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The Effects of Morphine Sulphate on Ovulation in the Immature Rat Treated with PMSG

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HULSE, G. K. AND G. J. COLEMAN. *The effects of morphine sulphate on ovulation in the immature rat treated with PMSG.* PHARMACOL BIOCHEM BEHAV **19**(2) 269–273, 1983.—Two experiments were carried out on the effects on ovulation of morphine sulfate administered prior to the preovulatory LH surge in the immature rat treated with PMSG. At the commencement of the experiments, rats were 30 days old. In Experiment 1 all rats were injected subcutaneously with 12 IU of PMSG at 1200 hr on day 30. Doses of 6, 12, 24 and 36 mg/kg of morphine were given IP at 1555 hr on day 32. Examination of oviducts on the morning of day 33 enabled the verification of ovulation as well as oocyte counts. Results suggest that the effect of morphine on ovulation is biphasic resulting in the stimulation of ovulation at low doses (6 mg/kg) and inhibition of ovulation at high doses (24 and 36 mg/kg). In Experiment 2, rats injected with a low dose of PMSG sufficient to result in ovarian maturation but not in a preovulatory LH surge, were injected on the eve of day 32 with either saline, 6 or 24 mg/kg morphine. The treatment of rats with 6 mg/kg morphine significantly increased mean ovulatory values compared with control and 24 mg/kg morphine conditions. Further, the percentage of 6 mg/kg treated rats ovulating was more than that of both control and 24 mg/kg morphine conditions. The failure of rats treated with 24 mg/kg morphine to display increments in ovulatory response similar to 6 mg/kg morphine injected rats suggests that increased ovulation is not due to the ability of morphine to cause adrenal progesterone release but is more probably the result of LH release at low doses of morphine.

Morphine sulphate PMSG Biphasic Ovulation

THE earliest investigations into the effect of opiates on the mammalian reproductive system produced contradictory results. Some researchers failed to observe any disturbance of the rat's estrous cycle, ovulation or fertility despite 132 days of chronic morphine treatment [6,14]. In contrast, others reported amenorrhea and sterility in human female morphine addicts [1]. The findings that spontaneous ovulation in the adult rat involves a cyclic hypothalamic factor [4,22] offered a new approach to the study of the effects of morphine on ovulation. With the use of neural blocking agents, it was demonstrated that the critical period at which this neurogenic stimulation occurs is between eight and ten hours following light onset in rats housed under a 14:10 light/dark cycle (LD 14:10) [4]. It has now been demonstrated that controlled room lighting is the most important single requirement in setting the luteinizing hormone (LH) and ovulatory critical period timing mechanism [5,27]. It has since been reported that ovulation could be blocked in female rats housed under a LD 14:10 cycle (lights on at 0600 hr) by a single large (50 mg/kg) administration of morphine sulfate injected subcutaneously (SC) eight to ten hours following light onset on the afternoon of proestrus, just prior to the critical period for the triggering of the LH surge [1]. Treatment with morphine sulfate after this period was, however, ineffective in blocking ovulation. It was suggested that the failure of others [6,14] to observe inhibition of ovulation following chronic morphine treatment was a consequence of the administration of morphine too early or too late in the day to affect the cyclic ovulatory timing mechanism [1].

In subsequent studies [21] it was demonstrated that blockage of ovulation by morphine administration was overcome by electrical stimulation of the median eminence, but not of other brain stem areas. This finding suggested that hypothalamic luteinizing hormone releasing factor (LRF) was present in sufficient quantities to induce ovulation but was not released as a result of the action of morphine. It was concluded that morphine interrupted the neurogenic stimulation of the hypothalamus which triggers the ovulatory discharge of LH. More recently this claim has been substantiated with the report that 20 mg/kg of morphine injected intraperitoneally (IP) on the afternoon of proestrus into female rats prevents the normal LH surge as well as ovulation [16].

Ovulation can be induced in the immature rat by a single treatment with a specific dose of pregnant mares serum gonadotrophin (PMSG). This single injection of PMSG into immature 30 day old rats results in an LH/follicle stimulating hormone (FSH) surge some 52–56 hr later on the eve of day 32 with ovulation occurring early on the morning of day 33 [24].

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Recently, a number of experimenters have utilized the immature rat treated with PMSG as a model for studying ovulation in the adult rat [9, 12, 15, 17, 18]. Their findings have suggested that much overlap exists between the morphological and biochemical changes induced in the PMSG primed immature rat and those occurring over the adult rat's estrous cycle, including the processes surrounding the preovulatory LH surge. These findings support the hypothesis that ovulation in the immature rat, as in its adult counterpart may be blocked by the administration of morphine prior to the preovulatory LH surge.

EXPERIMENT 1

The present experiment was designed to investigate the hypothesis that morphine sulfate administered prior to the preovulatory surge of LH in the PMSG primed immature rat would result in the blockage of ovulation.

METHOD

Animals

Subjects were sixty outbred, experimentally naive, Wistar derived, female rats aged 30 days and weighing 70–85 g at the commencement of the experiment. Groups of five rats selected at random were housed in boxes 500×330×160 mm in conditions of a 12:12 light dark cycle (lights on 0800 hr) and temperature 21±2°C. Clark King GR 2 + cubes and tap water were available ad lib.

Procedure

Rats were weaned at 21 days of age and placed into the controlled environment for 9 days prior to experimentation. At 1200 hr on day 30 rats were randomly assigned to one of six conditions and all conditions injected subcutaneously with 12 IU of PMSG freshly dissolved in 0.5 ml (0.154 M) NaCl. At 1555 hr on the afternoon on day 32, five of the six conditions were given a single injection of either 6, 12, 24, or 36 mg/kg of morphine sulfate or 0.5 ml (0.154 M) NaCl. All morphine was freshly dissolved in (0.154 M) NaCl and injected in a quantity of 0.5 ml. All morphine doses and saline were injected IP using a 1 ml syringe and 26 gauge needle. The remaining condition served as the non-injected control treatment. On the morning (0800 hr) of day 33 all rats were sacrificed, and oocyte counts made [20]. When no oocytes were retrieved, the ovaries were examined histologically. If there was a lack of follicular maturation, it was assumed that PMSG had failed to induce ovarian maturation. The ovulatory data of these rats were therefore excluded from statistical analysis. However, if mature follicles were present, the failure of ovulation was presumed to be due to the experimental treatment.

RESULTS

The effects of various doses of morphine or saline on the mean ovulation rates are shown in Fig. 1. A significant difference in mean ovulation rates for the six experimental treatments was found, $F(5,40)=10.04$, $p<0.05$, using a one-way analysis of variance [7].

Post hoc analysis by the Newman-Keuls procedure [7] demonstrated that the injection of 6 mg/kg of morphine resulted in mean ovulation values significantly greater (16.75 oocytes/rat) than those of both control (10.18 oocytes/rat) and saline injected rats (9.78 oocytes/rat).

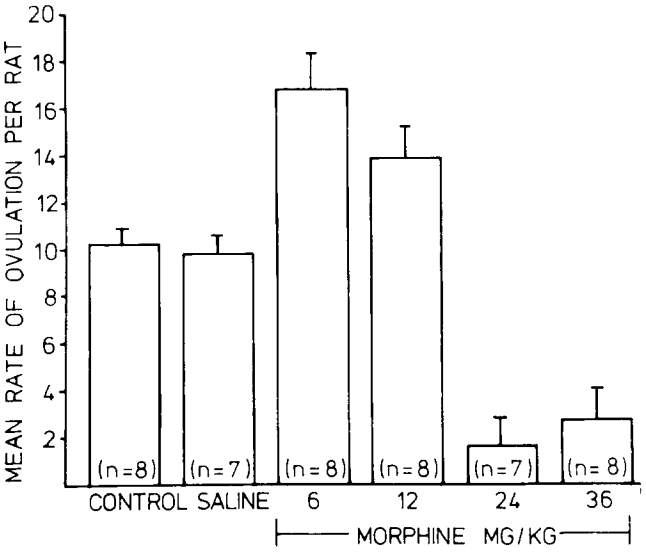

FIG. 1. The effect of various doses of morphine on the mean±SE ovulatory rate per rat in immature PMSG primed rats.

Similarly, the administration of 6 mg/kg of morphine also resulted in a significantly greater mean ovulation rate when compared to rats injected with either 24 (1.78 oocytes/rat) or 36 mg/kg morphine (2.75 oocytes/rat).

In contrast to the increased ovulatory responses of rats injected with 6 mg/kg morphine, the administration of 24 and 36 mg/kg of morphine resulted in mean ovulatory values being significantly smaller than saline and control conditions. *Post hoc* analysis revealed no differences between the mean ovulation values for control and saline injected rats.

The effect of various dose levels of morphine sulfate on the percentage of rats ovulating in each condition is shown in Fig. 2. All rats in control and saline conditions with mature ovarian follicles (N=8 and 7 respectively) were found to have ovulated. Similarly, all rats with mature follicles injected with 6 mg/kg of morphine (N=8) ovulated. In contrast, conditions receiving larger doses of either 12 (N=8), 24 (N=7) or 36 (N=8) mg/kg of morphine displayed a decrease in the number of rats with mature follicles ovulating (75%, 29% and 38% respectively).

EXPERIMENT 2

Ovulation occurs less frequently in immature rats when low doses of PMSG are administered [13,26]. Lack of ovulation associated with low PMSG doses is not due to the failure of ovarian follicles to mature, but is rather the result of the absence of the preovulatory LH surge [11]. Experiment 1 has demonstrated that morphine sulphate has a biphasic action on ovulation resulting in the blockage of ovulation at high doses and facilitation at low doses.

It is not known whether the low dose of morphine would be effective in inducing ovulation in immature rats primed with a dose of PMSG sufficient to produce follicular maturation but not sufficient to produce ovulation. If a low dose of morphine could produce ovulation under these circum-

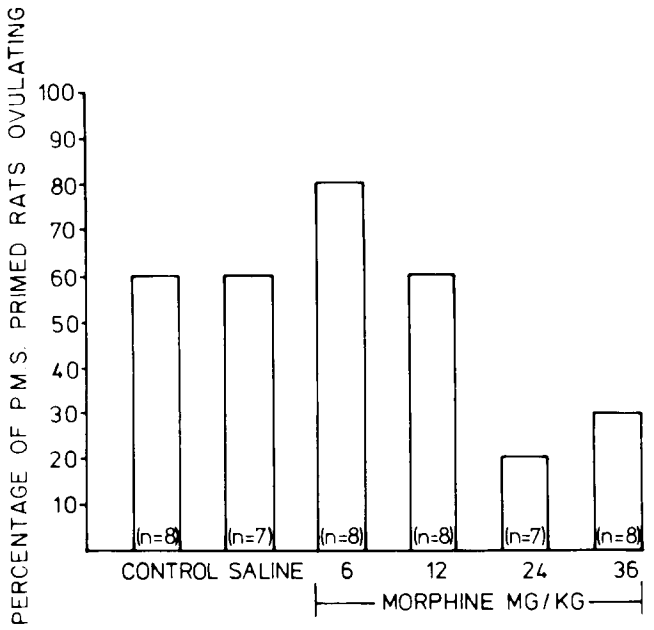

FIG. 2. The effect of various doses of morphine sulfate on the percentage of PMSG primed immature rats ovulating.

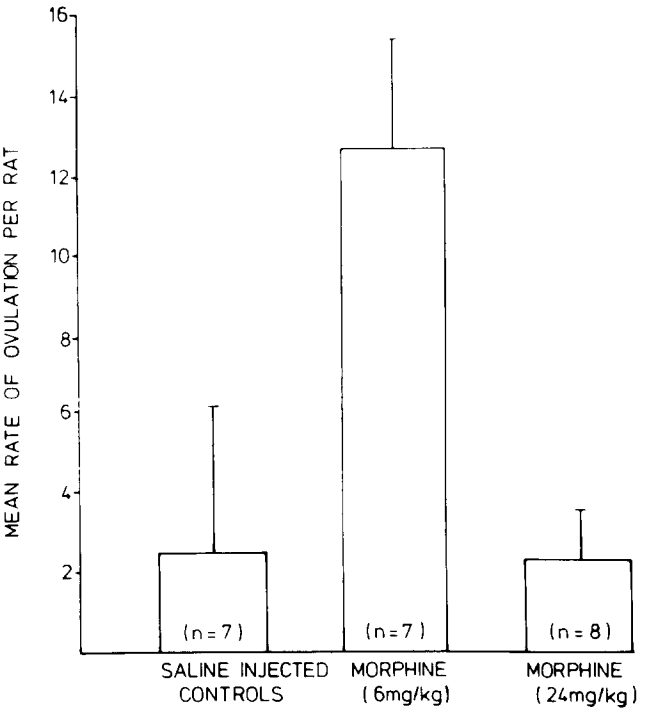

FIG. 3. Mean rate (±SE) of ovulation per rat following the administration of various doses of morphine sulfate in immature rats primed with a lower than optimal dose of PMSG.

stances, it would imply the release of either LH or progesterone or both. Similarly, if a high dose of morphine does not produce ovulation then it would imply that neither LH nor progesterone has been released. This further implies that the biphasic effect of morphine acts on LH or progesterone release.

The present experiment was designed to investigate the effect of large and small doses of morphine sulfate on the ovulatory response of immature rats primed with a lower than optimal ovulatory dose of PMSG.

METHOD

Animals

Subjects were of similar stock and age and housed under identical conditions to Experiment 1.

Procedure

Rats were randomly assigned to one of three conditions: saline, 6 mg/kg morphine or 24 mg/kg morphine.

At 1200 hr on the morning of day 30 each condition was injected with 6 IU of PMSG freshly dissolved in 0.5 ml (0.154 M) NaCl. At 1555 hr on the afternoon of day 32 the three conditions were injected IP using a 1 ml syringe and 26 gauge needle with either 6 mg/kg morphine, 24 mg/kg morphine or 0.5 ml (0.154 M) NaCl. Both morphine doses were freshly dissolved in (0.154 M) NaCl and injected in a quantity of 0.5 ml.

Rats were sacrificed, and oocyte counts made as in Experiment 1. Rats with no mature ovarian follicles were again excluded from analysis.

RESULTS

The effect of morphine sulfate (6 and 24 mg/kg) and saline on the mean ovulatory values is shown in Fig. 3.

One-way Analysis of Variance [7] revealed a significant difference in mean ovulatory rates for 6 mg/kg morphine, 24 mg/kg morphine and saline injected control treatments, $F(2,19)=8.82$, $p<0.01$.

Post hoc analysis using the Newman Keuls procedure [7] demonstrated that the administration of 6 mg/kg morphine significantly increased mean ovulatory values (12.6 oocytes/rat) compared to mean values for saline injected controls (2.4 oocytes/rat). Similarly the mean ovulatory rate for rats injected with a low (6 mg/kg) dose of morphine was found to be significantly higher than that of rats injected with 24 mg/kg morphine (2.1 oocytes/rat). The administration of 24 mg/kg morphine to rats resulted in a mean ovulatory value that was not significantly different from mean control values.

The effect of morphine sulfate and saline on the percentage of rats with mature ovarian follicles ovulating in each of the three treatments is shown in Fig. 4.

The percentage of saline injected rats with mature ovarian follicles ovulating (2/7) is markedly lower than the percentage of rats with mature ovarian follicles ovulating following the administration of 6 mg/kg morphine ($\chi^2=4.67$, $p<0.05$). Similarly the percentage of rats with mature follicles ovulating in the 24 mg/kg morphine condition (2/8) is markedly lower than in the condition injected with 6 mg/kg morphine ($\chi^2=5.53$, $p<0.05$). The injection of rats with 24 mg/kg morphine was found to result in a percentage of rats ovulating similar to control values.

GENERAL DISCUSSION

Major findings from Experiment 1 were that morphine has a biphasic effect on ovulation in PMSG primed immature

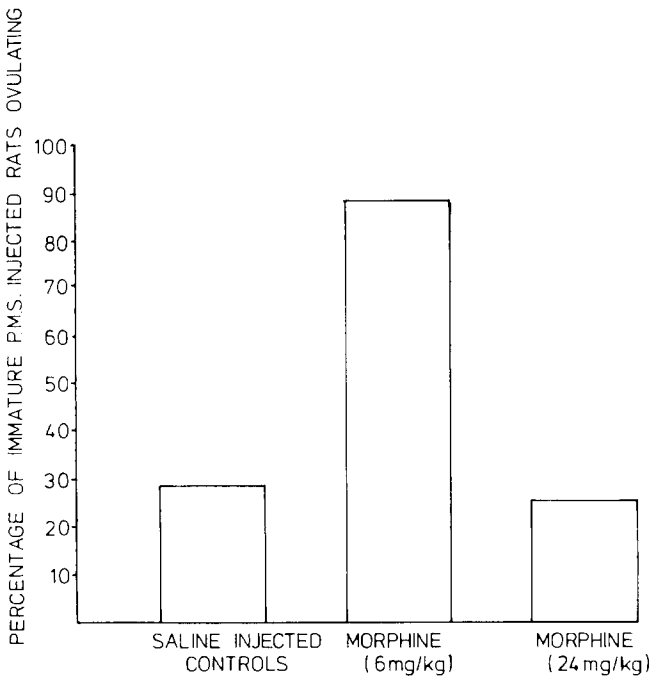

FIG. 4. The percentage of immature female rats primed with a lower than optimal ovulatory dose of PMSG ovulating following the administration of 6 and 24 mg/kg morphine sulfate.

rats, inhibiting it at high doses and increasing it at low doses. Experiment 2 demonstrated that low doses of morphine are also capable of inducing ovulation in immature rats primed with a dose of PMSG insufficient to induce ovulation on its own.

These findings are consistent with those reported in studies utilizing adult rats. It is well documented that a single large dose of morphine sulfate administered to adult cyclic female rats just prior to the LH proestrus surge results in the blockage of the LH surge, leading to the inhibition of ovulation [16,21]. It is reasonable to hypothesize that the inhibitory action of morphine on ovulation in the immature rats observed here and in adult cyclic rats observed by other experimenters is mediated by similar processes.

This hypothesis is supported in Experiment 1 by ovarian histological examination which reveals the presence of mature ovarian follicles in immature rats injected with 24 and 36 mg/kg of morphine. This, in turn, demonstrates that adequate stimuli necessary to induce complete follicular maturation (i.e., PMSG and FSH) has apparently been available to these non-ovulatory immature rats. Failure to ovulate may therefore be attributed to insufficient pituitary LH necessary to cause the rupture of these mature ovarian follicles.

There are at least two possible explanations for the facilitatory effects of low doses of morphine on ovulation reported here. First, at low doses, morphine increases the preovulatory release of LH in adult animals [16] and may therefore increase the number of oocytes ovulated. More recently it has been found that the injection of β-endorphin (1 or 5 mg), into the lateral ventricle of rats, elevates serum LH levels [25].

Second, the increased ovulatory response in rats treated with low levels of morphine (6 mg/kg) might be the result of progesterone release from the adrenals [18] in response to ACTH release [8]. In a review of the actions of progesterone on the pituitary and CNS [19], it was concluded that progesterone priming of rats, rabbits, frogs, toads, sheep, cows, and monkeys increased the ovulatory response (i.e., number of oocytes ovulated) of the ovary to LH. The period prior to ovulation during which progesterone had this effect was in the order of 24 hours.

If adrenal progesterone released as a result of morphine administration was responsible for the increased ovulatory response observed at low doses of morphine, then an equivalent or larger ovulatory response would be expected at higher morphine doses, since higher doses of morphine would (similarly to low doses) be expected to result in the release of adrenal progesterone. However, in Experiment 2, the administration of a high dose of morphine (24 mg/kg) failed to result in an increased ovulatory response.

Although progesterone administration alone has been shown to result in ovulation in PMSG primed immature rats, this ovulatory response was only small (3–4 oocytes/ovary) [13]. This further suggests that the large increments in ovulation and increased percentage of rats ovulating observed in Experiment 2 is unlikely to be the result of adrenal progesterone release.

Pituitary LH released in response to small doses of morphine remains a likely candidate to explain the increased ovulatory response observed in this study. Adrenal progesterone released as a result of morphine administration might further increase the ovulatory response due to this induced LH surge.

The results of both experiments demonstrate that the effect of morphine on ovulation is biphasic, possibly as a consequence of the stimulation of LH release at low doses (6 mg/kg) and the inhibition of LH release at high doses (24 and 36 mg/kg). The plausibility of this conclusion is increased by the fact that not only is morphine already known to exert biphasic effects on several physiological systems in the rat [23], but also that both excitation and inhibition of hypothalamic neurones, known to be associated with LH release, have been observed following acute morphine treatment [10].

Although the major objective of this study was to demonstrate that low doses of morphine can induce ovulation, it remains to be demonstrated by assay that the results reported here are primarily a result of increases in plasma LH.

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