0014-4754/96/030217-04$1.50 + 0.20

17β-estradiol in vitro affects Na-dependent and depolarization-induced Ca^{2+} transport in rat brain synaptosomes

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Received 5 April 1995; received after revision 14 July 1995; accepted 3 August 1995

Abstract. Effects of 17β-estradiol (E_2) in vitro on Na-dependent Ca^{2+} efflux from, and depolarization-induced Ca^{2+} uptake into, the nerve cell were studied with the use of synaptosomes isolated from the brain stem, mesencephalic reticular formation (MRF), caudate nucleus and the hippocampus of long-term ovariectomized adult female rats. It was found that E_2 (1) at a concentration of 10 nM or lower, stimulates Na-dependent Ca^{2+} efflux in the caudate nucleus and hippocampus, and does not affect the efflux in MRF and brain stem; (2) at concentrations above 10 nM has no effect on the Ca^{2+} efflux in any of the four structures investigated; and (3) produces a biphasic effect on the depolarization-induced Ca^{2+} uptake, increasing it in all structures except MRF at 10 nM concentration, and decreasing it at concentrations higher than 10 nM, irrespective of the structure investigated. These results suggest that E_2, acting at extranuclear sites, modulates synaptic transmission via alterations of Ca^{2+} transport mechanisms in nerve endings.
Key words. Estradiol; calcium transport; synaptosomes; brain; rat.

Steroid hormones have been shown to affect Ca^{2+} transport in various cells and tissues. For instance, in vivo estrogen, alone or in combination with progesterone, increases cellular Ca^{2+} uptake in the rabbit uterus[1], in vitro diethylstilbestrol inhibits the Ca^{2+}-ATPase activity of sarcoplasmic reticulum membranes[2], whereas progesterone and 17α-hydroxyprogesterone stimulate Ca^{2+} influx in human sperm[3,4]. Using isolated ventricular myocytes from guinea pig, Jiang et al.[5] have shown that inward Ca^{2+} current is changed after in vitro addition of E_2. We have recently demonstrated that estradiol 17β-benzoate and progesterone in vivo modulate depolarization-induced Ca^{2+} uptake[6] by, and Na^+-dependent Ca^{2+} efflux[7] from, rat brain synaptosomes. The described effects of the steroids, or at least some of them, appear to be non-genomic[8] and to be mediated via plasma membrane binding sites[9,10] of unknown identity. Deliconstantinos[11] found that testosterone increases, whereas progesterone decreases, the Ca^{2+}-stimulated ATPase activity in synaptic plasma membranes of rabbit brain. The aim of this work was to check other Ca^{2+}-transport mechanisms as possible targets for the central effects of gonadal steroids. Thus, influence of E_2 in vitro on Na-dependent Ca^{2+} efflux and depolarization-induced Ca^{2+} uptake was investigated in synaptosomes prepared from four rat brain regions.

Materials and methods

Three-month-old female Wistar rats, bilaterally ovariectomized (OVX) under ether anaesthesia three weeks before experiments, were used. They were maintained in large open colony cages (6/cage) at constant lighting (lights on: 0500–1700 h) and temperature (24 ± 2 °C) and had free access to food and tap water.

$^{45}CaCl_2$ (specific activity 68 mCi/nmol) was purchased from New England Nuclear Chemical Co. E_2 [1,3,5-(10)-estratriene-3,17β-diol, research grade] was obtained from Serva. All other chemicals were obtained from Sigma Chemical Co. or Calbiochem-Behring. Cellulos filters (pore size 0.45 μm) were purchased from Sartorius.

The animals were killed by decapitation with a guillotine (Harvard Apparatus), and fresh brain stems, MRF, caudate nuclei and hippocampi were dissected out and washed with ice-cold buffered sucrose (0.32 M sucrose containing 5 mM Tris-HCl, pH 7.6). The synaptosomes were isolated and purified from pools of brain tissue (6–12 brain region/pool), using a Ficoll gradient according to the method of Gray and Whittaker[12], as modified by Cotman and Matheus[13]. The purified synaptosomes were suspended in the buffered sucrose as above and diluted to a protein concentration of 1 mg/ml with ice-cold 'physiological' medium (PM) buffer containing (in mM): 4 KCl, 142 NaCl, 2 $MgCl_2$, 10 glucose and 25 Tris-HCl, pH 7.4. Protein concentration was determined by the method of Lowry et al.[14], and modified by Markwell et al.[15].

The synaptosomal suspensions were removed from ice and equilibrated at 36 °C for 10 min in PM buffer. The synaptosomes were preincubated for 15 min[16] in the presence of 10^{-9}, 4×10^{-9}, 10^{-8}, $3–6 \times 10^{-8}$ and 10^{-7} M E_2. The hormone was dissolved in 0.5% ethanol. Control samples were run in E_2-free medium containing

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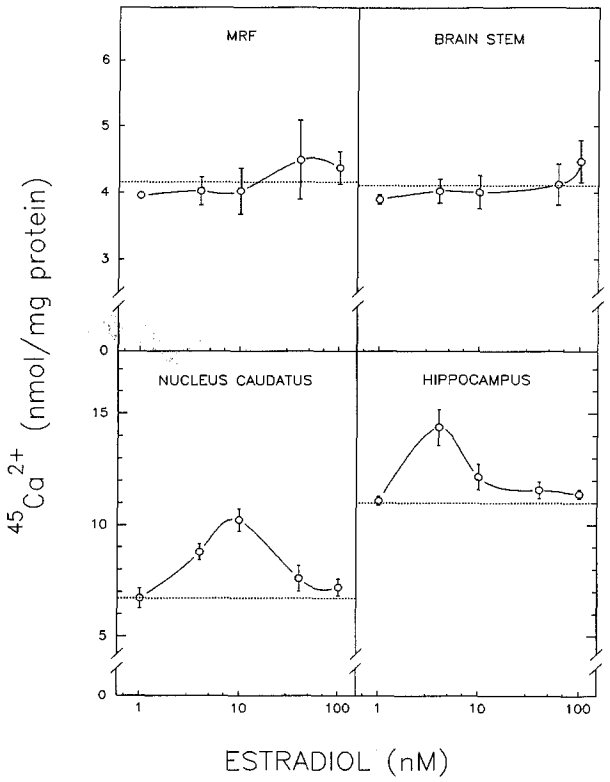

Figure 1. Effect of E_2 on Na-dependent Ca^{2+} transport in rat brain region synaptosomes. The synaptosomes were equilibrated in 142 mM NaCl and preincubated for 15 min in the presence of 10^{-9}, 4×10^{-9}, 10^{-8}, $3\text{–}6 \times 10^{-8}$ and 10^{-7} M E_2. Control samples (dotted lines) were run in E_2-free medium containing 0.5% ethanol. Following preincubation, Ca^{2+} uptake was allowed to proceed in a medium containing 4 mM KCl and 142 mM NaCl and, in addition, 0.6 µCi of $^{45}CaCl_2$. The uptake was carried out at 36 °C for 30 s and terminated by diluting the reaction mixture with 2 ml of ice-cold PM buffer (see 'Materials and methods') containing 2 mM $CaCl_2$. The synaptosomes were harvested by vacuum filtration on 0.45 µm pore size membrane filters which, after washing to remove free $^{45}Ca^{2+}$, were transferred into scintillation vials for radioactivity counting. Circles represent means of five experiments (triplicate determinations in each), and vertical lines represent ±SEM.

0.5% ethanol. After preincubation, samples were chilled on ice, and used immediately for Ca^{2+} uptake determination.

The Na-dependent Ca^{2+} uptake was measured in a medium containing 4 mM KCl and 142 mM NaCl[7]. The procedure was based on an earlier finding that, under resting conditions, Ca^{2+} uptake by synaptosomes is directly related to the Na^+-gradient formed during preincubation[17]. Since $[Na^+]_0$ in our experiments was constant, any change in the capacity of synaptosomes to take up $^{45}Ca^{2+}$ could be attributed to the hormone action during preincubation. The depolarization-induced Ca^{2+} uptake was induced by 50 mM KCl in the medium in which $[Na^+]$ was decreased by an equivalent amount.

The reaction mixtures for both Na-dependent and depolarization-induced Ca^{2+} uptake contained the same components as PM buffer and, in addition, 0.5 mM $CaCl_2$ (0.6 µCi of $^{45}CaCl_2$; specific activity 68 mCi/nmol). The uptake was initiated by the addition of 50 µl of synaptosomal suspension into 150 µl of the reaction mixture. The uptake was carried out at 36 °C of 30 s and was terminated by diluting the reaction mixture with 3 ml of ice-cold PM buffer containing 2 mM $CaCl_2$. The reaction mixtures were passed rapidly through 0.45-µm pore size membrane filters, which were then vacuum-washed with 5 ml of the same buffer and transferred from samples containing 4 mM KCl. Depolarization-induced Ca^{2+} uptake was estimated from the net ΔK-stimulated influx of Ca^{2+}, calculated by subtracting synaptosomal Ca^{2+} uptake in 4 mM KCl from the uptake in 50 mM KCl. The results were expressed as nmol Ca^{2+}/mg protein (mean ± SE of triplicate determinations), based on the specific radioactivity of $^{45}Ca^{2+}$ in the medium.

Results

Effect of E_2 on Na-dependent Ca^{2+} efflux. E_2 in near-physiological concentrations (up to 10 nM) influenced Na-dependent Ca^{2+} uptake only in synaptosomes isolated from caudate nucleus and hippocampus. Thus, significant increase of the Ca^{2+} uptake was seen following treatment of hippocampal synaptosomes with 4 nM E_2 (30% increase of the corresponding control value; fig. 1 lower right), and of caudate nucleus synaptosomes with 10 nM E_2 (50% increase; fig. 1 lower left), whereas these concentrations were ineffective in brain stem (fig. 1 upper right) and MRF (fig. 1 upper left). As can be seen from figure 1, higher concentrations of E_2 (up to 100 nM) had no effect on synaptosomol Ca^{2+} uptake in any of the investigated structures.

Effect of E_2 on depolarization-induced Ca^{2+} uptake. 10 nM E_2 increased synaptosomal Ca^{2+} uptake in hippocampus by 30% in comparison to the corresponding control value (fig. 2 lower right), in caudate nucleus by 93% (fig. 2 lower left) and in brain stem by 37% (fig. 2 upper right), but had no effects in MRF (fig. 2 upper left). As can be seen from figure 2, the presence of higher concentrations of E_2 in the incubation medium (up to 100 nM) caused a decrease of depolarization-induced Ca^{2+} uptake in all the structures. The percent decreases, in comparison to control values, were 33% in hippocampus, 32% in caudate nucleus, 26% in brain stem and 40% in MRF.

Discussion

The results presented in this work show that in vitro E_2 modulates Na-dependent and depolarization-induced Ca^{2+} transport in different regions of the rat brain.

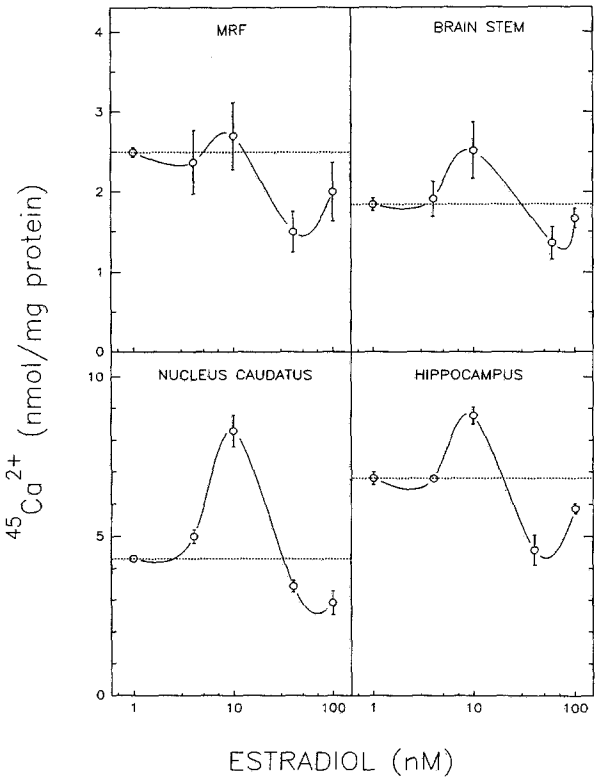

Figure 2. Effects of E_2 on depolarization-induced Ca^{2+} transport in synaptosomes isolated from the MRF, brain stem, caudate nucleus and hippocampus. Synaptosomes were equilibrated, preincubated and processed as described in the legend for figure 1, except that the Ca^{2+} uptake was induced by 50 mM KCl in the medium in which $[Na^+]$ was decreased by an equivalent amount. Depolarization-induced Ca^{2+} uptake was estimated from the net ΔK-stimulated influx of $^{45}Ca^{2+}$, calculated by subtracting synaptosomal Ca^{2+} uptake in 4 mM KCl from the uptake in 50 mM KCl. Circles represent means of five experiments (triplicate determinations in each), and vertical lines represent $\pm$SEM.

Since synaptosomes were used as experimental model, the described in vitro effects were extranuclear.

The considerably stronger effect of E_2 on depolarization-induced Ca^{2+} uptake in caudate nucleus than in hippocampus, although the two structures contain equal numbers of dihydropyridine-sensitive channels[18], supports the view that E_2 modulates depolarization-induced Ca^{2+} influx in synaptosomes via binding sites other than dihydropyridine-sensitive channels[19]. The possibility that these effects are mediated via specific binding sites stems from our recent finding that multiple types of E_2 membrane binding sites are present in rat brain regions[10]. Therefore, regional differences in the depolarization-induced Ca^{2+} transport as a response to E_2 treatment might originate from regional specificities in binding E_2. Two populations of binding sites, one having low and the other high capacity, were found in caudate nucleus, hippocampus, and brain stem[10]. The presence of the two populations of binding sites could account for the observed biphasic effect of E_2 on the Ca^{2+} transport in these structures: the higher concentrations of E_2, at which the hormone binds to the sites of high capacity, decreased the depolarization-induced Ca^{2+} influx, while lower E_2 concentrations, at which low capacity binding sites are saturated, increased the influx. On the other hand, only one population of binding sites, with high capacity, was detected in MRF[10], where E_2 proved to be effective only at higher concentrations.

Regional differences were also observed with respect to E_2 effects on Na-dependent Ca^{2+} tansports. The changes in this transport as a response of E_2 treatment were seen only in caudate nucleus and hippocampus, i.e. in the structures in which E_2 was shown to bind to the sites with low capacity and with positive cooperactivity[10]. E_2 did not affect Na-dependent Ca^{2+} transport in structures in which binding sites with low capacity are negatively cooperative (brain stem) or do not exist (MRF). Basal capacity of the Na^+/Ca^{2+} exchanger also differs between the four structures, as judged from considerably lower control values (without E_2) in brain stem and MRF than in the caudate nucleus and hippocampus.

Modulation of depolarization-induced and Na-dependent Ca^{2+} transport in rat brain region synaptosomes, shown in this study, together with the finding that testosterone increases and progesterone decreases the Ca^{2+}-stimulated ATPase activity in synaptic plasma membranes of rabbit brain[11], implicate gonadal steroids as ubiquitous modulators of Ca^{2+} transport mechanisms in the rodent brain, acting differently according to brain region. The question remains open as to whether these effects on Ca^{2+} transport mechanisms are direct, or secondary in the hormone action at some other site, such as the Na/K-ATPase[20].

Acknowledgments. This research was supported by the Serbain Research Foundation Grant No. 0303.

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