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Research report

Effects of 17β-estradiol on the spontaneous activity of substantia nigra neurons: Evidence for a non-genomic mechanism

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Abstract

Clinical and experimental evidence suggests that female sex hormones (estradiol and progesterone) affect structures in the central nervous system that are involved in the control of movement. Using conventional electrophysiological techniques to record extracellular action potentials in the substantia nigra of urethane-anesthetized rats, it was found that microiontophoretic applications of 17β-estradiol were able to modify the spontaneous activity of nigral neurons. 17β-estradiol produced significant changes in the firing frequency (excitation and inhibition) and increased the rhythmicity of the majority of cells studied. These changes appear to be influenced by the sex and the hormonal status of the animal. Effects are of short latency and are not blocked by the administration of tamoxifen. We conclude that estradiol produces changes in the firing rate and discharge pattern of nigral cells in the urethane-anesthetized rat via a non-genomic mechanism.

Theme: Endocrine and autonomic regulation
Topic: Neuroendocrine regulation: other

Keywords: Substantia nigra; Estradiol; Firing pattern

1. Introduction

Malfunctioning of the nigrostriatal system has been implicated in certain disorders such as Parkinson's disease [8,45], chorea, certain dyskinesias [47], schizophrenia and attention deficit hyperactive disorder (ADHD) [42,58].

There are several lines of evidence showing a relationship between the level of female sex hormones (estrogen and progesterone) and the appearance of these disorders. For instance, women show variations in the intensity of motor symptoms during the menstrual cycle [44,54], and a reversible type of chorea can be seen during pregnancy or contraceptive administration [16,26,48,49]. Furthermore, Sydenham chorea is more prevalent in women, especially pregnant women [31], tardive dyskinesia is more frequent in postmenopausal women than in men of comparable age [9,68,69], and Parkinson's disease is more prevalent in men than in women [5,38]. There have also been reports on sex differences in ADHD [4,58].

In vitro and in vivo experiments have shown that sex hormones have effects at different levels of the process of neurotransmission. They can affect neurotransmitter biosynthesis and release, produce allosteric modulation of membrane receptors and influence the density of membrane receptors. Such actions have been described both on hypothalamic and extrahypothalamic areas in the brain such as the hippocampus [32,65,66], mesencephalic dopaminergic systems [2,7,13,17,18,20], the cerebellum and the basal ganglia [6,9,27,35,61–63]. Furthermore, estrogens play a key role in maintaining the integrity of nigral cells [34,58].

Although the mechanism by which these actions take place is not clear, it could be related to the well known effect of steroid hormones on nuclear receptors, the so called genomic effect [40,41]. More recent evidence indicates that

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these hormones can have non-genomic effects probably exerted on the plasma membrane itself [28,50,52] or through interaction with membrane receptors such as the $GABA_A$ receptor [9,27,33,35–37].

As the substantia nigra (SN) appears to be implicated in several motor disorders (see above) and seems to be a target of estrogenic action in the brain [12–14], it was decided to explore the possible effects of iontophoretically applied 17β-estradiol on the discharge pattern of SN cells and look further into the mechanism by which these effects could be mediated.

2. Materials and methods

Fifty Sprague–Dawley rats, 19 male and 31 female, weighing 250–350g were used. Females were subdivided into two groups; 23 intact rats in different phases of the estrous cycle and 8 rats ovariectomized 6 to 8 weeks before the experiments. Animals were housed 4 to a cage with free access to food and water and kept under a 12/12 h light/dark cycle with lights on at 06:00. In intact female rats, the phase of the estrous cycle was assessed by vaginal smears [53]. Experiments were conducted between 8:00 a.m. and 8:00 p.m.

Animals were anesthetized with urethane (1g/kg, I.P.). In experiments in which tamoxifen was administered, the right jugular vein was cannulated. A craniotomy was performed, which allowed recording in the SN at coordinates AP = −4.8 to −5.8; L = 1.5 to 3.0 and V = 7.0 to 8.5 referred to bregma [51]. A cisternal puncture was made to minimize brain movement. Temperature was monitored with a rectal thermometer and kept at 37 ± 0.5 °C by means of a homeothermic blanket.

2.1. Electrophysiology

Recordings were made by means of five-barreled micropipettes made of borosilicate glass; the central barrel of the assembly was filled with a solution of Pontamine Sky Blue in 2% sodium acetate and was used for recording and marking of the recording site at the end of the experiment. The remaining barrels were filled with one of the following solutions: 17β-estradiol hemisuccinate 0.25 mM, pH 7.4; 0.25 mM sodium succinate pH 7.4; or NaCl 4 M. Compounds were applied by means of a constant current source, with neutralization current capabilities (Dagan 6400), using the appropriate polarities to eject or retain the substances.

Neuronal activity was recorded using conventional electrophysiological methods, cells were identified as belonging to SN by their characteristic waveform and discharge pattern [22–24,30], spikes were converted to constant pulses by means of a Schmitt trigger circuit, and these pulses were saved for subsequent analysis using the RTIME software [25]. Discharge patterns were analyzed off line using the Neuropak software [19].

Once a suitable cell was found, the recording protocol consisted of a control period in which spontaneous activity was assessed; only cells with less than 10% spontaneous variation in firing rate were studied. Then, 17β-estradiol hemisuccinate was ejected with a negative current of 30–40 nA for a 10 min period; thereafter, the neuronal activity was monitored for 20 more minutes. Appropriate controls were made using sodium succinate and by applying currents of the same polarity and magnitude through the NaCl barrel. All recording sites were marked by the ejection of Pontamine Sky Blue.

In some experiments, the anti-estrogen tamoxifen was used to see whether it could block the effects produced by 17β-estradiol. In these experiments, tamoxifen was administered I.V. (5 mg/kg) 2 h before the experiment and then after estrogen effects were found in a cell; subsequently, the effects produced by estradiol were re-evaluated in the same unit. At the end of the experiments, the animals were perfused through the heart with a formalin solution, the brains were removed, and a histological analysis was made in order to corroborate the recording sites.

2.2. Statistical analysis

Average frequencies for each period (control, estradiol application and recovery) were compared using the Wilcoxon test. Changes in the firing pattern were evaluated by means of interspike interval histograms, autocorrelation analysis, autospectral analysis and/or joint interval histograms.

3. Results

A total of 73 SN neurons from both pars compacta (PC) and pars reticulata (PR) were studied. Of these, 27 were found in male rats, 37 in intact female rats and 9 in ovariectomized rats. Neurons were identified by electrophysiological criteria and stereotaxic coordinates, and their location was later corroborated by histological analysis.

As has been described [24,67], there are two populations of nigral cells. One population shows a higher average frequency, in the present experiments 21.19 ± 1.84 spikes/min and is located predominantly in the pars reticulata. The other population fires with a lower frequency, 4.51 ± .36 spikes/min and is found in the pars compacta. Both cellular types were found in male and intact female rats, whereas only low frequency cells were found in ovariectomized rats.

3.1. Effects of 17β-estradiol hemisuccinate on the firing rate of SN neurons

Responses to 17β-estradiol could be grouped in various types: (1) Short-latency excitations, in which the firing rate increased during the first seconds after the application began and was maintained throughout its duration, returning to the