Evidence for suppression of Na-dependent Ca^{2+} efflux from rat brain synaptosomes by ovarian steroids in vivo

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Summary. The possibility that intracellular Ca^{2+}, which mediates neurotransmitter release, regulation of membrane permeability, microtubule polymerization and axonal transport, is influenced by gonadal steroids via a Na-Ca exchange mechanism was examined. The resting Ca^{2+} uptake into synaptosomes was measured using crude synaptosomal pellets (P_2 fraction), isolated from the brain stem, mesencephalic reticular formation (MRF), nucleus caudatus (NC) and the hippocampus of intact, long-term ovariectomized (OVX) and OVX plus progesterone (P) or estradiol-17β benzoate (EB) treated adult female rats. Irrespective of the brain structure investigated, the uptake was 1) markedly increased in synaptosomes from OVX animals in comparison to intact controls, and 2) reduced to near control values in synaptosomes from OVX rats treated s.c. with a single dose of 2 mg P or 5 µg EB. Since Ca^{2+} influx into synaptosomes was shown earlier to depend on external sodium concentration, which was the same in all experiments described in this work, the results obtained indicate that ovarian steroids modulate basal synaptic activity in the rat brain by suppressing Na-dependent Ca^{2+} efflux from the nerve cell.
Key words. Calcium uptake; synaptosomes; rat brain; progesterone; estradiol.

There is evidence that ovarian steroids interact with mechanisms controlling the transport of neurotransmitters and ions across nerve cell membrane. Thus, in vitro estrogens interfere competitively with the high-affinity uptake of norepinephrine [1], dopamine and serotonin [2] by synaptosomes from the rat cerebral cortex. Changes in estradiol levels of female rats may alter the high-affinity transport process regulating acetylcholine synthesis in the cerebral cortex [3]. Estrone and P in vitro decrease the specific activity of the Na^+, K^+-ATPase of synaptosomal plasma membranes from young dog brain [4]. We have recently demonstrated that P and EB affect the voltage-dependent Ca^{2+} uptake by synaptosomes from different rat brain regions [5]. In this work, the influence of P and EB in vivo on Na-dependent Ca^{2+} transport across rat synaptosomal membranes isolated from the brain stem, MRF, NC and the hippocampus was investigated. The experiments were performed on intact, OVX and OVX plus P or EB treated animals, always using the same high [Na^+] during preincubation. This procedure was based on an earlier finding that [Na^+] during preincubation is proportionally related to subsequent Ca^{2+} influx into rat brain synaptosomes [6]. We assumed that, if the ovarian hormone status of the animal was varied, and [Na^+] in the preincubation medium was maintained constant at a high level, any change in the Ca^{2+} influx following preincubation could be attributed to action of hormones in vivo on Na-dependent Ca^{2+} translocation.

Materials and methods

3-month-old female Wistar rats from a local colony were bilaterally ovariectomized under ether anesthesia. 3–4 weeks later, each animal was injected s.c. with a single dose of 5 µg EB in 0.1 ml olive oil or 2 mg P in 0.2 ml olive oil. Control OVX rats received 0.1 ml or 0.2 ml of the carrier alone. 72 h and 24 h following EB and P treatments, in the morning, the animals were decapitated with a guillotine (Harvard Apparatus). The same steroid treatment scheme was shown earlier to be effective on depolarization-induced Ca^{2+} uptake by rat brain region synaptosomes [5].

Brain stems, MRFs, NCs and the hippocampi were dissected from fresh brains and pooled (5/pool) for immediate preparation of synaptosomes. Crude synaptosomal pellet (P_2 fraction) was prepared as described elsewhere [7]. The final synaptosomal pellet was suspended in 0.32 M sucrose at a protein concentration of approximately 10 mg/ml. The synaptosomes were diluted to a protein concentration of 1 mg/ml with a physiological medium (PM) containing (in mM): 4 KCl, 142 NaCl, 2 $MgCl_2$, 10 glucose and 25 Tris-HCl, pH 7.4. Then, at 2-min intervals, the synaptosomal suspensions were preincubated for 10 min at 36 °C, chilled on ice and used for the resting Ca^{2+} determinations. The reaction mixture contained the same components as PM plus 0.5 mM $CaCl_2$ (0.6 µCi of $^{45}CaCl_2$; specific activity 68 mCi/nmol). The reaction was initiated by adding 50 µl of the preincubated ice-cold synaptosomal suspension into 150 µl of the reaction mixture. Uptake was allowed to continue for 30 s at 36 °C, and then terminated by diluting the mixture with 2 ml of ice-cold PM containing 2 mM $CaCl_2$. The reaction mixtures were rapidly vacuum-filtered through 0.45-µm pore size membrane filters, which were then vacuum-washed with 5 ml of ice-cold PM containing 2 mM $CaCl_2$ and transferred to scintillation vials for radioactivity counting. The entire wash procedure lasted approximately 1 min per sample. The results are expressed as nmol Ca/mg protein (mean ± SEM of triplicate determinations), based on the specific radioactivity of $^{45}Ca^{2+}$ in the medium.

Protein concentration was determined by the method of Lowry et al.[8], as modified by Markwell et al.[9]. Effects of treatments on basal $^{45}Ca^{2+}$ synaptosomal uptake were analysed by one-way analysis of variance in combination with Newman-Keuls test, using pools of triplicate determinations from two similar experiments. Differences between treatment means were considered significant at $p \leqq 0.05$.

Results

Effects of long-term ovariectomy. In intact animals, the basal $^{45}Ca^{2+}$ uptake by synaptosomes (in nmol/mg protein) was 3.33 for the brain stem, 3.29 for MRF, 3.45 for NC and 3.97 for the hippocampus (fig. 1). Bilateral ovariectomy resulted in a marked increase in the $^{45}Ca^{2+}$ synaptosomal uptake in all the brain structures investigated. The values were 5.15 nmol/mg protein for brain stem (55% increase of the corresponding value for intact controls; $p < 0.05$), 6.25 for MRF (90%; $p < 0.05$), 7.15 for NC (107%; $p < 0.05$) and 6.36 for hippocampus (60%; $p < 0.05$) (fig. 1).

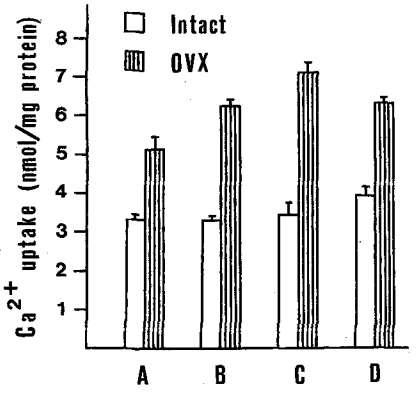

Figure 1. Basal Ca^{2+} uptake by synaptosomes prepared from the brain stem (A), MRF (B), NC (C) and the hippocampus (D) of long-term ovariectomized rats. Columns represent means of two similar experiments with triplicate determinations and lines represent SEM.

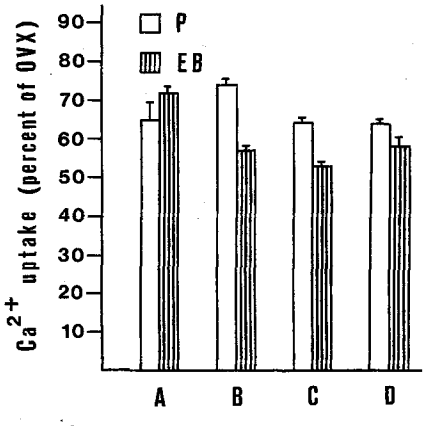

Figure 2. Basal Ca^{2+} uptake by synaptosomes prepared from the brain stem (A), MRF (B), NC (C) and the hippocampus (D) of long-term ovariectomized rats following a single s.c. injection of 2 mg P or 5 µg EB. Columns represent means of two similar experiments with triplicate determinations and lines represent SEM.

Effects of in vivo administration of P or EB. Single s.c. injection of 2 mg P into OVX rats 24 h prior to synaptosome preparation resulted in a pronounced decrease of the $^{45}Ca^{2+}$ uptake by synaptosomes from the brain stem (3.37 nmol/mg protein; 35% decrease in respect to the corresponding value for OVX rats; $p < 0.05$), MRF (4.62; 26%; $p < 0.05$), NC (4.56; 36%; $p < 0.05$) and the hippocampus (4.06; 36%; $p < 0.05$) (fig. 2). Significant inhibition of the basal $^{45}Ca^{2+}$ uptake by synaptosomes from all four structures was also seen following administration of 5 µg EB into OVX animals 72 h before sacrifice; the values obtained were: 3.69 for the brain stem (28% decrease in respect to the corresponding value for OVX rats; $p < 0.05$), 3.56 (43%; $p < 0.05$) for MRF, 3.76 (47%; $p < 0.05$) for NC and 3.67 (42%; $p < 0.05$) nmol/mg protein for the hippocampus (fig. 2).

Discussion

$^{45}Ca^{2+}$ influx into rat brain synaptosomes under resting conditions depends on external sodium concentration during preincubation[6]. Since the $[Na]_o$ was the same in all experiments described in this work, the observed differences in the synaptosomal Ca^{2+} uptake between OVX animals on one side, and intact controls and OVX plus P or EB treated animals on the other, suggest interference of the ovarian steroids in vivo with mechanisms controlling Ca^{2+} translocation. In the absence of ovarian steroids, the extrusion of Ca^{2+} during preincubation increased, resulting in an increased capacity of synaptosomes from OVX rats subsequently to accumulate $^{45}Ca^{2+}$. The suppressive effect of both P and EB on the extrusion of Ca^{2+} from synaptosomes under resting conditions was common for the brain stem, MRF, NC and hippocampus, and was also clear in OVX rats following in vivo P or EB treatment, which brought the synaptosomal Ca^{2+} influx back to near control values.

It seems unlikely that the pronounced differences in the Ca^{2+} uptake can be accounted for by the ATP-dependent Ca-pump, because of its low capacity[10]. Therefore, the Na^+-Ca^{2+} exchanger could be considered as the target for the action of ovarian steroids in maintaining the resting intrasynaptosomal Ca levels. Further studies may reveal whether the observed effect is a direct one on the Na-Ca exchanger, or is mediated via inhibition of Na^+,K^+-ATPase[4]. In conclusion, it appears from this work that ovarian steroids interfere with carrier mechanisms responsible for the transport of calcium ions, in addition to their well-known effects on carrier mechanisms for neurotransmitter uptake[1-3].

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Glycogenolytic effect of vasoactive intestinal peptide in the rat in vivo

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Summary. The effects of VIP (300 pmol/kg), injected via the portal vein, on the glycogen content of the liver and on glycemia, were studied in the rat in vivo. VIP enhanced glycogenolysis and caused hyperglycemia in a time-dependent manner.
Key words. Glycogen; rat; VIP.

Vasoactive intestinal peptide (VIP), a highly basic octacosapeptide originally isolated from porcine small intestine [1], is widely distributed in neurons throughout the body [2]. There is experimental evidence indicating a metabolic role for VIP. In vitro studies have shown that VIP stimulates glycogenolysis in rabbit [3] and rat [4] liver slices, and isolated rat hepatocytes [5-7]. In vivo studies have shown that VIP causes hyperglycemia when administered i.v. to dogs [3, 8] and to man [9]. However, although most metabolic effects of VIP have been studied in rat hepatocytes (in vitro), little is known about its role in the rat in vivo. Therefore, the aim of this paper was to study the glycogenolytic effect of VIP in the rat in vivo. The peptide was injected directly into the superior mesenteric vein of anaesthetized rats to study its effect on hepatic glycogen content, blood glucose levels, and basal plasma insulin and glucagon levels.

Materials and methods

VIP was obtained from Peninsula Laboratories Europe (Merseyside, UK); bovine serum albumin (BSA) from Sigma Chemical (St Louis, Mo, USA). The peptide was dissolved in 0.9% NaCl-1% BSA. Male Wistar rats weighing 250–350 g were used. The animals were fed a standard diet ad libitum. The experiments were performed on anesthetized rats (pentobarbital sodium, 50 mg/kg i.p.) after a short-term fast (4–6 h) in the postabsorptive state. Anesthetized rats were injected with 1 ml VIP solution (300 pmol/kg). Control rats were injected with 0.9% NaCl-1% BSA. As described before [10], pieces of hepatic lobes from the same rat, weighing approximately 0.3 g, were tied off and rapidly excised before injection and at 10 and 20 min after injection, and immediately processed to obtain the glycogen by alcoholic precipitation. Blood samples (0.9 ml) were taken from the jugular vein before and at 5, 10 and 20 min after the injection. The blood obtained was heparinized and 20 µl were taken to measure blood glucose levels, the remaining blood was immediately centrifuged and plasma separated and stored at −20 °C. An ELISA kit (Boehringer Mannheim GmbH, W. Germany) was employed to measure plasma insulin. Radioimmunoassay (kit from Medgenix, Brussels, Belgium) was employed to measure plasma glucagon. Glycogen was determined by enzymatic conversion (amyloglycosidase from Boehringer Mannheim GmbH, W. Germany) to glucose which was determined by the glucose oxidase method. Blood glucose levels were also determined by this method. Student's t-test was used to test the degree of significance.

Results and discussion

For the first time the glycogenolytic effect of VIP in the rat in vivo has been investigated. The injection into the superior mesenteric vein of VIP (300 pmol/kg) produced a decrease in glycogen content of liver. This dose is similar to that employed by other authors for in vitro studies to obtain a maximal glycogenolytic effect [6, 7]. The basal content of hepatic glycogen after 4-6 h fasting was 51.3 ± 5 mg/g liver. The results of glycogenolysis are ex-