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K. ASŁANOWICZ-ANTKOWIAK, W. Z. TRACZYK

OCCURRENCE OF OVULATION AFTER INTRACEREBROVENTRICULAR INFUSION OF SUBSTANCE P IN 6-OHDA PRETREATED FEMALE RATS

Department of Physiology, Institute of Physiology
and Biochemistry,
Medical University of Lodz.
90-131 Łódź, Poland

Substance P (SP) infused into the third cerebral ventricle blocks spontaneous ovulation in female rats, probably through catecholaminergic neurons. The studies presented in this paper were undertaken to investigate whether SP exerts its suppressing effect on ovulation in 6-hydroxydopamine (6-OHDA) pretreated female rats. After 8—12 days following 6-OHDA pretreatment female rats were infused, on the day of proestrus, with vehicle and all animals were found to ovulate or with a solution containing 5 nmol of SP and 89% of female rats were then found to ovulate. In the group pretreated with vehicle and subsequently infused with SP, ovulation was found to occurr only in 25% of animals. The obtained results indicate that spontaneous ovulation in 6-OHDA-pretreated female rats cannot be blocked by i. c. v. administration of SP, and it may be concluded that SP exerts its suppressing effect through the monoaminergic neurons.

Key words: *ovulation, intracerebroventricular infusion, Substance P (SP), 6-hydroxydopamine (6-OHDA)*

INTRODUCTION

In spite of many biological effects of Substance P (SP) on the central and peripheral nervous system, the role of this undecapeptide in the hypothalamo-pituitary system still remains unclear. A prolongation of the estrus phase of the cycle in female rats after implantation of Hexa-SP_{6-11} into the diencephalon above the hypothalamic hypophysiotropic area was observed (1). The disturbances of the estrus cycle in female rats were also observed in the case of Hexa-SP_{6-11} infusion into the lateral cerebral ventricle (2). An

inhibition of spontaneous ovulation was found after Hexa-SP$_{6-11}$ infusion into the third cerebral ventricle (3). In these in vivo experiments intracerebroventricular infusion of Hexa-SP$_{6-11}$ decreased the number of oocytes at each cycle and also prolonged the diestrus stage from 2 to 13 days. These results indicate that SP may exert an inhibitory effect on LH release from the pituitary. The role of SP as a modulator controlling gonadotropin activity of the pituitary gland at the hypothalamic level was suggested after observations of different SP content in the median eminence during diestrus and estrus (4). Simultaneous decrease or increase of SP and LH-RH content in this structure, dependent on the stage of the cycle was also observed (5).

Spontaneous ovulation seems to be Substance P dependent in contrast to reflex ovulation induced usually by copulation. Female rats infused i. c. v. with SP and then placed in a cage with fertile males on the night of proestrus were found to be pregnant (6).

Different neurotransmitters have been postulated to be of importance in the modulation of the pulsatile secretory pattern of several pituitary hormones, particularly LH (7). The secretion of this hormone seemed to be modulated by the noradrenergic system. Injection of noradrenaline (NA) into the 3rd ventricle has been shown to induce ovulation in rats made anovulatory by various experimental procedures (8). NA infusion into the 3rd cerebral ventricle induced ovulation in pseudopregnant rats (9), but this effect was completely abolished when topical aplication of NA was preceded by SP infusion. The suppressing effect of intraventricularly infused SP on NA-induced ovulation was observed in all pseudopregnant rats, indicating an inhibitory activity of SP-ergic neurons exerted on NA-ergic neurons.

The aim of the present study was to find whether SP exerts its inhibitory effect on the occurrence of ovulation in the case of monoaminergic pathways destruction by 6-OHDA pretreatment.

The preliminary results were presented at the 17th Congress of the Polish Physiological Society (10).

MATERIALS AND METHODS

The experiments were carried out on 25 female rats weighing from 210 to 260 g, being the first (F_1) generation of crossbred Wistar females and Buffalo males from the stock of the Institute of Oncology in Gliwice. The animals were kept under controlled conditions of temperature and lighting (14 h light, 10 h darkness). They were given free access to standard laboratory pellets and tap water.

All the infusions were carried out via a needle cannula introduced into the third cerebral ventricle through a chronically implanted guide tube. A stainless steel guide tube of 0.6 mm external and 0.4 mm internal diameter, was equipped with a platinum wire mandrin 0.3 mm in diameter, removed before each infusion.

On the day of proestrus the guide tube was implanted in a stereotaxic apparatus under i. p. hexobarbital anaestesia (80 $mg \cdot kg^{-1}$ b. w.) according to the stereotaxic atlas (11) in the median sagittal plane, 7 mm anterior to the zero frontal plane, determined by the line connecting both external auditory meatuses. The tip of the guide tube was implanted to a depth of 3 mm from the skull surface. The guide tubes were fixed to the skull by dental cement.

The needle cannula, made of stainless steel and protruded 2.5 mm from the end of the guide tube, was connected by polyethylene tubing with a gas-tight microsyringe (Hamilton 1705-1001, Bonaduz, Switzerland).

In all the experimental groups the first infusion was performed immediately after guide tube implantation, and the second at 13.00 h on the day of proestrus, 8 to 12 days following the first one. 6-Hydroxydopamine (Regis Chemical CO) was dissolved in 0.9% NaCl containing additionally ascorbic acid 1 mg/1 ml. SP (Laboratory of Peptides, Faculty of Chemistry, University of Warsaw) was dissolved in 0.9% NaCl.

The days of proestrus and estrus were assessed on the basis of examination of vaginal smears taken between 09.00 h — 10.00 h. In order to assess the occurrence of ovulation at 11.00 h on the day of estrus (22 hours after the second infusion), the rats were injected intraperitoneally with 20% solution of urethane in a volume of 5 $ml \cdot kg^{-1}$ b. w., the oviducts were isolated and the ova were counted in the lumen of the oviduct ampoule with the use of a stereoscopic microscope. Then 10 µl of a 1% solution of trypan blue was infused into the third cerebral ventricle, the skull was cut off, placed in 10% formaldehyde solution and after ten days the brains were removed, frozen and sliced in frontal plane (50 µm), in order to assess the correct positioning of the cannula. Only those animals were taken into account which had clear staining of the 3rd ventricle lining.

The experiments were carried out on three groups of female rats: the 1st. group — 8 rats were pretreated (i. c. v.) with 20 µl of a solution containing 200 µg of 6-OHDA (1st infusion) and as 2nd infusion received 10 µl 0.9% saline; the 2nd group — 9 rats were pretreated (i. c. v.) with 6-OHDA (1st infusion) in the dose and volume as the animals in the 1st group, and as 2nd infusion received 10 µl of a solution containing 5 nmol of SP; the 3rd group — 8 rats were petreated (i. c. v.) with 10 µl of 0.9% saline (1st infusion) and as 2nd infusion received 10 µl of a solution containing 5 nmol of SP. Statistical analysis of the obtained results was carried out with the use of Wald-Wolfowitz nonparametric runs test (12).

RESULTS

Some disturbances in the length of the first estrus cycle after guide cannula implantation were noted in the control as well as in the two experimental groups. The second 4-day estrus cycle appeared in most of the animals and on the day of proestrus which starts the third estrus cycle a typical cytologic picture of the vaginal smears was found. In the first control group and in the second experimental group of animals suppression of ovulation was not observed. Inhibition of ovulation was found in the third group of female rats. Among all the tested animals in this group only 25% were found to ovulate. Statistical analysis carried out with the use of runs test demostrated that the differences in the number of ova in groups I and II females were not significant ($\psi = 0.05$). Comparison of the number of ova in all the females of groups II and III demonstrated ($\psi = 0.05$) that the differences were significant.

Table 1 Occurrence of ovulation in female rats after intracerebroventricular SP and 6-OHDA infusion.

Group	n	Intracerebroventricular administration on the day of proestrous		The day of estrous — 22 hrs after treatment	
		Pretreatment	Treatment after 8—12 days	No of ovulating/ tested rats	No of ova per rats ovulating (mean)
1st	8	6-OHDA 200 µg/20 µl	0.9% NaCl 10 µl	8/8	13.1
2nd	9	6-OHDA 200 µg/20 µl	SP 5 nmol/10 µl	8/9	13.6
3rd	8	0.9% NaCl 10 µl	SP 5 nmol/10 µl	2/8	11.5

DISCUSSION

In female rats Substance P infused into the lateral cerebral ventricle (2) or implanted into the diencephalon (1) caused disturbances of estrus cycle. SP introduction into the third cerebral ventricle resulted in the inhibition of spontaneous ovulation (3). Injection of anti-SP antibodies caused an increase of the blood LH level (13). All these facts support the hypothesis that SP exerts an inhibitory effect on LH release from the pituitary. The presented results are in accordance with the study mentioned above, 89% of females which underwent infusion of SP into the third cerebral ventricle, preceded by 6-OHDA infusion were found to ovulate. This suggests that the inhibitory effect of SP on ovulation is probably exerted by involvement of the noradrenergic pathways. Ljungdahl et al. (14) found that all the catecholaminergic neurons in the CNS of the rat were surrounded by SP-like immunoreactive nerve terminals. Catecholaminergic neurons may influence pituitary gonadotropin secretion, and of the two major catecholamines, DA and NA, NA is thought to stimulate the release of luteinizing hormone (9, 15—18).

6-Hydroxydopamine (6-OHDA) is found to induce a long lasting depletion of noradrenaline from the rat brain (19). Intraventricularly injected 6-0HDA is able to deplete the transmitter content in the NA and DA nerve terminals lying close to the ventricles. Transmitter depletion is followed by a degeneration of central NA and DA neurons (20). 6-OHDA infused into the lateral ventricles of rats significantly reduced NA levels in the hippocampus, hypothalamus and septum despite pretreatment with desmethylimipramine (DMI) commonly considered to block 6-OHDA-induced depletion of NA (21). Bilateral injections of 6-OHDA in the ventral tegmental area caused depletion of DA content in

the forebrain areas, and the areas innervated by the noradrenergic bundle were also depleted of noradrenaline (22).

The results obtained in the first group of females (infused with 6-OHDA and vehicle) are in accordance with the study of Nicholson et al. (23), who found resumption of estrus cycles and ovulation (24) in animals treated with 6-OHDA. In these animals ovulation returned, in spite of the very low LH surge, below the normal level. These findings confirm the previous interpretation that only a fraction of the preovulatory LH is sufficient for induction of ovulation.

Martinovic and McCann (25) demonstrated that bilateral injection of 6-OHDA into the ventral noradrenergic bundle of proestrus rats caused a complete block of preovulatory LH release and significantly reduced NA level in the median eminence and the anterior hypothalamus 24 h after microinjection. Nicholson et al. (23) observed reduction of NA concetration in the hypothalamus and preoptic area after bilateral injection of 6-OHDA into the ventral pons. Lesions of the ventral noradrenergic bundle had no effect on serum LH, and regular estrus cycles were observed after a transient period of irregularities. A significant increase of LH level was observed after a subthreshold dose of NA infused into the third cerebral ventricle in male rats pretreated with 6-OHDA (26). Probably depletion of hypothalamic NA after 6-OHDA treatment causes hypersensitivity of NA receptors to residual NA.

Hancke and Wuttke (24) suggested that the signal triggering preovulatory hormone release is not noradrenergic because regular estrus cycles were observed after a reduction of diencephalic NA concentrations. However recent studies (27) postulate noradrenergic stimulation of ovulatory LH surges and LH-RH pulse generation. Infusion of NA into third cerebral ventricle, however, induced ovulation in pseudopregnant animals and this effect was abolished by preceding the NA-infusion by infusion of SP (9). These data may indicate a noradrenaline-dependent inhibitory effect of SP.

Our results indicate that Substance P infusion preceded by 6-OHDA infusion is not able to inhibit spontaneous ovulation. Infusion of 6-OHDA into the third cerebral ventricle, as mentioned erlier leads to a selective and long lasting depletion of noradrenaline and degeneration of noradrenergic nerve terminals. It is possible that SP-ergic neurons have an inhibitory influence on the NA-ergic ones. In the case of NA-ergic neurons' destruction the suppressing effect of Substance P is not observed.

On the basis of earlier studies it would appear that there is more than one site of action of SP to control gonadotropin release. Substance P seems to participate as inhibitory neuromodulator or neurotransmitter in the control of LH and FSH secretion (28). It is possible that SP acts directly on LH-RH-ergic neurons or indirectly through monoaminergic neurons. In both cases the inhibitory effect of SP may be postsynaptic or presynaptic.

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Author's address: Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Łódź, ul. Lindleya 3, 90-131 Łódź, Poland.