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Na^+-DEPENDENT Ca^{2+} ION FLUX INHIBITION BY 17 beta-ESTRADIOL IN CAUDATE NUCLEUS MITOCHONDRIA

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Abstract

In this study the Ca^{2+} ion flux modulation in the synaptosomal mitochondria isolated from caudate nucleus (CN) of the ovariectomised rats was examined. 17 beta-estradiol (E2), E2-conjugated to bovine serum albumin (E-BSA), estradiol receptor α (ERα) agonist 4,4',4"-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT), ERβ agonist 2,3-bis(4-hydroxyphenyl)-propionitrile (DNP) and ERα/β antagonist 7α,17β-[9[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17-diol (ICI 182,780) were used. The Ca^{2+} efflux inhibition of about 27% was detected in the presence of 0.5 nmol/l E2, and of about 20% in the case of E-BSA. DNP (10 nmol/l) was as much potent Ca^{2+} efflux inhibitor as E2, while PPT (10 nmol/l) hardly had any inhibitory effect (9% efflux decrease). When E2 binding to ERα and ERβ was prevented by 1 μmol/l ICI 182,780, the Ca^{2+} efflux inhibition of about 15% was detected. Our results suggest that E2 prevents Ca^{2+} efflux from synaptosomal mitochondria due to ERβ activation rather than ERα. The involvement of the external E2 binding site on the mitochondrial membrane probably different from ERα/β should not be excluded because of Ca^{2+} efflux inhibition detected in the presence of E-BSA and ICI 182,780. The Ca^{2+} efflux modulation could be the mechanism through which E2 exerts its neuromodulatory role in specific brain structures.

Introduction

Due to their Ca^{2+} transport mechanisms mitochondria are organelles critical for Ca^{2+} buffering in neuronal cells. The concentration of Ca^{2+} in mitochondrial matrix ($[Ca^{2+}]_m$) regulates the respiration rate, ATP production, reactive oxygen species generation, $\Delta\Psi_m$ collapse occurrence and also, it is a critical trigger for the permeability transient pore opening and therefore for apoptosis [1].

The CN is an important brain structure necessary for learning and memory, particularly regarding feedback processing. Also, it prevents explosive activation of excitatory synapses by measuring the general activity of cerebral cortex and controlling the threshold potential. Permanently high neuronal activity in this brain region could be the reason for intensive movements of ions, especially Ca^{2+} ions.

The steroid hormone E2 could modulate the activity of two main mitochondrial Ca^{2+} transport mechanisms: I) Ca^{2+} influx by the ruthenium red (RR) sensitive uniporter and II) Ca^{2+} efflux by the Na^{+}/Ca^{2+} exchanger. In our previous work using synaptosomal mitochondria isolated from whole rat's brains, the Na^{+}-dependent Ca^{2+} efflux inhibition by physiological E2 concentrations was detected [2]. Therefore, the aim of present study was to determine if E2 could influence mitochondrial Ca^{2+} transport in CN and to estimate the contribution of ERα and/or ERβ in Ca^{2+} transport modulated by E2, using ERs antagonist and specific agonists. The observed effects on mitochondrial functions as well as on mitochondrial Ca^{2+} sequestration might be a consequence of membrane binding sites and/or mitochondrial estradiol receptors (ERα and/or ERβ) activation.

Experimental

Synaptosomal mitochondria used for Ca^{2+} transport measurements were isolated from the CN of ovariectomised (3 weeks prior to use) female rats. For Ca^{2+} transport monitoring mitochondria were preincubated at 22°C for 10 min in medium containing: 300 mmol/l mannitol, 10 mmol/l KCl, 1 mmol/l maleate, 5 mmol/l glutamate, 10 mmol/l Tris-HCl, pH 7.4. The influx of Ca^{2+} to synaptosomal mitochondria was initiated by adding 0.2 mmol/l $CaCl_2$ (0.6 μCi $^{45}CaCl_2$). The reaction lasted 5 min and stopped by RR (17.5 μg/mg protein). For Ca^{2+} efflux monitoring, mitochondria were loaded with Ca^{2+} in the same way and after adding RR, the Ca^{2+} efflux was initiated by adding NaCl (20 mmol/l) and 0.2 mmol/l EDTA and lasted 5 min. The different agents effects on Na-dependent Ca^{2+} efflux were measured by incubating Ca^{2+}-preloaded mitochondria with 0.5 nmol/l E2 (10 min), 10 nmol/l DNP (10 min), 10 nmol/l PPT (10 min) and 1 μmol/l ICI 182,780 (20 min) before efflux initiation.

Results and discussion

In the present study the Ca^{2+} movements through the CN mitochondrial membrane were monitored in order to determine the direct effect of E2 on mitochondrial Ca^{2+} flux.

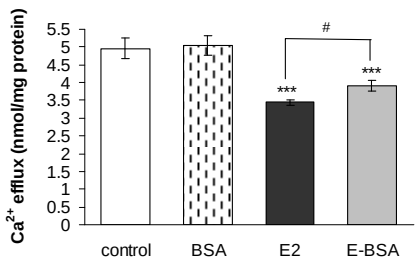

Fig.1. Effects of E2 and E-BSA *in vitro* on mitochondrial Na^{+}-dependent Ca^{2+} efflux.

In our model system no effect of E2 on mitochondrial Ca^{2+} influx that occurs through the RR-sensitive uniporter was detected (data not shown). To verify if E2 affects Na^{+}-dependent Ca^{2+} efflux by acting directly on the mitochondrial membrane, the Ca^{2+}-preloaded mitochondria with 0.5 nmol/l E2 or membrane-impermeable E-BSA before efflux initiation were incubated. The efflux in control mitochondria and in the presence of E2, BSA or

E-BSA is presented in Figure 1. The Ca^{2+} efflux was decreased by 27% in the presence of E2. BSA did not affect Ca^{2+} efflux, while the same concentration of E-BSA reduced Ca^{2+} efflux by 20%, indicating inhibition due to an external binding site on the mitochondrial membrane, independent of E2 diffusion into mitochondrial matrix.

The involvement of mitochondrial ERα/β during E2 modulation of Ca^{2+} efflux, also, was tested. When E2 binding to mitochondrial ERα and ERβ was prevented by ICI 182,780 pretreatment, the E2 inhibitory effect on Ca^{2+} efflux was lowered, but the Ca^{2+} efflux decrease of 15% comparing to control still persisted (Fig. 2). The mitochondria incubation with ERα agonist, PPT, led to no change in Ca^{2+} efflux with respect to control. However, the ERβ agonist DPN was almost as much potent inhibitor as E2 and inhibited Ca^{2+} efflux by 25% (Fig. 3).

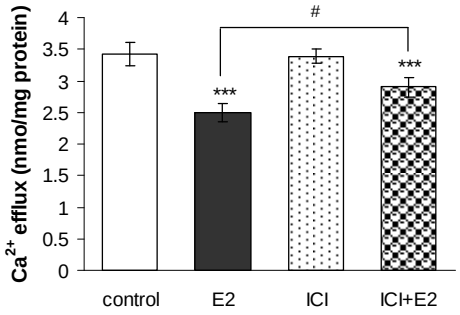

Fig.2. Effects of E2 and ICI *in vitro* on mitochondrial Na^+-dependent Ca^{2+} efflux.

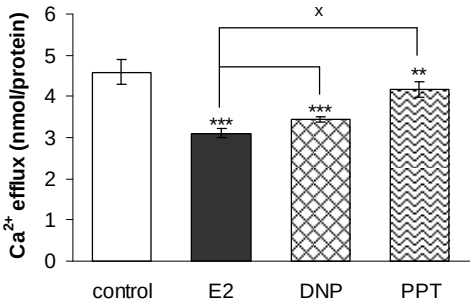

Fig.3. Effects of E2, DNP and PPT *in vitro* on mitochondrial Na^+-dependent Ca^{2+} efflux.

Conclusion

In summary, our results confirm that E2 prevents Ca^{2+} efflux from caudate nucleus synaptosomal mitochondria by I) direct acting on still unknown membrane E2 binding sites (about 20% inhibition) and II) ERβ activation (about 25% inhibition), rather than ERα. Most likely, these are the mechanisms through which E2 could exert its neuromodulatory role in specific brain structures.

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