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EFFECTS OF PROGESTERONE ON SOME BRAIN NEUROTRANSMITTERS IN INTACT RATS

S. K. CHAUDHURI*†, R. N. CHATTOPADHYAY**, S. K. MAITRA**, S. RAY** AND SRABANI CHAUDHURI*

* *Department of Physiology, Medical College, Calcutta*

and

** *Department of Pharmacology, School of Tropical Medicine, Calcutta*

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Abstract: Effects of progesterone on four neurotransmitters (viz, noradrenaline, 5–HT, dopamine and histamine) of brain were seen in rats with *intact ovaries*. It was found that progesterone lowers the noradrenaline concentration in medulla, pons, midbrain, hypothalamus, thalami and pituitary, uniformly, when the rats were killed within 4 hours of progesterone injection. At longer intervals (48 hrs) effects of progesterone were seen when progesterone in heavy dose was administered to rats pretreated with estrogen. It is likely that one of the modes of action of the oral contraceptives may be the reduction of noradrenaline content in selected areas of brain, by progesterone. It is also suggested, therapeutic usage of progesterone carries the risk of development of depression in the user.

Key words: progesterone neurotransmitters noradrenaline depression oral contraceptives

INTRODUCTION

Receptors of progesterone occur fairly extensively in the brain (1, 2). Progesterone has well recognized effects on the different aspects of metabolism of various neurotransmitters of brain, like, noradrenaline (NA), dopamine (DA) and 5–hydroxytryptamine (5-HT) (3, 4, 5).

The neurotransmitters like NA, DA, 5–HT and histamine (H) are implicated in the etiology of various psychiatric disorders and alterations of mood, like depression, schizophrenia and anxiety states (6). It is possible that the exogenous progesterone, used in oral contraceptive pills, dysfunctional uterine bleeding, Pickwickian syndrome and in other conditions have some effects on the neurotransmitters of the brain which may cause alteration of mood and behaviour. It is reminded that while in some therapeutic regimes, the dose of exogenous progesterone is small, in some others, as in the first day of dysfunctional uterine bleeding, the dose may be stupendous and as high as 60 mgms in the first 24 hrs (7).

Aims of this work included, the study of the effects of progesterone in animals on the levels of NA, DA, H and 5–HT, in brain, which are known to be involved in the etiology of psychiatric disorders. A knowledge of this will forewarn the clinician, planning to use progesterone for therapeutic purposes.

METHODS

216 adult female virgin rats, weighing between 140 to 180 gms were used. The rats were divided into 12 groups. Each group (consisting of 18 rats) was divided further into two sub-groups of 9 rats each. For each group, one subgroup was treated as control and the other as experimental.

In six (shown under A in Table I) groups no pretreatment with estrogen was given (as the rats were intact, some endogenous estrogen was presumed to be present). The experimental subgroups (each subgroup consisting of 9 rats) of all these 6 groups received progesterone intraperitoneally in a vehicle of propylene glycol. The rats of control subgroups received only propylene glycol.

† Corresponding Author

All the rats of the rest of the six groups (B in Table I) received estrogen as pretreatment. 48 hrs after the pretreatment by estrogen, the rats of the experimental subgroups received progesterone in propylene glycol by IP route while the controls received only propylene glycol IP.

Rats were killed 2, 4 and 48 hrs after progesterone (experimental) or propylene glycol (control) treatment. Rats were sacrificed betwen 12 noon to 2 p.m. to avoid any diurnal variation of concentration of NA.

Brain was dissected out, wiped quickly and divided into two portions, (i) medulla, pons, mid brain, thalami, hypothalamus and the pituitary (the *'brain stem - hypothalamus – pituitary'* portion) and (ii) cerebral hemispheres and the cerebellum.

Each portion was cut into small pieces (each piece of about 2 × 2 × 2 mm size), stored in the freezer of a refrigerator (–8.0°C) immediately, till the chemical estimation of the neurotransmitters, which was not later than 24 hrs, was made.

Estimation of the NA, DA, 5–HT and H was done by the method of Sadavongvivad (8).

Drugs : 'Ovocyclin P' (estradiol), in dose of 20 µgms/100 gm body wt, was given intrapertioneally (IP) to pretreat with estrogen. Two doses of progesterone, *viz,* a high and a low dose were used. Rats receiving high dose received 0.23 mg/100 gm body wt, and those receiving low dose received 0.06 mg/100 gm body wt. of progesterone in the form of 'primolut N' (norethisterone) given IP, in propylene glycol.

RESULTS

Table I shows the effects of progesterone on NA, DA, 5-HT and H concentrations. In 9 out of 12 subgroups where the rats were treated by progesterone, the NA concentration of the brain stem-hypothalamus-pituitary segment fell as compared with the controls. All the three subgroups where NA levels did not show any alteration, were those where the rats were killed after 48 hrs and either had not received any pretreatment with estrogen or had received a low dose of progesterone. Administration of progesterone caused fall of [NA] in the brain stem-hypothalamus-pituitary segment in *every* group whether (a) the progesterone was given without any pretreatment or with estrogen or (b) whether the progesterone was given in high dose or low dose, *provided,* the rats were killed within 4 hours of the progesterone treatment. On the other hand, [NA] was restored to normalcy within 48 hours of progesterone treatment, unless the dose of progesterone was high and also the rats had been pretreated with estrogen.

If therefore, we examine the results of progesterone treatment on the [NA] of the brain stem-hypothalamus-pituitary segment of the brain in the 2nd and 4th hours alone, it will be seen that progesterone causes a significant fall of [NA] in 100% of rats.

On other neurotransmitters (DA, 5-HT and H) progesterone treatment produced inconsistent effects.

TABLE I : Effects of progesterone injection on the *brain stem hypothalamus - pituitary segment* of brain of rats with intact ovaries.

A. Rats without any pretreatment by estrogen.

Dose	Hr. of sacrifice	Neurotransmitter concentrations (in µgms/gm)							
		NA		DA		5-HT		H	
		CONTROL	EXPT.	CONTROL	EXPT.	CONTROL	EXPT.	CONTROL	EXPT.
LD	2	0.52±0.06*	0.32±0.04 P<0.02	0.06±0.01	0.07±0.02	0.17±0.02	0.19±0.02	0.54±0.04	0.50±0.05
LD	4	0.49±0.02*	0.44±0.02 P<0.05	0.13±0.01	0.14±0.01	0.34±0.01	0.35±0.01	0.55±0.02	0.58±0.02
LD	48	0.54±0.05	0.53±0.04	0.08±0.02*	0.13±0.01 P<0.05	0.25±0.02	0.25±0.02	0.83±0.04	0.81±0.07
HD	2	0.52±0.02*	0.27±0.01 P<0.001	0.17±0.02	0.14±0.03	0.44±0.03*	0.33±0.01 P<0.05	0.41±0.03	0.45±0.05
HD	4	0.49±0.04*	0.29±0.01 P<0.001	0.09±0.01	0.10±0.01	0.45±0.03	0.49±0.03	0.52±0.04	0.59±0.03
HD	48	0.45±0.02	0.40±0.02	0.14±0.01	0.13±0.01	0.45± 0.02	0.48±0.02	0.55±0.03	0.59±0.03

B. Rats after pretreatment by estrogen.

Dose	Hr. of sacrifice	Neurotransmitter concentrations (in μgms/gm)							
		NA		DA		5-HT		H	
LD	2	0.41±0104* P<0.01	0.26±0.02	0.09±0.02	0.09±0.02	0.21±0.02	0.24±0.01	0.45±0.02	0.44±0.04
LD	4	0.46±0.02* P<0.01	0.36±0.02	0.09±0.02	0.10±0.02	0.24±0.01* P<0.05	0.21±0.01	0.46±0.03	0.44±0.02
LD	48	0.23±0.02	0.21±0.02	0.07±0.01	0.07±0.01	0.38±0.02	0.36±0.02	0.59±0.02* P<0.001	0.46±0.02
HD	2	0.72±0.02* P<0.001	0.53±0.03	0.03±0.02	0.02±0.01	0.29±0.01	0.27±0.01	0.90±0.02* P<0.01	0.73±0.05
HD	4	0.40±0.05* P<0.01	0.24±0.02	0.06±0.01* P<0.05	0.03±0.01	0.20±0.02	0.25±0.02	0.59±0.02	0.60±0.04
HD	48	0.25±0.01* P<0.001	0.13±0.02	0.05±0.01	0.03±0.01	0.15±0.01* P<0.05	0.18±0.01	0.30±0.06	0.31±0.02

P = progesterone, E= estrogen, LD = Low dose of progesterone, HD = high dose of progesterone, * = difference is significant.

DISCUSSION

Our results (that progesterone causes a consistent fall of NA concentration in some areas of brain) thus support the works of Tonge and Greenglass (9), who noted in their ovariectomized rats that estrogen and progesterone treatment causes a fall of [NA] in the mid portion of rat brain (consisting of hippocampus, hypothalams, thalamus and striatum).

In our experiment, although the concentrations of DA, H and 5-HT changed occassionally, no consistent pattern was seen. This may be due to the (i) uncertainty posed by the endogenous estrogen or progesterone, or, (ii) dilution effect (we took rather too big blocks in the shape of brain stem-hypothalamus-pituitary segment, as well as in the form of entire cerebral hemisphere and cerebellum). But a third possibility also exists: we used rats with intact ovaries. Owing to the presence of various chemicals some of which have hormone like activities and might have (feed back) effects on the hypothalamus, like, ovarian inhibin, testosterone and relaxin (10,11) the final effect becomes much more uncertain.

Depression is associated with fall of [NA] in some areas of brain. Oral contraceptives are known to produce depression; indeed, about 6% users of oral contraceptive pills develop sufficient depression which needs discontinuation of the oral contraceptive therapy (12). Our results (that progesterone causes fall of NA concen-tration) suggets strongly that depression might be due to presence of progesterone in the oral contraceptive pills. Also, there are evidences to suggest that clinically, women receiving progesterone are susceptible to worsening of the mood (13).

The current practice in the treatment of dysfunctional uterine leeding is to administer a loading dose (between 30 to 60 mg) of progesterone on the first day followed by a low maintenance dose of 5 to 10 mg/day (7). Our work suggests that this heavy initial dose is attended with the risk of depression. It will be seen that our low dose, *viz* 0.06 mg/100 gm body wt (ie, 36 mg in a women of 60 kg body wt.) falls on the lower side of the advocated loading dose (prescribed for the patients) on the first day.

Reports indicate that increase in the noradrenergic (NAgic) activity or the rise of [NA] in the hypothalamus can lead to LH surge, ovulation and appearance of sex behaviour in the female animals, whereas NA depletion or suppression of NAgic activity can cause ovulation failure (14,15). It is also known that a high rise of progesterone lead to blockade of LH surge and thus ovulation (16) and indeed that is why no LH surge or ovulation is seen in the secretory phase of the menstrual cycle. Probably the termination of the sex behaviour in animals is also due to the rise of progesterone (5). If all these data are integrated with our finding, a tentative picture like the following emerges:

Treatment by progesterone leads to NA depletion which in turn leads to inhibition of LH surge and ovulation. *This may be one of the possible ways how*

the oral contraceptive pill acts as ovostatic agent.

It will be seen that [NA] varied somewhat unusually, between themselves in some subgroups serving as controls. Whereas the mean [NA] in most (9 out of 12) subgroups of control were between 40 to 54 µgms/gms, in the rest three subgroups the mean [NA] were sharply different. The explanation at this stage, is not clear.

However, all these 3 groups were those where pretreatment with estrogen was made. One possible explanation of such wide fluctuation is, pretreatment with estrogen changed their [NA] of the brain. It may be recalled that estrogen treatment is expected to cause alteration of NA concentration in hypothalamus, as estrogen causes efflux of NA from the hypothalamus (17). Also, NA turnover in the hypothalamus increased in proestrus (18). As estrus development requires about 36 hours, it is possible that the endogenous progesterone also appeared in the animals further influencing the [NA]. That is, other influencing factors (like administration of a heavy dose of estrogen) acting concomitantly produced the aforesaid aberrations in the [NA] of the controls in those 3 subgroups. Anyway, this reinforces our suspicion, whatever be the status of the endogenous estrogen or progesterone, exogenous progesterone overrides the influence of the other factors and causes a reduction of [NA] in the brain stem - hypothalamus - pituitary segment of the brain.

Finally, it will be seen that instead of taking the hypothalamic block only (like the classicist), we took rather a wide area as a block which consisted of many other parts of the brain and even the whole pituitary in our brain stem-hypothalamus-pituitary block. Our justification was, the classical hypothalamic block excludes the locus ceruleus and lateral tegmentum, ie, areas from where the noradrenergic fibers arise. Brain stem-hypothalamus-pituitary segment also included the recticular activating system where the Nagic fibres are present. Inclusion of pituitary ensured that dopaminergic fibers entering the pituitary (which are concerned with the prolactin inhibition) are not left out.

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