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EFFECTS OF SEROTONIN ON SPONTANEOUS OVULATION: A THEORY FOR THE DUAL HYPOTHALAMIC CONTROL OF OVULATION

By

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ABSTRACT

In an attempt to study the inhibitory effects of serotonin on spontaneous ovulation, the monoamine was administered subcutaneously to rats with 4-day oestrous cycles. Administration (50 mg/kg) at 5.00 p.m. on the day before pro-oestrus interfered with ovulation without affecting vaginal cornification, uterine ballooning or mating. This effect on ovulation could be overcome with methysergide, a specific antagonist of serotonin. Administration, at appropriate times, of LH or oestradiol benzoate or the stimulus provided by mating prevented the inhibitory effects of serotonin, implicating a central rather than a peripheral mechanism in interference with ovulation. This was further confirmed by the persistence in the serotonin-treated animals of high levels of pituitary LH, comparable to pro-oestrous levels. It is probable that serotonin blocked ovulation by augmenting the inhibitory effects of serotoninergic fibres in the hypothalamus. It is postulated on the basis of the present results and those reported in the literature that the hypothalamus exercises a dual control over ovulation, inhibitory influences being transmitted through serotonin-linked neurones while stimulatory effects are delivered via catecholaminergic fibres to neurones which synthesize releasing factor(s) for the ovulating hormone. It is postulated that a certain degree of balance in favour of the catecholaminergic system is necessary for the occurrence of ovulation. Inhibition of ovulation occurs whenever the serotoninergic system gains dominance over catecholaminergic system. The theory can account for the effects on ovulation of a multitude of chemically diverse agents reported in the literature.

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It has become apparent from recent studies that a complex series of neuroendocrine events precede ovulation. Some of the components of this series have been studied in this laboratory. These include positive oestrogen feedback (Labhsetwar 1970a,b) and its site of action (Bainbridge & Labhsetwar, in press), LH-RF (Labhsetwar 1970a,b) and LH and/or FSH as a final stimulus for the rupture of follicles (Labhsetwar 1970c). It is known that a multitude of chemically diverse agents interfere with spontaneous ovulation, including progestagens (progesterone (Rothchild 1965); oral contraceptives (Pincus 1965)); anti-oestrogens (clomiphene, ICI 46,474; MER-25 etc. (Labhsetwar 1970a,b; Shirley et al. 1968)); tranquillizers (reserpin, chlorpromazine (Barruclough & Sawyer 1957); haloperidol (Labhsetwar, in press)); anaesthetics (barbiturates (Everett 1964); halothane (Labhsetwar, unpublished)); a-adrenergic blockers (phenoxybenzamine, dibenamine (Everett 1964; Moore 1961; Ratner 1970)); ethanol (Keiffer & Ketchel 1970); and alkaloids (ergocornine (Kraicer & Strauss 1970)). Some of these agents are known to act centrally. The exact neural pathway by which the input of these agents is funnelled to a common link in the chain, i.e. hypothalamic neurones which synthesize releasing factor(s) for ovulating hormone (an as yet undetermined combination of LH and FSH) is not known. The median eminence region is particularly rich in serotonin (5-HT)-containing neurones (Fuxe & Hökfelt 1969). The possibility, therefore, exists that this monoamine may function as a chemical mediator at synaptic junctions between 5-HT fibres and neurones which synthesize releasing factors. The present paper examines the effects of 5-HT on spontaneous ovulation in the 4-day cyclic rat and then attempts to present an unified concept for the dual hypothalamic control of ovulation. A preliminary report has appeared elsewhere (Labhsetwar 1971).

MATERIALS AND METHODS

Rats. – Adult, sexually mature females weighing approximately 180 to 220 g (2 to 4 months old) were of the Alderley Park Strain I, originally derived from Wistar rats and had been bred for several generations under specific pathogen free conditions. Animals were maintained under temperature (22°C) and light (from 6.00 a.m. to 8.00 p.m.) controlled conditions and allowed free access to a standard diet and tap water. Vaginal smears were taken daily and all animals included in the experiment had at least one 4-day oestrous cycle. Individual days of the oestrous cycle were designated as oestrus, metaoestrus, dioestrous and pro-oestrus.

Compounds. – 5-HT (5-hydroxytryptamine creatinine sulphate), 5-HTP (5-hydroxy-L-tryptophan), tryptophol (3-hydroxyethyl indole-3-ethanol) and melatonin (N-acetyl-5-methoxytryptamine) were obtained from Sigma Co., London. Methysergide (methysergide hydrogen maleate) was from Sandoz Co.; mebanazine was from ICI; while oestradiol benzoate was supplied by B.D.H., London. Ovine luteinizing hormone (LH-S-14) was a gift from the Endocrine Study Section, National Institutes of Health, Bethesda, USA).
All compounds except oestradiol, which was given in oil, were made up in saline. In initial experiments 5-HT was mixed with physiological saline and ball milled overnight. In the later experiments the monoamine was dissolved in saline made slightly acidic with hydrochloric acid. All injections were made in 0.25 or 0.5 ml saline either subcutaneously or intraperitoneal at a specific time in the oestrous cycle as indicated under results. Other compounds were similarly injected in saline.

Rats were killed, unless stated otherwise, on the morning of expected oestrus and tubal ova per rat determined (Labhsetwar 1970a). In some experiments ovarian and uterine weights were recorded and pituitary glands kept frozen for bioassay of LH by the method of Parlow (1961) modified as already described (Labhsetwar 1969).

Appropriate controls were run simultaneously. For some unknown reason the effect of 5-HT on ovulation tended to vary from one batch of rats to another. Because of this we have always included a 5-HT-treated group as a control when other treatments were superimposed.

RESULTS

5-HT exerted sedative effects which appeared transient. The changes in body weight during the 48 h between 5-HT injection and autopsy were not much different than those found in the controls. Therefore, at the 50 mg/kg dose used in this study, toxicity is unlikely to play any significant role.

Effects of 5-HT on ovulation (Table 1)

A single subcutaneous injection of 5-HT (50 or 75 mg/kg) at 5.00 p.m. on the day before pro-oestrus interfered with ovulation but a lower dose (10

Table 1.
Effects of 5-HT on ovulation and organ weights in 4-day cyclic rats. All rats were killed on the morning of expected oestrus. (Mean ± se).

<table>
<thead>
<tr>
<th>Dose (mg/kg, sc)</th>
<th>Day of administration</th>
<th>Rats ovulating</th>
<th>Ova ovulating rat</th>
<th>Uterine wt. (mg)</th>
<th>Ovarian wt. (mg)</th>
<th>Rats with cornified smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Oestrous control</td>
<td>5/5 (100)</td>
<td>9.4 ± 1.7</td>
<td>327 ± 12</td>
<td>79 ± 3</td>
<td>5/5</td>
</tr>
<tr>
<td>0</td>
<td>Pro-oestrous control</td>
<td>0/5 (-)</td>
<td>-</td>
<td>348 ± 25*</td>
<td>78 ± 3</td>
<td>0/5</td>
</tr>
<tr>
<td>75</td>
<td>Dioestrus</td>
<td>1/5 (20)</td>
<td>12</td>
<td>306 ± 33</td>
<td>81 ± 4</td>
<td>4/5</td>
</tr>
<tr>
<td>50</td>
<td>5.00 p.m.</td>
<td>3/11 (27)</td>
<td>1</td>
<td>326 ± 8</td>
<td>84 ± 4</td>
<td>11/11</td>
</tr>
<tr>
<td>50</td>
<td>Pro-oestrous, 9.30 a.m.</td>
<td>1/10 (10)</td>
<td>11</td>
<td>310 ± 18</td>
<td>78 ± 3</td>
<td>10/10</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>3/4 (75)</td>
<td>8.3 ± 1.7</td>
<td>272 ± 11*</td>
<td>81 ± 3</td>
<td>3/4</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>3/4 (75)</td>
<td>6.3 ± 0.8</td>
<td>281 ± 11*</td>
<td>88 ± 10</td>
<td>3/4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>3/4 (75)</td>
<td>10.3 ± 1.9</td>
<td>319 ± 17</td>
<td>87 ± 6</td>
<td>3/4</td>
</tr>
</tbody>
</table>

* P < 0.05 when compared with oestrous control.
mg/kg) only reduced the ovulation rate, without affecting the incidence of ovulation. When the experiment with 50 mg/kg was repeated essentially similar results were obtained. The monoamine proved inactive when administered on the morning of pro-oestrus (i.e. before the critical period), when neural blocking agents are known to block ovulation.

With a few exceptions 5-HT did not cause significant changes in the uterine weight nor did it affect ovarian weight. Vaginal smears in virtually all the treated rats were cornified at autopsy and were indistinguishable from those in controls. When rats pre-treated with 5-HT were killed at 12:00 on the day of pro-oestrus the uterine weight was not significantly different from that in the controls (389 vs 398 mg, P > 0.05).

**Specificity of 5-HT** (Table 2)

When rats were pretreated with methysergide (25 mg/kg o.p.) a specific antagonist of 5-HT half an hour before the subcutaneous injection of 5-HT on the day before pro-oestrus, no interference with ovulation was encountered.

### Table 2.
Specificity of 5-HT in blocking ovulation as demonstrated by reversal of blockage by antagonist of 5-HT-methysergide and failure of several related compounds to inhibit ovulation. All rats were killed on the morning of expected oestrus.

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Dose (mg/kg)</th>
<th>Additional treatment (Dose mg/kg and route)</th>
<th>No. rats ovulating Total no. (%)</th>
<th>Ova ovulating rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. None</td>
<td>–</td>
<td>–</td>
<td>5/5 (100)</td>
<td>11.4 ± 0.51</td>
</tr>
<tr>
<td>B. 5-HT</td>
<td>50</td>
<td>–</td>
<td>1/6 (16)</td>
<td>1</td>
</tr>
<tr>
<td>C. 5-HT</td>
<td>50</td>
<td>Methysergide&lt;sup&gt;b&lt;/sup&gt; (25, ip)</td>
<td>4/4 (100)</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>D. –</td>
<td>0</td>
<td>Methysergide&lt;sup&gt;b&lt;/sup&gt; (25, ip)</td>
<td>4/4 (100)</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>E. 5-HTP</td>
<td>50</td>
<td>–</td>
<td>4/4 (100)</td>
<td>11.2 ± 0.7</td>
</tr>
<tr>
<td>F. Tryptophol</td>
<td>50</td>
<td>–</td>
<td>3/4 (75)</td>
<td>11.7 ± 0.7</td>
</tr>
<tr>
<td>G. Parachloro-phenylalanine</td>
<td>50</td>
<td>–</td>
<td>3/4 (75)</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>H. Melatonin</td>
<td>50</td>
<td>–</td>
<td>4/4 (100)</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>I. MAO&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10–20</td>
<td>–</td>
<td>4/11 (36)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.2 ± 1.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> All treatments applied at 5.00 p.m. on dioestrus, sc.

<sup>b</sup> Half an hour before 5-HT.

<sup>c</sup> Mehanazine (intravenous).

<sup>d</sup> All rats had cornified smears except one.
(Group C). The antagonist alone in the absence of 5-HT was inactive (Group D) as were 5-HP, a precursor of 5-HT (Group E) or tryptophol, which is an intermediate metabolite of the same (Group F). Since 5-HT is an intermediate in the formation of melatonin, which is known to have inhibitory effects on secretion of gonadotrophins (Fraschini 1969), the possibility that the effects of the monoamine may be mediated through its conversion to melatonin was examined in Group H. A dose comparable to 5-HT (50 mg/kg) failed to interfere with ovulation. Parachlorophenylalanine, a specific depletor of 5-HT, also proved inactive (Group G). Mebanazine, a monoamine oxidase inhibitor which prevents metabolic degradation of 5-HT, inhibited ovulation in 64% of rats (Group I) without affecting vaginal cornification.

**Restoration of ovulation** (Table 3)

Ovulation blocked by the administration of 5-HT at 5.00 p.m. on the day before pro-oestrus could be restored by the intravenous injection of LH (Expt. A). Similarly concurrent administration of oestradiol benzoate (Expt. B)

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Dose of 5-HT (mg/kg, sc)</th>
<th>Restoring treatment (Dose, route)</th>
<th>No. rats ovulating (Mean ± se)</th>
<th>Ova ovulating rat (Mean ± se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>–</td>
<td>5/5</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>–</td>
<td>3/11</td>
<td>1¹</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>LH (25 µg, iv)</td>
<td>3/4</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>–</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Oestradiol benzoate (400 µg, sc)</td>
<td>5/5</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>–</td>
<td>1/3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Mating stimulus¹</td>
<td>4/5</td>
<td>10.3 ± 0.9</td>
</tr>
</tbody>
</table>

¹ Rats were caged with males on the night of pro-oestrus. All had spermatozoa in vaginal smears on the following morning.

² One rat each had one egg without cumulus.
prevented the inhibitory effect of 5-HT. When rats pretreated with 5-HT were caged with males on the night of expected pro-oestrus, all females mated as indicated by the presence of spermatozoa in the vaginal smear the next morning and all but one rat had a normal complement of tubal ova (Expt. C). By contrast, only one of the 3 concurrently run controls treated with the amine, but not caged with males, had ovulated. Thus the stimulus provided by mating was effective in reversing the effect of the monoamine.

**Pituitary LH content** (Table 4)

The hypophyseal level of LH in rats pretreated with 5-HT at 5.00 p.m. on dioestrus and killed on the morning of expected oestrus, when no tubal ova were found, was comparable to levels found on the morning of pro-oestrus in the control rats. Thus the depletion of pituitary LH stores which occurs in association with ovulation was blocked by the monoamine.

**Anti-oestrogenic property of 5-HT**

Vaginal cornification induced by exogenous oestradiol (1 µg/kg for 3 days) in spayed rats was not blocked by the simultaneous administration of a single injection of 5-HT (50 mg/kg).

### DISCUSSION

There was no significant loss in body weight during the experimental period and no untoward side effects were apparent apart from transient sedation. Furthermore, methysergide, a specific antagonist of 5-HT prevented the effects of the monoamine, and other compounds related to 5-HT given in comparable doses failed to inhibit ovulation (Table 2). These results demonstrate a high

<table>
<thead>
<tr>
<th>Group</th>
<th>% Inhibition of ovulation</th>
<th>LHb concentration</th>
<th>Total content</th>
<th>λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrous controla</td>
<td>0</td>
<td>0.79 (0.46–1.34)</td>
<td>8.96</td>
<td>0.253</td>
</tr>
<tr>
<td>Pro-oestrous</td>
<td>–</td>
<td>3.20 (1.87–5.50)</td>
<td>24.67</td>
<td>0.253</td>
</tr>
<tr>
<td>Serotonin</td>
<td>100</td>
<td>3.89 (2.74–5.47)</td>
<td>43.1</td>
<td>0.158</td>
</tr>
</tbody>
</table>

(50 mg/kg)

a Data from earlier study Labhsetwar (1970b).

b µg equivalents of NIH-LH-S-14/mg wet pituitary.
degree of specificity of 5-HT and rule out toxicity as a cause of the inhibition of ovulation.

Inhibitory effects of 5-HT on gonadotrophin-induced ovulation in immature mice (Brown 1967) and rats (O'Steen 1965; Jaitly et al. 1967) and more recently on spontaneous ovulation in the adult rat (Endersby et al. 1970) have been reported. However, conclusive evidence of a central mechanism of action of the amine in all cases is not available. In fact, in the last study, 100 mg/kg of 5-HT blocked ovulation even when administered after the "critical-period". An earlier study from the same laboratory showed that 5-HT did not interfere with gonadotrophin-induced ovulation in hypophysectomized-immature rats (Jaitly et al. 1967). Similarly, a peripheral action of 5-HT is unlikely to play a significant role in the present study since (a) LH restored ovulation in the blocked rats (even a threshold dose of LH (5 µg) has been found effective in this respect (unpublished observations)); (b) pituitary LH stores in those rats which were blocked remained at pro-oestrous levels and (c) the mating stimulus prevented the effect of the monoamine. The last observation suggests (since the mating stimulus is transmitted through the central nervous system) that the anterior pituitary was sensitive to LH-RF and the ovaries were responsive to endogenous LH (and FSH). Collectively, these results imply a central rather than a peripheral action of 5-HT in interfering with ovulation. It is probable that the monoamine interfered with ovulation by augmenting the inhibitory effects of 5-HT fibres. That such fibres normally exercise restraint over the secretion of LH has been recently demonstrated by an injection of 5-HT into the third ventricle (Kamberi et al. 1968; Schneider & McCann 1970). Our results additionally suggest that the decrease can be such that ovulation fails to occur. Indeed, it has already been demonstrated that the superovulatory response to exogenous gonadotrophins in immature rats can be reduced by treatments designed to raise hypothalamic levels of 5-HT (Kordon et al. 1968; Kordon 1969).

It must, however, be noted that doses of 5-HT required to block ovulation are exceedingly high in relation to the amount of the monoamine likely to be present in the hypothalamus which from our unpublished observations is less than 5 µg. This may be due to the failure of systemically administered 5-HT to cross the blood-brain barrier (Weil-Malherbe et al. 1961). The precursor of 5-HT, 5-HTP, is known to cross the barrier easily, but proved inactive; perhaps the dose used was too low or the time of injection may have been inappropriate.

The existence in the hypothalamus of a stimulatory system linked to a catecholamine (CA) – either epinephrine (Markee et al. 1952; Rubinstein & Sawyer 1970) or dopamine (Kamberi et al. 1968; Schneider & McCann 1970) – has been reported. These reports taken in conjunction with the present data suggest that the hypothalamus exercises a dual control over ovulation, the
inhibitory influences being transmitted through 5-HT fibres while the stimulatory ones via CA fibres. In fact, such a dual hypothalamic control has been suggested earlier from the study of super-ovulation in immature rats (Kordon & Glowinski 1969) and spontaneous ovulation in hamsters (Lippman 1968). We postulate that a certain degree of balance between these contrasting influences determines the occurrence of ovulation. In the reproductive cycle, blood LH levels in several species studied appear to remain more or less constant except in association with ovulation when they rise sharply (see Rosenberg 1968). This suggests that during most of the reproductive cycle, 5-HT fibres dominate over the CA ones, except at the time of ovulation when the balance is altered in favour of CA system by an active intervention of some unknown factor(s). Although the exact nature of this factor(s) is not known at the present time, it is likely to be the positive feedback of oestrogen in the spontaneously ovulating species (Ferin et al. 1969; Labhsetwar 1970a,b) and the mating stimulus in the induced-ovulating species such as the rabbit etc. (Hammond & Marshall 1925).

The theory suggests that inhibition of ovulation occurs whenever 5-HT fibres gain dominance over the CA ones. This can be brought about either by augmenting the inhibitory influences of 5-HT fibres as is likely to occur following exogenous administration of 5-HT (Table 1), or by treatments designed to raise the endogenous level of 5-HT (Kordon 1969; Kordon et al. 1968; Lippman 1968) and/or by reducing the stimulatory influences of CA system. Thus, haloperidol or chlorpromazine are believed to interfere with dopaminergic transmission by blocking post-synaptic receptors (see Fuxe & Hökfelt 1969), and both of these compounds can block ovulation following administration late in dioestrus (Barraclough & Sawyer 1957; Harrington et al. 1966; Labhsetwar, in press). Inhibitors of CA synthesis are also known to interfere with induced ovulation in immature rats (Coppola et al. 1966; Kordon & Glowinski 1969). CA transmission can also be interrupted by a-adrenergic blockers, since phenoxybenzamine (Ratner 1970) or dibenamine (Everett 1964) can block spontaneous ovulation. Thus the multitude of chemically diverse agents mentioned in the introduction may interfere with ovulation either by augmenting the inhibitory influences of 5-HT fibres and/or by interfering with CA transmission, leaving the 5-HT system in a state of dominance. At the present time the precise contribution of each system in case of every ovulation inhibitor is not known.

Conversely, ovulation should occur, according to the theory, whenever the balance favours the CA system. This may be brought about by stimulation of adrenergic system. Experiments are currently being done to test this hypothesis but the success of such a treatment would obviously depend on how selectively the agent stimulates a-adrenergic receptors, since β-receptors play very little role in ovulation (unpublished observations). Specific inhibitors of
5-HT synthesis, by eliminating serotonergic influences, should leave the CA system in dominance, but so far we have failed to trigger ovulation by means of such agents. Thus, neither parachlorophenylalanine, a specific depletor of 5-HT, nor methysergide – a specific antagonist of 5-HT, caused ovulation in pseudopregnant rats. Perhaps the balance did not shift following these treatments to an optimum degree.

The theory of dual hypothalamic control can be applied to account for several varied observations in the literature (Fig. 1). Thus oestrogen or the mating stimulus may overcome the inhibitory effects of 5-HT by shifting the balance in favour of the CA system by directly stimulating the latter (the monoamine by itself had no demonstrable oestrogenic or anti-oestrogenic property). The stimulation of the CA system can also account for induction of ovulation in immature (Hohlweg 1937) or pregnant rats (Everett & Nicholas 1968; Brown-Grant 1969) by a single injection of oestrogen. However, chronic administration of oestrogen (as in oral contraceptives) is known to exert inhibitory influences on ovulation. Perhaps when CA neurones are chronically

![Diagram of hypothalamic control of ovulation](image_url)

**Fig. 1.**

A diagrammatic representation of the theory of dual hypothalamic control of ovulation. It is postulated that stimulatory and inhibitory influences are transmitted to LH-RF synthesizing neurones in the median eminence region by catecholamine and serotonin-dependent mechanisms respectively, and a certain degree of balance in favour of the catecholaminergic system triggers ovulation while the opposite interferes with it. A wide variety of agents are believed to affect ovulation simply by shifting the balance between the contrasting influences in an appropriate direction and to an optimum degree.
exposed to oestrogen, they lose their sensitivity. It is also possible to explain
the failure of ovulation in persistent oestrous rats as being due to a permanent
maintenance of balance in favour of the 5-HT system, brought about by neo-
natally administered androgen or other treatments. Clomiphene, then, may in-
duce ovulation in these animals (Docke 1969) as well as in women (Greenblatt
1961) simply by shifting the balance in favour of the CA system. The mild
oestrogenicity of clomiphene may stimulate CA system, while its anti-oestro-
genic property may depress 5-HT system leaving the former in a state of
optimal domination to trigger ovulation.

Admittedly additional evidence is required to further substantiate the theory;
but the theory does permit designing meaningful experiments to check its
validity.

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