Acetylcholine-Induced Relaxation of Rat Aorta Is Greatest during Estrus in the Sexual Cycle

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ABSTRACT—Acetylcholine (ACh)-induced relaxation in the aortae precontracted with norepinephrine was significantly enhanced in the aortae from estrus (E) rats, compared with that in those from metestrus (D-1), diestrus (D-2) and proestrus (PE) rats. *N*^G-Nitro-L-arginine methyl ester (L-NAME) inhibited the endothelium-dependent relaxation in E rats. These results suggest that there is a difference in ACh-induced relaxation of the thoracic aorta during the sexual cycle of rats, and the relaxation is greatest in E of the sexual cycle; this may be due to a difference in nitric oxide synthesis in the endothelium in the sexual cycle.

Keywords: Sexual cycle, Nitric oxide, Aorta

Pregnancy in humans and different mammalian species, including rats, results in a decreased sensitivity to various pressor substances (1, 2). It is also reported that the contractility of blood vessels in response to vasoactive agents is blunted in pregnant sheep (3), guinea pigs (4) and rats (5–7). Recently, we reported that the endothelium-dependent relaxation induced by acetylcholine (ACh) was significantly enhanced in aortae from pregnant rats, compared with that in aortae from non-pregnant rats (8). It is well-known that the levels of serum sexual hormones change during the sexual cycle of rats as well as during pregnancy. Therefore, the aim of the present study was to determine if there is a difference in ACh-induced relaxation during the sexual cycle of rats.

Eight-week-old female Wistar rats in metestrus (D-1), diestrus (D-2), proestrus (PE) and estrus (E) were anesthetized by ether and sacrificed by bleeding. The thoracic aorta was isolated and placed in Hepes solution (pH 7.4) having the following composition: 120.3 mM NaCl, 4.8 mM KCl, 1.8 mM CaCl₂, 1.3 mM MgSO₄, 1.2 mM KH₂PO₄, 5.6 mM glucose and 10.0 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) at 37°C gassed with 100% O₂. The pH was adjusted to 7.4 by adding 1.0 N NaOH. The tissue was cleaned by removing connective tissue. The thoracic aorta was cut into rings about 4-mm-long. Contraction was measured by suspending the rings between two stainless-steel hooks, one of which was attached to the end of a bathing tube and the other connected to a force transducer (45196A; NEC San-ei, Tokyo). The resting tension was 0.5 g, and each

preparation was equilibrated in the 10-ml bathing solution for 90–120 min before the experiment. Isometric tension changes were recorded on a polygraph (LECT-HORIZ-8K, NEC San-ei). After the equilibration, the rings were exposed to 50 mM KCl. When the contractile responses plateaued, the rings were rinsed with Hepes solution and allowed to equilibrate for an additional 60 min before the application of 3×10^{-7} M norepinephrine (NE). For the relaxation studies, submaximal tone (approximately 80% of the maximum tone) was induced with 3×10^{-7} M NE and then ACh or sodium nitroprusside (SNP) was added in a cumulative fashion. *N*^G-nitro-L-arginine methyl ester (L-NAME) was added to the solution 5 min before treatment with NE to observe the effect of L-NAME on ACh- or SNP-induced relaxation. ACh chloride, SNP and L-NAME (Sigma, St. Louis, MO, USA) were dissolved in distilled water. Other chemicals were of analytical grade and obtained from Wako Pure Chem. Co., Ltd. (Osaka). Values were expressed or plotted as the mean $\pm$ S.E., and statistical analysis was performed with the multiple Tukey test. Differences were considered significant at $P < 0.05$.

The addition of ACh (10^{-8} – 3×10^{-5} M) produced dose-dependent relaxation in the rings from D-1, D-2, PE and E rats (Fig. 1). ACh-induced relaxation was significantly enhanced in the thoracic aortae from E rats compared with that in aortae from D-1, D-2 and PE rats. The negative logarithm of ED₅₀ values for efficacy (defined as percentage of relaxation per dose of agonists divided by maximum relaxation achieved in that arterial

Fig. 1. Cumulative relaxation to acetylcholine (ACh) of the aortic rings precontracted with norepinephrine in female rats. $\square$: D-1, metestrus; $\blacksquare$: D-2, diestrus; $\circ$: PE, proestrus; $\bullet$: E, estrus. Data are expressed as a percentage of the response to norepinephrine. Each value is the mean $\pm$ S.E. from 11–24 experiments. * $P < 0.05$, ** $P < 0.01$ from E.

Fig. 2. Cumulative relaxation to sodium nitroprusside (SNP) of the aortic rings precontracted with norepinephrine in female rats. $\square$: D-1, metestrus; $\blacksquare$: D-2, diestrus; $\circ$: PE, proestrus; $\bullet$: E, estrus. Data are expressed as a percentage of the response to norepinephrine. Each value is the mean $\pm$ S.E. from 11–24 experiments. * $P < 0.05$, ** $P < 0.01$ from E.

ring) calculated from 17–24 determinations in D-1, D-2, PE and E rats were 7.03 ± 0.097 , 6.92 ± 0.101 , 7.04 ± 0.085 and 7.37 ± 0.073 , respectively. The negative

logarithm of ED_{50} values for ACh was significantly higher in E rats than in D-1 ($P < 0.01$), D-2 ($P < 0.05$) and PE rats ($P < 0.05$). E was associated with increased efficacy of

Fig. 3. Individual experimental traces of acetylcholine (ACh)- and sodium nitroprusside (SNP)-induced relaxation of aortic rings precontracted with norepinephrine (NE), and influence of indomethacin (Indo) and N^G -nitro-L-arginine methyl ester (L-NAME) on the relaxation in estrus rats. Figure indicates negative logarithm of drug concentration.

ACh for the thoracic aorta.

In contrast to ACh, the relaxation to cumulative SNP was decreased in E rats compared with those from D-1, D-2 and PE rats (Fig. 2). The negative logarithms of ED₅₀ values for efficacy calculated from 11–15 determinations in D-1, D-2, PE and E rats were 8.70 ± 0.097 , 8.50 ± 0.125 , 8.33 ± 0.087 and 8.22 ± 0.137 , respectively. The negative logarithm of the ED₅₀ value for SNP was significantly lower in E rats than in D-1 rats ($P < 0.05$).

The relaxation induced by ACh was abolished by L-NAME (10^{-4} M) but did not affected by indomethacin (10^{-5} M) (Fig. 3). However, the relaxation induced by SNP was not affected by L-NAME (10^{-4} M) or indomethacin (10^{-5} M).

Our present results indicate that there was a difference in ACh-induced relaxation during the sexual cycle of rats. The relaxation induced by ACh was significantly enhanced in the aortae from E rats, compared with those from D-1, D-2 and PE rats. There may be a possibility that the muscarinic receptors are altered, but to our knowledge, there is no report that functions of muscarinic receptors in the aortae were altered during the sexual cycle of rats. The relaxation induced by ACh in E rats was abolished by L-NAME but was not affected by indomethacin, suggesting that the enhanced relaxation by ACh was due to the increase of nitric oxide (NO) activity. It was reported that pregnancy enhanced the endothelium-dependent relaxation induced by ACh in guinea pig uterine artery (9) and rat aorta (8), suggesting that the enhancement by pregnancy was due to estradiol (8, 10). In these studies, non-pregnant animals were used without consideration of the sexual cycle, so it was considered that there were no great difference in the endothelium-dependent relaxation induced by ACh between non-pregnant and pregnant animals because there was a difference in ACh-induced relaxation during the sexual cycle.

According to Watanabe et al. (11), the level of plasma estradiol in PE, one day before E, is the highest during the sexual cycle of rats. It is of interest that ACh-induced relaxation is higher in E rats than in PE rats even though the level of serum estradiol is higher in PE rats than in E rats. More than several hours of the high level of serum estradiol may be needed to enhance ACh-induced relaxation. Indeed, in pregnant rats, the level of serum estradiol progressively increased and reached more than tenfold on day 18, 3 days before the observation of the enhanced endothelium-dependent relaxation induced by ACh (8). The enhancement of ACh-induced relaxation in both E and pregnant rats may be due to the increase in NO syn-

thase (NOS) activity that resulted from the enzyme induction by estradiol. Weiner et al. (10) suggested that the increase in NOS activity was the result of augmented enzyme synthesis since it was accompanied by the increase in the specific mRNAs for eNOS in skeletal muscle from estradiol-treated guinea pigs. The decreased response to SNP in E rats may be due to the down-regulation after the increased basal release of NO.

In conclusion, there is a difference in the ACh-induced relaxation of the thoracic aorta during the rat sexual cycle, and ACh-induced relaxation is greatest in E. It may be related to the enhancement of NO activity in the endothelium.

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