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EFFECT OF MEDIAN RAPHE NUCLEUS ELECTROCOAGULATION AND SEROTONIN SYNTHESIS BLOCKADE ON OESTROGEN RECEPTOR CONTENT OF RAT UTERUS

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Waloch, M., Chrapusta, S. and *Paszko, Z.*: Effect of median raphe nucleus electrocoagulation and serotonin synthesis blockade on oestrogen receptor content of rat uterus. Acta physiol. pol., 1981, 32 (6): 651—658. The number of oestrogen receptors (OR) in rat uterus changes in relation to the cyclic function of the ovary. In the dioestrus phase the OR content of rat uterus is significantly greater than in the oestrus phase. Electrocoagulation of the median raphe nucleus causing in rats increased release of gonadotrophins and consequent disturbances in the cyclic ovarian function is associated also with changes in OR content of the uterus. These changes are, however, not analogous to those observed in the normal sexual cycle. In the oestrus phase a very high accumulation of OR is found in the uterus, while in the dioestrus phase OR are barely detectable.

Serotonin synthesis blockade with p-CPA, which is associated with an increase in the number of maturating and mature follicles in the ovary and dioestrus phase prolongation leads to a striking increase in the number of OR in the uterus.

A hypothetical model of neurohormonal regulation assumes that the centres of release of hypothalamic hormones are subject to the action of two opposite systems: catecholaminergic and serotoninergic respectively stimulating and inhibiting the release of hypothalamic factors regulating the gonadotropic function of the pituitary.

In histofluorescent investigations it has been found [1, 2] that serotonin-producing cell bodies are situated in the raphe nuclei from where ascending fibres run to numerous structures in the prosencephalon and hypothalamus.

It has been demonstrated that electrolytic damage of the median raphe nucleus (MR) leads to increased secretion of luteinizin hormone in castrated females of rats [19] and to disturbances of the sexual cycle of female rats indicating increased oestrogen activity [17] These changes were most intense 10—14 days after the procedure and were associated with a fall of serotonin level (5-HT) in the prosencephalon.

The action of oestrogens in uterine cells is mediated by a specific cytoplasmatic receptor which after binding to the hormone penetrates into the cell nucleus [11, 12]. The content of oestrogen receptors (OR) in the target organs changes as a result of the action of exogenous oestrogens and progesterone [4, 15] as well as during the sexual cycle [20].

Hormonal disturbances observed after damage to the median raphe nucleus might be reflected in changes of OR number in the uterus. We tried also to establish whether reduced 5-HT level in the brain caused by p-chlorphenylalanine (p-CPA) an inhibitor of 5-HT synthesis, exerts a similar effect on OR content as that observed after electrolytic damage to the centre of 5-HT synthesis.

MATERIAL AND METHODS

Female rats weighing 170—190 g from a randomized breed were used for the investigations. The animals were kept on a standard diet, in a room with a stable temperature, with regulated light-darkness cycle (light from 7.00 to 19.00).

Median raphe nucleus electrocoagulation was performed as described elsewhere [17—19]. The uteruses of these animals were studied in which MR lesion had been varified histologically. In the experimental animals vaginal smears were studied for selecting two groups of rats — in the oestrus phase and in the dioestrus phase.

Another group comparised female rats receiving after 36 hours p-CPA intraperitoneally 100 ng/kg daily. The injections were done in two series of three injections each with an interval of 48 hours between these series. The animals were killed 48 hours after the last dose of p-CPA. In all animals only the dioestrus phase was found in vaginal smears. The control group contained female rats not operated upon which were in the oestrus and dioestrus phase.

Oestrogen receptor determination in rat uterine cytosol

Materials: 1) ethylene glycol, POCh Gliwice; 2) disodium versenate (Na_2EDTA), POCh Gliwice; 3) tris-hydroxymethylaminomethane, Austranal; 4) 17β-oestradiol E, Organon; 5) (2,4,6,7-^{3}H)-17β-oestradiol, spec. act. 100 Ci/mmol, Amersham; 6) activated charcoal washed with acid, Sigma; 7) dextran 60 000, Pharmacia; 8) p-chlorophenylalanine (p-CPA), Koch-Light.

The animals were decapitated, the uterus was removed, weighed and frozen in solid carbon dioxide (dry ice). The tissue was ground to powder in a porcelain mortar cooled with liquid nitrogen, and the powder was suspended in cold (+2°—+4°C) TED buffer (Tris 10 mM, Na_2EDTA 1.5 mM saccharose 0.25 M). After 15 minutes the suspension was centrifuged for 30 min at 20 000× g. The obtained supernatant (cytosol) was stored at −25°C. The OR in the cytosol fraction was determined by the method described by *Paździk* et al. [15]. The results of the determinations of specific receptor bonds were subjected to Scatchard's analysis for calculating the number of sites binding specifically oestradiol and determining the constant of binding dissociation (K_d). The number of sites binding specifically oestradiol was expressed in pM E$_2$ per mg of DNA in the uterus. Protein was determined by the method of Lowry [14], and DNA by the method of Burton in the modification of *Giles* and *Myers* [6].

RESULTS

Fig. 1 shows the site of MR damage.

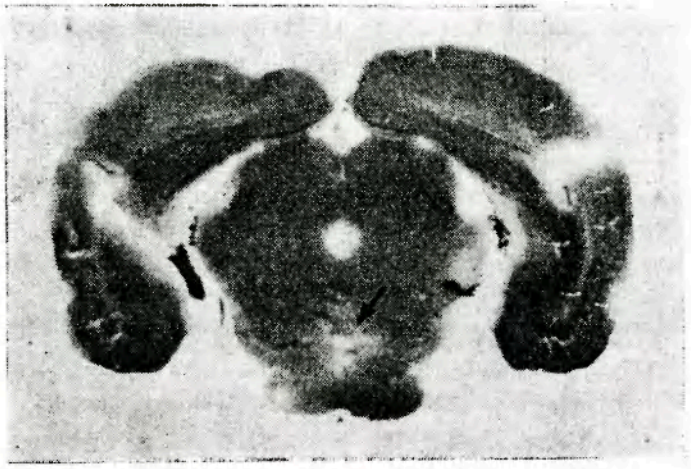

Fig. 1. Frontal cross-section of the brain showing the site of lesion in the median raphe nucleus (MR).

The results of oestrogen receptor determinations in rat uterus on the 10th and 30th days after MR damage, after p-CPA administration, and in control groups are shown in Table T and Fig. 2, 3 and 4.

OR content in the uterus of rats in the control group in the oestrus or dioestrus phase

Table 1. Effect of median raphe nucleus electrocoagulation and blockade of serotonin synthesis on the content of oestrogen receptors in rat uterus in the phases of oestrus and dioestrus

Sexual phase cycle	Experimental group	No of binding sites pM E_2/mg DNA	Dissociation constant (K_d)
Oestrus	control	0.66	2×10^{-10}
	10 days after MR damage	1.91	3×10^{-9}
		2.16	1.4×10^{-10}
	1 month after MR damage	0.61	1.1×10^{-9}
		0.54	0.9×10^{-9}
Dioestrus	control	2.24	1.7×10^{-9}
	10 days after MR damage	0.08	1.6×10^{-10}
	1 month after MR damage	1.02	0.7×10^{-9}
	p-CPA	4.06	1.5×10^{-10}

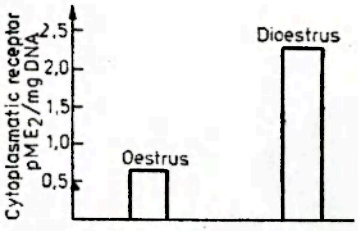

Fig. 2

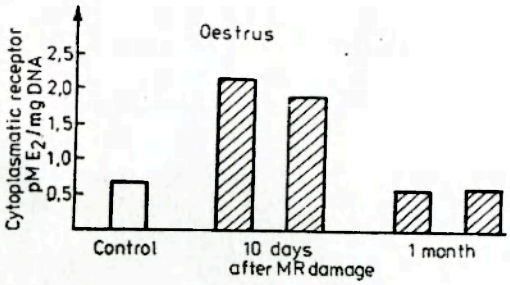

Fig. 3

Fig. 2. Oestradiol (E_2) binding by cytosol of rat uterus in the oestrus and dioestrus phase.

Fig. 3. Oestradiol (E_2) binding by rat uterus cytosol in the oestrus phase 10 and 30 days after MR damage in relation to the control groups.

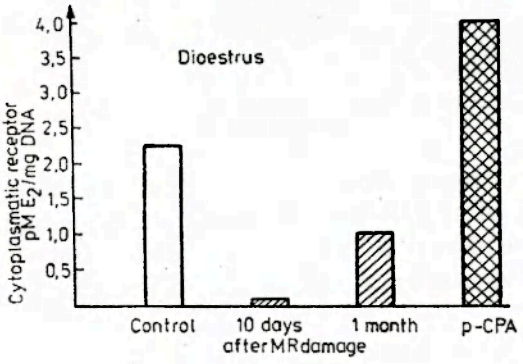

Fig. 4. Oestradiol (E_2) binding by rat uterus cytosol in the dioestrus phase 10 and 30 days after damage to the median raphe nucleus (MR) or administration of p-CPA.

It was demonstrated that the uterus cytosol in the oestrus phase bound specifically 0.66 pM E_2/mg of DNA, while the cytosol from the dioestrus phase bound 2.2 pM E_2/mg of DNA. The results are presented in Fig. 2.

Effect of MR lesion on oestrogen receptor content in the cytosol of rat uterus in the oestrus phase

On the 10th day after MR damage rat uterine cytosol bound specifically 2.1 pM E_2/mg of DNA, on the average (Tab. 1, Fig. 3), that is three times as much as in the control group.

One month after the procedure uterine cytosol bound 0.57 pM E_2/mg of DNA, on the average. In this time period the OR level in rat

uterus was nearly identical as in the control animals in the oestrus phase. Evaluating the effect of MR damage on the number of oestrogen receptors in rat uterus in the oestrus phase it was found that in an early time period after the procedure this number increased significantly, while after one month it fell again to the initial value.

Effect of MR damage on the number of oestrogen receptors in rat uterus in the dioestrus phase and after p-CPA administration

In the study of the effect of MR damage on the OR number in rat uterus in the dioestrus phase a striking fall of its value was observed on the 10th day after the procedure (0.08 pM E_2/mg of DNA) in relation to the control group. Determinations one month after the procedure showed a significant increase in the number of OR (1.02 pM E_2/mg of DNA) as compared with the value on the 10th day after the procedure. Nevertheless, the number of OR one month after MR damage was about half that in the controls.

Thus MR damage caused early after the procedure a striking fall of OR number in the rats in the dioestrus phase, but one month later a tendency for return to the initial value was marked (Fig. 4).

Administration of p-CPA to normal rats caused a significant rise in OR number (4.08 pM E_2/mg of DNA), exceeding the value in the control group (in the dioestrus phase) as well as the value found after the procedure in the animals in the dioestrus phase.

DISCUSSION

The main subject of the investigations presented in this paper are changes in the number of oestrogen receptor in rat uterus after damage to the MR nucleus.

It is believed that oestradiol increased and progesterone decreased the OR number in the uterus [3, 4, 9, 13]. Reports have been published also that OR number is influenced by prolactin which is thought to raise the uptake of ^{3}H-oestradiol by rat uterus [13].

The results presented here confirm earlier observations of other authors that OR number changes depending on the phase of the sexual cycle [20]. In the dioestrus phase the uterus of the normal rat contains three times as many OR than in the oestrus phase.

Earlier investigations demonstrated that electrocoagulation of the median raphe nucleus caused a rise in plasma luteinizing hormone level in castrated female rats. Which was detectable on the 10th as well as on the 30th day after the procedure [19]. Simultaneously, characteristic

changes were found in the ovaries indicating stimulation of their morphotic elements [17].

In connection with the changes in the secretory activity of the hypophysis and ovaries developing after MR lesion evident differences are observed in the number of OR in the uterus in the phase of oestrus as well as dioestrus. In the oestrus phase in the initial time period after MR damage the OR number in the uterus increased on the 10th day after the procedure in relation to controls. Only on the 30th day after MR damage the OR number returned to the value observed in normal rats in the oestrus phase. In the rats with the dioestrus phase developing after MR damage the OR number in the uterus was significantly decreased. In an initial time period it was barely detectable, and later on (on the 30th day) it was raised without, however, reaching the value found in normal rats in the dioestrus phase.

Generally speaking, the rats with MR lesions had in the oestrus phase a greater number of OR in the uterus than control rats, while in the dioestrus phase the OR number was significantly reduced.

It may be supposed that excessive secretion of the ovaries due to increased release of gonadotrophins after MR damage is responsible for these changes. This caused the appearance of prolonged oestrus in most animals, increased number of follicles and corpora lutea in the ovaries [17, 18]. It should be supposed that in this situation excessive secretion of oestrogens and progesterone develops. In the later period of 30 days, when corpora lutea prevail significantly in the ovaries, the number of oestrogen receptors decreases in the uterus.

In a part of the rats with damage to the MR prolonged dioestrus phase develops. In these animals shortly after the procedure (10 days) stimulation of follicles and a rise in their number is observed in the ovaries without a significant rise in the number of corpora lutea. It is not understandable why in these rats the number of OR in the uterus is reduced and not increased. This may be due, perhaps, to prolactin deficiency which may develop after MR damage with consequently reduced production of 5-HT [7]. However, in the rats receiving p-CPA, an inhibitor of serotonin synthesis, prolonged dioestrus phase is observed, with a rise in the number of ovarian follicles without a change in the number of corpora lutea, and with a striking increase in the number of OR in the uterus.

The described changes may be explained in part by the investigations of other authors on the effects of exogenous and endogenous sex hormones and gonadotrophins on the OR number in the uterus. *Jacobson* et al. [10] found that the number of OR decreased in false pregnancy but increased after lutectomy. This observation was confirmed by the re-

ports of *Leung* et al. [13], *Brenner* et al. [3] who demonstrated that exogenous progesterone can exert an action antagonistic to that of oestrogens by reducing the number of specific receptor sites for oestrogens. Similarly *Hsueh* et al. [9] reported that massive doses of progesterone decreased specific binding of oestradiol in the uterus. *Paszko* et al. [16] demonstrated that administration of cestrogens caused initially a striking increase in the number of OR while after prolonged stimulation by oestrogens the number of OR showed no further increase and it even decreased. A similar effect on the OR number is exerted by gonadotrophins. Initially gonadotrophin administration is associated a great rise in OR number, but later on this number falls steeply to barely detectable levels [16]. This example most resembles the experimental model used in this work. Analogous situations existed when the ovaries stimulated by excess amounts of gonadotrophins produced, depending on the duration of the stimulation, either a rise or a fall in the number of uterine OR. The number of OR at a given time depends, most likely, on the ratio of oestrogens to progesterone.

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