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The effect of estrogen and progesterone on spreading depression in rat neocortical tissues

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Although gender differences in the incidence of migraine with aura appear to be related to high circulating levels of ovarian hormones, the underlying mechanisms are not yet fully understood. Several studies have suggested a major role for spreading depression (SD) in the pathogenesis and symptomatology of migraine with aura. To investigate a possible role of SD in the association of high female hormones and attacks of migraine with aura, the effects of β-estradiol and progesterone on SD were studied in rat neocortical tissues. Application of both hormones enhanced the repetition rate as well as the amplitude of SD in neocortical slices treated with hypotonic artificial cerebrospinal fluid. β-Estradiol and progesterone also dose dependently increased the amplitude of SD induced by KCl microinjection. Both hormones exhibited a pronounced, persisting, and significant enhancement of long-term potentiation of the field excitatory postsynaptic potential in the neocortical tissues. The changes in SD characteristics in the presence of estrogen and progesterone may responsible for increased migraine with aura attacks associated by high female hormones. These hormones may exert their effects on SD via facilitation of synaptic transmission.

Keywords: Migraine; Synaptic transmission; Sexual hormones; Headache

Introduction

Spreading depression (SD), a self-propagating front of depolarization with characteristic ionic, metabolic, and hemodynamic changes accompanied by transient suppression of neuronal activity (Leao, 1944; Bures, 1999), is believed to play an essential role in some neurological disorders including migraine with aura (Somjen, 2001; Buzzi and Moskowitz, 2005). Propagation of a SD-like phenomenon in human neocortical tissues generates aura symptoms in migrainous patients (Hadjikhani et al., 2001) and several animal studies suggested a link between SD and migraine pain (Moskowitz et al., 1993; Bolay et al., 2002; Gorji et al., 2004).

Some attention has been paid to SD effects on the brain reactivity to hormones. SD can exert a modulatory effect on pituitary function that is dependent on the hormonal background conditions (Colombo et al., 1973, 1975; Colombo and Sawyer, 1973, 1975). SD initiates a release of luteinizing hormone in rats (Colombo et al., 1975; Taleisnik and Caligaris, 1962) and modulates both active and passive components of mating behavior in males as well as females rats (Beach, 1940, 1944; Larsson, 1962). Pretreatment with estrogen or progesterone, can enhance the females passive components of mating behavior by SD in ovariectomized rats (Clemens et al., 1967). These studies suggest a possible interaction between SD and the ovarian hormones.

There is ample evidence for a link between female hormones and migraine (Welch, 1997; Silberstein, 2001). Ovarian hormones significantly influence migraine headache throughout the female life span. The sexual prevalence of migraine greatly favors women. A higher female prevalence of migraine after puberty, with lifetime prevalence of 25% compared with 8% in men was reported (Rasmussen et al., 1991). This difference between sexes becomes greater with age, peaking early in the fifth decade of life and then declining. The pattern of migraine headache in women alters by hormonal changes including menarche, menstruation, pregnancy, menopause, oral contraceptive use and hormonal replacement therapy (Silberstein, 2001). Changes in ovarian hormones can be associated with an increase or decrease in the frequency of migraine headaches (Somerville, 1971; MacGregor, 2004). Although gender differences in the incidence of migraine appear to be related to circulating levels of female hormones, the underlying mechanisms are not yet fully understood (Bousser, 2004). One possibility is that gonadal hormones may influence SD phenomenon (Gorji, 2001). Therefore, the effect of estrogen and progesterone on SD phenomenon in neocortical tissues was investigated in the present study.

Material and methods

The experiments were carried out on adult female rat (250–350 g) somatosensory neocortical slices. Male and female rats were used in control experiments (see Experimental protocols). The brain was removed under deep methohexital anaesthesia and placed in cold (1–4°C) artificial cerebrospinal fluid (ACSF) pre-

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equilibrated with 5% CO_2 in O_2 to give a pH of 7.4. The ACSF contained (in mmol/l): NaCl 124, KCl 4, $CaCl_2$ 1.0, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10. The somatosensory neocortices were dissected and cut into slices of 500 μm thickness. The slices were incubated in ACSF solution for >1 h at 28°C. After 30-min incubation, $CaCl_2$ was elevated to 2.0 mmol/l. Slices were transferred to an interphase-type experimental chamber and superfused with ACSF at 32°C (1.5–2 ml/min).

Electrophysiological recordings

Extracellular field potentials were recorded with glass microelectrodes (150 mmol/l NaCl; 2–10 M) connected to the amplifier by an Ag/AgCl–KCl bridge in the third layer of neocortical tissues. Field potentials were traced by an ink-writer and recorded by a digital oscilloscope.

Induction of SD

Two methods were used to elicit SD: 1. KCl microinjection: a glass electrode filled with 2 M KCl was fixed in a special holder connected with plastic tube to a pressure injector and the tip inserted into the sixth layer of the neocortical slices. A high-pressure pulse was applied to inject an amount of K^+ in the tissue sufficient to induce SD (tip diameter: 2 μm; injection pressure 0.5–1.0 bar applied for 200–300 ms, up to two separate injections, 1–3 nl per pulse). The number of injections was always constant during a single experiment and for all conditions. 2. Slices were exposed for 60 min to low-NaCl solutions (hypo-osmotic medium), in which 50% of NaCl was omitted from ACSF while other ingredients and pH were unchanged (Chebabo et al., 1995). SD-like events were evaluated with respect to their repetition, amplitude, duration and velocity rates. SD duration was defined as the interval between the time of half-maximal voltage shift during onset and recovery of the negative DC potential deflection.

Long-term potentiation

Single pulses of electrical stimulation were applied through a bipolar platinum electrode attached to the white matter perpendicular to the recording electrodes. Evoked field excitatory postsynaptic potentials (fEPSP) were recorded in the third layer of neocortical slices. The fEPSP was elicited by adjusting the intensity of stimulation to 50% of that at which population spikes after fEPSP began to appear. The amplitude of fEPSP 1 ms after the onset was measured for data analysis. In long-term potentiation (LTP) experiments, the cortex was sequentially stimulated once every minute. Ten trains of four pulses (pulse duration 0.1 ms; interpulse interval 50 ms; intensity 5 V) were repeated at intervals of 10 s. LTP was operationally defined as the mean change in fEPSP amplitude in response to five stimuli given 30 min after tetanic stimulation compared with the mean response to five test pulses applied immediately before the stimulation. Thus % potentiation = [(posttetanus amplitude of fEPSP/baseline amplitude of fEPSP) 1] 100. Tetanic stimulation was applied 60 min after application of estrogen (25 μmol/l) or progesterone (10 μmol/l).

Experimental protocols

Three different experimental protocols were used, each of which consisted of several periods.

The first experimental protocol consisted of four periods as follows: (a) control period, neocortical slices were superfused with ACSF (30 min), tested for spontaneous SD; (b) KCl injection, induction of SD (SD1); (c) application of β-estradiol (0.01–50 μmol/l) or progesterone (0.1–50 μmol/l, 60 min) the second injection of KCl (SD2); (d) washout of estrogen or progesterone with ASCF (45 min, second control period), third injection of KCl (SD3). Only a single concentration of estrogen or progesterone was used in a given slice. In control experiments, DMSO was added to the bath solution after the first KCl injection (60 min) and washed with ASCF (45 min) after the second and before the third KCl application.

The second experimental protocol consisted of four periods as follows: (a) control period, neocortical slices were superfused with ACSF (30 min), tested for spontaneous SD; (b) application of β-estradiol (25 μmol/l) or progesterone (10 μmol/l, 60 min) before the first injection of KCl (SD1); (c) KCl injection, induction of SD (SD1); (d) washout of estrogen, progesterone, with ASCF (45 min) before the second injection of KCl (SD2).

The third experimental protocol consisted of three periods as follows: (a) control period, neocortical slices were superfused with ACSF (30 min), tested for spontaneous SD; (b) application of β-estradiol (25 μmol/l), progesterone (10 μmol/l, 60 min) or ACSF; (c) superfusion of low NaCl ASCF with or without β-estradiol or progesterone (60 min); (d) washout with ACSF, second control period.

Drugs

β-Estradiol and progesterone, both purchased from Sigma, were dissolved in DMSO. The final concentration of DMSO was less than 0.5%. All solutions used in control periods contained the same concentration of DMSO.

All data are given as mean ± SEM. The data were statistically analyzed using the Mann–Whitney rank sum test. Multiple comparisons were performed by analysis of variance test (ANOVA) for repeated measures followed by a Duncan's or Holm-Sidak's test. Significance was established when the probability values were less than 0.05. The investigations were approved by the local ethics committee (Tierversuchsgenehmigjung, Ethikkommission der Bezirksregierung Münster, Deutschland, AZ: 50.0835.1.0, G79/2002).

Results

The effect of female gonadal hormones on SD

Microinjection of KCl

Local application of KCl in the sixth layer of neocortical tissues induced negative DC potentials followed by positive waves (amplitude of 14.4 ± 1.9 mV; duration of 95 ± 4 s). SD waves propagated opposite to the direction of the ACSF flow at propagation velocity of 3.1 ± 0.2 mm/min. The effect of six different concentrations of β-estradiol (0.01, 0.1, 1, 10, 25, 50 μmol/l; $n = 6$ to 8 for each concentration) and progesterone (0.1, 1.25, 2.5, 5, 10, 25, and 50 μmol/l; $n = 6$ to 8 for each concentration) was tested on potassium-evoked SD in neocortical tissues. The ratio between the second and first DC potential waves (SD2/SD1) was calculated in control slices and slices treated with β-estradiol or progesterone. Sixty minutes of β-estradiol application at 0.01 μmol/l had no significant effect on different cha-