondria was initiated by adding 0.2 mM $CaCl_2$ (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 (20mM) and 0.2mM EDTA and lasted 5 min. Aliquots of 1ml, before and after addition of Na/EDTA, were vacuum-filtered and

washed on cellulose-nitrate filters. The influx of Ca^{2+} (pmol/mg protein) in mitochondria was calculated from radioactivity counting in samples just after addition of ruthenium red. The 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 EDTA). The effect of estradiol on Na-dependent Ca^{2+} efflux was measured by incubating Ca^{2+}-preloaded mitochondria with estradiol 5pM and 0.5nM for 10 min and initiating efflux by NaCl and EDTA for 5 min.

Results and Discussion

In this study, the movement of calcium ions through the mitochondrial membrane was monitored. Mitochondria were isolated from synaptosomes of the whole brain and hippocampus of OVX female rats with the aim to explore the *in vitro* effect of estradiol on Ca^{2+} flux. The hippocampus is the structure of interest since it is filogenetically an old cortical structure, directly involved in memory functions and therefore the brain region with extremely high neuronal activity which could be the reason for intensive movements of ions, especially Ca^{2+} movements.

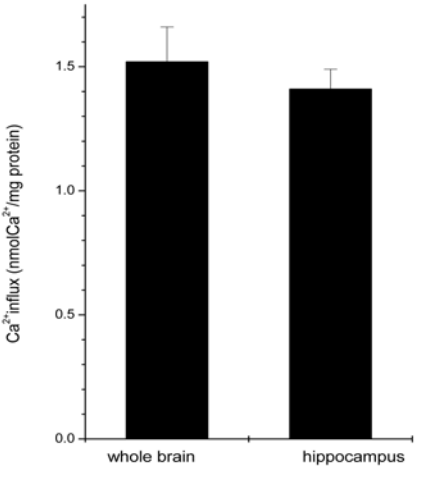

Figure 1. Influx of Ca^{2+} in synaptosomal mitochondria.

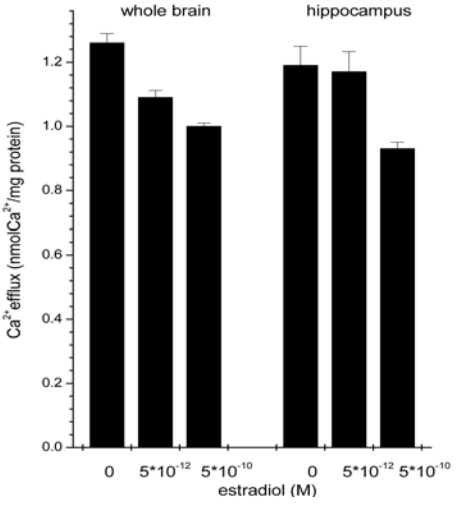

Figure 2. Na-dependent Ca^{2+} efflux from synaptosomal mitochondria.

As presented in Fig. 1, the influx of Ca^{2+} ions in mitochondria from hippocampus (1.41 nmol Ca^{2+}/mg protein) is pretty similar to the one measured in mitochondria from the whole brain (1.52 nmol Ca^{2+}/mg protein). These results may be expected and compared with our previous results on mitochondria from synaptosomes of the whole brain of intact female rats (1.1 nmol Ca^{2+}/mg protein) [6] where a certain decrease in

influx activity is noticeable. Estradiol *in vitro* did not affect the influx of investigated ions (data not shown).

Na-dependent Ca^{2+} efflux was measured in $^{45}Ca^{2+}$ preloaded synaptosomal mitochondria isolated from the whole brain and hippocampus in the presence and absence of estradiol *in vitro*. As shown in Fig. 2. in the absence of estradiol, there was no significant difference in released calcium, 1.26 nmol Ca^{2+}/mg protein for whole brain and 1.19 nmol Ca^{2+}/mg protein for hippocampus, which is in both cases about 85% of preloaded Ca^{2+} content. Estradiol had a different effect on ion efflux depending on its concentration. While the concentration of 5 pmol/l decreased Ca^{2+} efflux in the whole brain mitochondria about 15%, there was no effect in the case of the hippocampus. At 100x higher concentration (0.5 nmol/l) estradiol decreased Ca^{2+} efflux in mitochondria from both sources about 25%.

Conclusion

These results suggesting that the transport of Ca^{2+} ions through ruthenium red sensitive channels and by antiporter, Na^{+}/Ca^{2+} exchanger, in synaptosomal mitochondria isolated from the whole brain and hippocampus are nearly the same under basal conditions (absence of estradiol). Na-dependent Ca^{2+} efflux in hippocampus are probably less sensitive to estradiol when presented in small concentrations. The inhibition of Na^{+}/Ca^{2+} exchanger by 0.5 nmol/l concentration of estradiol can be expected to increase ability of mitochondria to buffer changes in cellular Ca^{2+}.

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References

[1] S.J. Smith, G.J. Augustine,Trends Neurosci. 1988, 11, 458.
[2] M.R. Duchen, M. Valdeolmillos, S.C. O'Neill, D.A. Eisner, J. Physiol. (London) 1990, 424, 411.
[3] H. Rottenberg, M. Marbach, Biochim. Biophys. Acta 1990, 1016, 77.
[4] T.E. Gunter, D.R. Pfeiffer, Amer. J. Physiol. 1990, 258, C755.
[5] J.C.K. Lai, J.B. Clark, Methods Enzymol. 1970, 55, 51.
[6] A. Horvat, S. Petrović, N. Nedeljković, J. Martinović, Gen. Physiol. Biophys 2000, 19, 59.