J. Endocrin. (1967), **37**, 327–333
Printed in Great Britain

THE EFFECT OF 5-HYDROXYTRYPTAMINE AND TWO OF ITS ANTAGONISTS ON OVULATION IN THE MOUSE*

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(*Received* 24 *August* 1966)

SUMMARY

Immature mice were induced to ovulate by a single injection of pregnant mare serum gonadotrophin (PMS). Additional treatment with 5-hydroxytryptamine (5-HT) during the final 24 hr. of the experiment caused significant alteration in the dose-response relationship for PMS, the ovulatory response to low doses of PMS being increased and that to high doses reduced. Both lysergic acid diethylamide and methysergide caused a consistent and significant reduction in the incidence of ovulation induced under the same test conditions.

Neither 5-HT nor its antagonists influenced spontaneous ovulation in adult mice of a different strain under the conditions tested. Therefore, if 5-HT is involved at some stage in the processes leading up to ovulation when this is induced in immature mice, either it does not have the same importance or its effect is less easily modified under the physiological conditions of spontaneous ovulation in mice of the strain used.

INTRODUCTION

Previous experiments concerned with the effects of drugs on the induction of ovulation by two injections of gonadotrophin in immature mice (Brown, 1966) showed that large doses of 5-hydroxytryptamine (5-HT) 2 hr. after the second injection of gonadotrophin consistently increased the number of mice ovulating. The present paper describes the effects of 5-HT on ovulation in two other experimental situations. The first of these was the induction of ovulation in immature mice by a single injection of gonadotrophin given 2 days before 5-HT. The aim of these experiments was to see whether 5-HT acted only if given within a few hours of the injected gonadotrophin, the absorption and distribution of which it might alter. The second situation was that of spontaneous ovulation in adult mice to see if 5-HT would influence normal ovulation. Antagonists of 5-HT were also tested, both on induced

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and spontaneous ovulation, to explore the possibility that 5-HT is necessarily involved in the processes leading up to ovulation.

MATERIALS AND METHODS

The effect of drugs was tested in the following experimental situations.

(1) *Induction of ovulation in immature mice by a single injection of gonadotrophin*

Mice of the Schofield strain, aged 25 ± 1 days, were given a single subcutaneous injection of pregnant mare serum gonadotrophin (PMS) (Gestyl, Organon) at 13.00 hr. on the first day. This injection was in a volume of 0·5 ml. 0·9 % NaCl solution; all other injections mentioned in this paper were in 0·1 ml. The mice were killed at 10.00 hr. on the fourth day and response was measured by the number of mice in a treatment group showing ova surrounded by cumulus cells in the uterine tubes. Differences in the number of mice ovulating were compared by the χ^2 test, and dose-response lines after the probit transformation of percentages were compared according to Gaddum (1953). The drugs tested were injected 24, 20 and 16 hr. before the mice were killed. The effect of a barbiturate was also tested to discover the extent to which endogenous gonadotrophin was likely to be contributing to the ovulatory response. Schofield mice were used in these experiments because attempts to induce ovulation with a single injection of PMS in mice of the OS 1 strain (which was used in a different situation in a previous study (Brown, 1966)), were not successful.

(2) *Spontaneous ovulation*

Adult female OS 1 mice were caged in pairs and vaginal smears were examined every morning between 08.00 and 09.30 hr. After normal cyclic activity had been demonstrated, a mouse was selected for use on the first day the vaginal smear was free of leucocytes. Drugs were injected at various times on this day and the mice were killed at a mean time of 09.30 hr. the next day. The uterine tubes were examined for ova which were counted if present. Absence of leucocytes is a simple and relatively objective criterion, and has been shown to be the most useful one to assess response in assays of oestrogen (Biggers & Claringbold, 1954). The selection of mice in this way has the advantage that, in our colony, from $\frac{1}{2}$ to $\frac{2}{3}$ of control mice examined the next day will have ovulated. It should therefore be possible to demonstrate the effect of a drug which inhibits ovulation, such as a barbiturate, by a decrease in the proportion ovulating. If, on the other hand, a drug induces ovulation prematurely, this should also be demonstrable.

Drugs. 5-Hydroxytryptamine creatinine sulphate (referred to as 5-HT but doses stated in terms of the creatinine sulphate) was dissolved in 0·9 % NaCl solution and injected subcutaneously. Lysergic acid diethylamide (LSD) and the 1-(hydroxymethyl) propylamide of 1-methyl-(+)-lysergic acid hydrogen maleate (methysergide bimaleate, MS) were dissolved in distilled water and injected intraperitoneally. Solutions of 5-HT were used within an hour of preparation, but in some experiments solutions of the antagonists were stored in a dark refrigerator for up to 8 hr. if

multiple injections were made. In all experiments control mice were injected with the appropriate vehicle.

Body weight. Body weights of immature mice were taken when they were killed. The mean weight of control mice was 19·4 g. and in no experiment did the mean body weight of mice treated with drugs differ significantly from the control mean. Adult mice were weighed before being injected with drugs and again when they were killed next day. When 400 μg. 5-HT were given twice in the day there was a small but significant ($P < 0{\cdot}02$) fall in weight. None of the other treatments caused significant change. The mean weight of mice before treatment was 41·6 g.

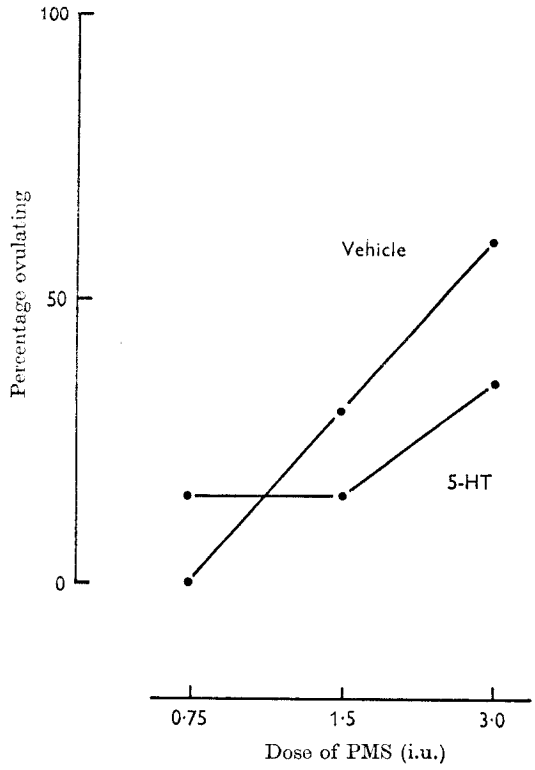

Fig. 1. The effect of 5-hydroxytryptamine (5-HT) (three injections of 100 μg. each) on the ovulatory response to a single injection of pregnant mare serum gonadotrophin (PMS) in immature mice. Lines relating percentage ovulating to log dose of PMS are drawn for mice injected with 5-HT and for mice injected with the vehicle. There were 20 mice in each treatment group.

RESULTS

Induction of ovulation in immature mice: effect of 5-HT

Preliminary experiments suggested that 100 μg. 5-HT, given three times on the day before the mice were killed, produced an increase in the percentage ovulating. This dose of 5-HT was therefore used in a large-scale experiment in which all the mice were treated with a small dose of PMS (1 i.u.) which caused six out of 46 (13 %) of the control mice to ovulate. In mice treated with 5-HT, 13 out of 45 (29 %) ovulated. Although this increase represented more than a doubling of the number of

mice ovulating it was not statistically significant owing to the low over-all level of response.

In the next experiment the same dose of 5-HT was tested on groups of mice given graded doses of PMS. The results (Fig. 1) showed that 5-HT increased the percentage of mice ovulating at the lowest dose-level of PMS (0·75 i.u.), as it had done in the previous experiment. At higher doses of PMS (1·5 and 3·0 i.u.), however, treatment with 5-HT caused a reduction in the number of mice ovulating. The line relating response to log dose of PMS was significantly less steep for mice treated with 5-HT than for mice injected with the vehicle ($P < 0{\cdot}001$).

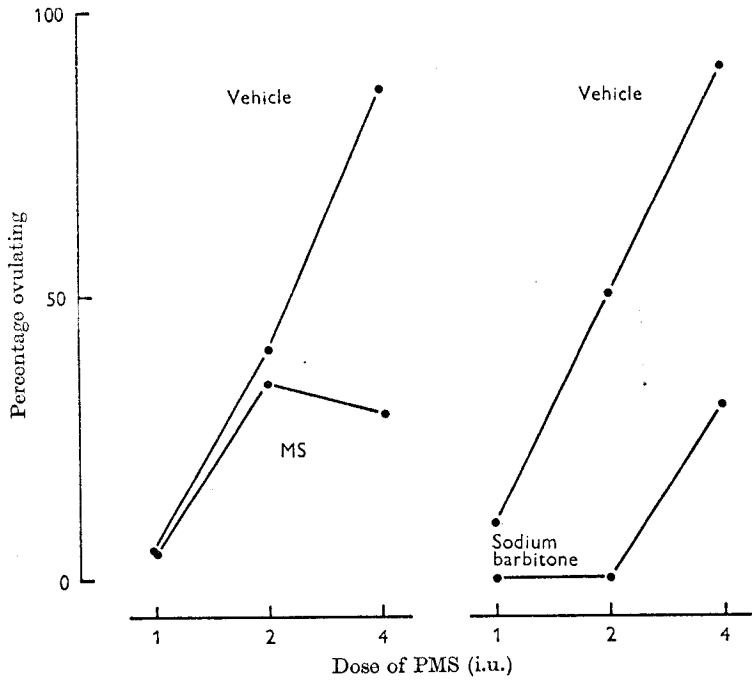

Fig. 2. The effect of methysergide bimaleate (MS) (three injections of 20 μg. each) and sodium barbitone (three injections of 2 mg. each) on the ovulatory response to a single injection of pregnant mare serum gonadotrophin (PMS) in immature mice in two separate experiments. Presentation as in Fig. 1. There were 19 to 21 mice/group in the experiment with MS and 10 mice/group in that with sodium barbitone.

It was concluded that 5-HT caused a considerable change in the dose-response relationship and that it appeared to do this by increasing low responses and decreasing high responses.

Induction of ovulation in immature mice: effect of 5-HT antagonists

Figure 2 shows the results obtained when groups of mice injected with graded doses of PMS were also treated with 20 μg. MS or its vehicle three times in the day. Treatment with the drug produced a striking reduction in the response to the highest dose of PMS, which resulted in a significant ($P < 0{\cdot}02$) fall in the slope of the dose-response line for PMS.

Table 1 shows the results of further experiments in which mice were given a standard dose of PMS and, in addition, 5-HT antagonists or their vehicles. A single dose of 20 μg. MS caused a significant reduction in ovulation if given at 14.00 hr. on the day before the mice were killed. The same dose at 10.00 or 18.00 hr. reduced the incidence of ovulation but not significantly. In another experiment, a significant reduction was obtained with a single dose of 10 μg. MS, while 5 μg. produced a non-significant reduction. In a final experiment, a single dose of 30 μg. LSD at 14.00 hr. caused a significant reduction.

It was concluded that 5-HT antagonists caused a consistent reduction in ovulation. This was best seen when a high proportion of the control mice ovulated, but it occurred also when the control response was only 43 %. For comparison, the alteration in response produced by the injection of three doses of 2 mg. sodium barbitone is shown in Fig. 2.

Table 1. *The effect of 5-hydroxytryptamine antagonists on ovulation induced by a single injection of gonadotrophin*

Drug	Dose (μg.) 10.00 hr.	14.00 hr.	18.00 hr.	Mice (ovulating/ total)	% ovulating	Significance of difference from control (P)
MS	0	0	0	13/30	43	—
	20	0	0	8/30	27	$>0{\cdot}05$
	0	20	0	5/31	17	$<0{\cdot}05$
	0	0	20	7/31	23	$>0{\cdot}05$
MS	—	0	—	26/41	63	—
	—	5	—	17/37	46	$>0{\cdot}05$
	—	10	—	12/36	33	$<0{\cdot}02$
LSD	—	0	—	20/31	65	—
	—	30	—	11/32	34	$<0{\cdot}05$

MS = methysergide bimaleate; LSD = lysergic acid diethylamide.

Spontaneous ovulation in adult mice: effects of 5-HT and its antagonists

Adult mice were injected with drugs either once or twice at various times between 11.30 and 17.30 hr. on the day before they were killed. Doses of 5-HT ranged up to two doses of 400 μg. each; those of MS up to two doses of 50 μg. or a single dose of 100 μg.; and those of LSD up to two doses of 100 μg. each. These drugs caused neither significant alteration in the percentage of mice ovulating nor in the number of ova produced by those which did. It was thought possible that a first dose at 11.30 hr. was too late to influence ovulation. This assumption was partially supported by the finding that although the injection of 5 mg. sodium barbitone at 11.30 and again at 17.30 hr. caused a significant reduction in the percentage of adult mice ovulating, it did not prevent ovulation entirely. A final experiment was therefore performed in which 400 μg. 5-HT or 50 μg. MS or their vehicles were injected at 08.30 hr. Again, there was no significant effect on ovulation.

DISCUSSION

In previous experiments, when ovulation was induced in immature mice by two injections of PMS, treatment with 5-HT increased ovulation at all levels of response (Brown, 1966). Its effects on induced ovulation in the experimental situation described in this paper were more complex, and they increase rather than decrease the difficulties in interpretation. The dose of 5-HT used was large and the observed effects may well have been secondary to the general disturbance caused. If, however, the site of action of 5-HT is in the central nervous system, the concentration of 5-HT attained there may be much reduced by difficulty in passing the blood-brain barrier. McCann, Taleisnik & Friedman (1960) showed that an intravenous injection of 25 μg. 5-HT into immature rats prepared as for the assay of luteinizing hormone (LH) according to the method of Parlow (1958) caused a significant depletion of ovarian ascorbic acid, presumably by the release of LH. This dose would be expected to cause proportionately less general disturbance in the rat and, again, the concentration reached in the brain might be relatively low. It remains possible, therefore, that 5-HT may exert some effect on the release of pituitary gonadotrophin.

Antagonists of 5-HT consistently reduced the ovulatory response to a single injection of PMS in immature mice, suggesting that 5-HT may be involved at some stage in the processes leading to ovulation under these circumstances. The effect of LSD could be secondary to the general disturbance it causes, the doses used in immature mice producing detectable changes in behaviour for about 20–30 min. However, no such changes were seen with MS. The antagonists could modify ovulation by suppressing endogenous gonadotrophin secretion. The alteration in ovulation caused by barbitone has been used to illustrate how effective such a suppression can be and the importance of endogenous gonadotrophin in immature mice has been discussed previously (Brown, 1966). The response to barbitone is more marked in this test situation than in that used previously when ovulation was induced by two, as opposed to one, injections of PMS (Brown & Wells, 1965). This accords with the unpublished finding that LSD and MS had similar, though less marked and statistically non-significant, effects on the ovulation induced by two injections of PMS in this other system.

The experimental findings in immature mice are, therefore, consistent with the hypothesis that 5-HT is involved at some stage in the processes leading up to the secretion of pituitary gonadotrophin. If this is so, it might be expected that 5-HT and its antagonists would have a detectable effect on spontaneous ovulation in the adult mouse, and such an effect was looked for but not found. This may mean that mechanisms involving 5-HT are not important in spontaneous ovulation. Alternatively such mechanisms may be differently, and perhaps more critically, timed in the mature animal and failure to demonstrate them may have been due to the limitations of the experimental design. One drawback in design was that mice of different strains had to be used in the two situations being compared. Contrasting results may, therefore, reflect strain differences.

I am grateful to Dr D. S. Freestone and Sandoz Products Limited for the gift of MS and LSD.

REFERENCES

Biggers, J. D. & Claringbold, P. J. (1954). Criteria of the vaginal response to oestrogen. *J. Endocrin.* **11**, 277–284.

Brown, P. S. (1966). The effect of reserpine, 5-hydroxytryptamine and other drugs on induced ovulation in immature mice. *J. Endocr.* **35**, 161–168.

Brown, P. S. & Wells, M. (1965). Factors which influence assays of gonadotrophin based on the induction of ovulation in mice. *J. Endocrin.* **33**, 507–514.

Gaddum, J. H. (1953). Simplified mathematics for bioassay. *J. Pharm. Pharmac.* **6**, 345–358.

McCann, S. M., Taleisnik, S. & Friedman, H. M. (1960). LH-releasing activity in hypothalamic extracts. *Proc. Soc. exp. Biol. Med.* **104**, 432–434.

Parlow, A. F. (1958). A rapid bioassay method for LH and factors stimulating LH secretion *Fedn Proc. Fedn Am. Socs exp. Biol.* **17**, 402.