EFFECTS OF REPEATED MORPHINE ADMINISTRATION
ON COPULATION AND ON THE HYPOTHALAMIC-
PITUITARY-GONADAL AXIS OF MALE RATS

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Abstract—Adult male rats were administered morphine twice a day for 45 days and
the effects of morphine on the copulation rate, the weight of various organs, and on
the hypothalamic-pituitary-gonadal axis were examined. Morphine administered
rats showed a loss of weight, hypertrophy of the adrenals, decreased weight of accessory
sex organs, low sperm count, and decreased copulation rate. The contents of the
luteinizing hormone releasing hormone in the hypothalamus and the luteinizing hormone
in the pituitary remained unchanged. Serum luteinizing hormone and testosterone
levels decreased, but serum follicle-stimulating hormone levels increased. These
results suggest that morphine inhibits the hypothalamic-pituitary-gonadal axis and
causes a diminution in the number of fertilizations of the partner females.

While investigating the possible teratogenicity of morphine, we found that the copulation
rate of morphine-tolerant male rats was less than that of saline-treated control males sug-
uggesting that the repeated administration of morphine inhibits gonadotropin secretion and
causes regressive changes in the reproductive organs of rats (1). The present study was
undertaken to determine whether or not morphine does affect the hypothalamic-pituitary-
gonadal axis in male rats. Some of these data have already been reported (2).

MATERIALS AND METHODS

Twenty sexually mature male Wistar rats, 16 weeks of age, weighing from 330 to 380 g
at the start of morphine administration, and 40 adult female virgin rats of the same strain
were used. The morphinized and the control groups each consisted of 10 males and 20
females. Morphine hydrochloride was administered s.c. twice daily to the male rats only.
The initial dose was 20 mg/kg and the dose was increased by 20 mg/kg every three days.
After the 16th day, the dose was fixed at 100 mg/kg twice daily. Control male rats were
given the same amount (5 ml/kg) of physiological saline solution. After the 16th day of
administration of morphine or physiological saline, each male rat was put with 2 female
rats. Confirmation of copulation was made by examining vaginal smears for sperm every
morning. Each male rat was housed with a virgin female in a cage until copulation for
12 days at the longest. After copulation or after 12 days' housing with its partner, when
copulation was not evident, the first female was taken away and a second new female was
put into the cage and copulation was checked again. The female was examined for pregnancy
3 weeks later to obtain the gestation rate. When sperms were found in the vaginal smears
of the second female rat, or, after 12 days of housing with a second female rat if no sperms were found in the smear, the male rat was isolated from the female for 5 days to restore the sperm, and was decapitated 2 hr after the last administration of morphine or physiological saline. The hypothalamus, pituitary, adrenals and male reproductive organs were weighed and the reproductive organs were examined histologically. The serum was kept frozen at -70°C until the assay of hormones. To count the number of sperm in the epididymis, the caudal part of one epididymis was minced in 1 ml of physiological saline solution for 5 min and the number of sperm in the resultant cloudy solution was counted. The hypothalamus was isolated by the method of Glowinski and Iversen (3) and was homogenized in 2 ml of 0.2 M acetic acid. The supernatant obtained by 10 min centrifugation at 2,000 × g was lyophilized and kept at -70°C until the assay of luteinizing hormone releasing hormone (LHRH). Hypothalamic LHRH was determined by double antibody radioimmunoassay (4) using kits kindly supplied by Dr. T. Makino, Tokyo Dental College. Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined by double antibody radioimmunoassay kits supplied by NIAMDD. The assays of gonadotropins were carried out according to the standard supplied with the kits. The values of gonadotropins were expressed in terms of the standards provided. Pituitary samples were homogenized in 5 ml of phosphate buffered saline (0.01 M phosphate, 0.15 M NaCl, 0.01% NaN₃, pH 7.6) and the homogenate was used for LH assay. Serum testosterone was determined by radioimmunoassay using kits obtained from Teikokuzoki, Co., Tokyo, after the procedure of Makino (5). The intra-assay coefficients of variation for LHRH, LH, FSH and testosterone were 11, 13, 8, and 11% of the values determined respectively. All samples from each experiment were measured within a single assay. The results were statistically evaluated using Student's t-test or chi-square test.

RESULTS

Effects of morphine on copulation and gestation rates

Table 1 shows that the saline-treated male rats mated with 90% of all female partners and that 80% of these rats were impregnated. The morphine-treated male rats mated with only 40% of all females and only 25% became pregnant. Both rates of morphinized male rats were significantly lower than those of controls. The ratio of gestation to copulation

| Table 1 | Effect of morphine on the rate of copulation and gestation |
|---------|----------------------------------------------------------|
|         | No. of female rats tested | Copulation rate (%) | Gestation rate (%) |
| Physiological saline (control) | 20 | 90 | 80 |
| Morphinized | 20 | 40* | 25* |

a, Both the control and the morphinized groups included 10 male and 20 female rats. The copulation rate is the percentage of copulated female rats. The gestation rate is the percentage of pregnant rats. Only male rats were given morphine or physiological saline.

b, P < 0.001
### Table 2  Body weight, sperm count and wet weight of organs

|                        | Body weight (g) | Sperm count (×10⁶/mm²) | Pituitary (mg) | Adrenals (mg) | Testes (g) | Epididymides (mg) | Seminal vesicles (g) | Prostate (mg) |
|------------------------|-----------------|------------------------|----------------|---------------|------------|-------------------|----------------------|--------------|
| **Physiological saline** |                 |                        |                |               |            |                   |                      |              |
| (control) N=10         | 359 ± 5         | 160 ± 20               | 9.8 ± 0.5      | 74.0 ± 4.0    | 2.85 ± 0.19| 251 ± 8           | 1.25 ± 0.15          | 681 ± 46     |
|                        | (17.4 ± 0.9)    |                        |                | (0.65 ± 0.02) | (58 ± 2)   |                   | (0.32 ± 0.02)         | (161 ± 13)   |
| **Morphine**           | 351 ± 6         | 59 ± 19b               | 9.2 ± 0.5      | 97.7 ± 4.5b   | 2.35 ± 0.13a| 162 ± 14b         | 0.71 ± 0.09b          | 437 ± 44a    |
| N=10                   | (27.7 ± 1.6b)   |                        |                | (0.66 ± 0.03) | (45 ± 3b)  |                   | (0.19 ± 0.02b)        | (122 ± 11a)  |

Mean ± S.E.  a, P<0.05,  b, P<0.01.
Relative weight of organs (wt./100 g body wt.) is indicated in parentheses.

### Table 3  Hypothalamic content of LHRH, pituitary content of LH, serum LH, FSH and testosterone levels

|                  | LHRH (ng/hypothalamus) | LH (pituitary) (ng/mg wet wt.) | LH (serum) (ng/ml) | FSH (serum) (ng/ml) | Testosterone (serum) (ng/ml) |
|------------------|------------------------|--------------------------------|-------------------|--------------------|-----------------------------|
| **Physiological saline** (control) | 2.86 ± 0.24 (N=9) | 40.5 ± 3.4 (N=9) | 64.4 ± 7.9 (N=6) | 293 ± 26 (N=9) | 3.27 ± 0.46 (N=10) |
| **Morphine**     | 2.63 ± 0.15 (N=9)    | 45.6 ± 6.1 (N=10) | 33.4 ± 6.0b (N=9) | 397 ± 40b (N=9) | 1.14 ± 0.26b (N=10) |

a, P<0.05  
b, P<0.01  
Mean ± S.E.
rate was lower in morphinized rats than in the controls.

Effects of morphine on body weight, sperm count, and weight of various organs

Table 2 shows that the final body weight of morphinized rats was significantly less than that of the controls. The weight of the pituitary of morphinized rats was almost the same as that of the controls. The adrenals of the morphinized males were heavier than those of controls. The relative weights of the organs (weight of organ per 100 g body weight) were calculated in the morphinized and control rats because the body weight of the morphinized rats was less than that of the control rats and we deemed it necessary to compare the wet weight of organs on the same body basis. Except for the testes, the relative weight of epididymides, seminal vesicles and prostate of morphinized rats was significantly less than that of controls.

We assumed that the number of sperm in the minced epididymal solution would correlate with the number in the semen, therefore, the former was determined. The sperm count of the epididymis from saline control rats was significantly higher than that from morphinized rats.

Effects of morphine on hormone levels

The effects of repeated administrations of morphine on the hypothalamic-pituitary-gonadal axis of male rats are shown in Table 3. The serum LH and testosterone levels of morphine-treated rats were significantly less than those of controls. On the other hand, the serum FSH levels of morphinized rats were higher than those of controls. The hypothalamic content of LHRH in control rats was within the range of the reported value, but the pituitary LH content was slightly higher than already reported (6). Neither the hypothalamic content of LHRH nor the pituitary content of LH was changed by morphinization.

Histological examination

Histological examination of the seminal vesicles from morphinized rats disclosed that the epithelial folds were decreased in number and that their height was also moderately decreased, but there were no changes in the amount of secretory materials in the cytoplasm of the epithelia. Testes of morphinized rats were normal except for one of the 10 males in which diffuse interstitial fibrosis of testes was seen. Spermatogenesis was well preserved in all of the males. Pathological changes in the other organs were nil.

DISCUSSION

The hypothalamic-pituitary-gonadal axis has become increasingly comprehensive and it would appear that the production of androgens by the testes is under the control of pituitary LH and that the secretion of pituitary LH is influenced by the hypothalamic LHRH (7). Although we did not examine the time course of the changes of the serum LH or testosterone levels after morphine administration, the serum levels of both hormones in morphinized rats were decreased 2 hr after morphine administration. This result suggests that morphine decreases the serum testosterone levels by diminishing the serum LH levels. Even though morphine was administered to male rats of the same age and strain, the body weight of
morphinized rats was less than that of the control rats at the time of sacrifice. The hypertrophy of adrenals and the diminished weight gain reconfirmed previous data (8). There appears to be more than just a causal relationship between the decreased weight of male accessory sex organs and the general malnutrition as the result of morphine because the relative weights of the male accessory reproductive organs of morphinized rats were less than those of the controls.

Cicero et al. (9) reported that a 3 day implantation of morphine pellets in male rats produced atrophy of the male accessory reproductive organs and low plasma testosterone levels. Our experimental design differs from Cicero's experiment in that we used rats which were fully mature sexually and were administered morphine repeatedly for a longer period of time. Nevertheless, our study showed similar inhibitory effects of morphine on the reproductive system of male rats. Although all the morphinized male rats but one had microscopically normal testes, their low sperm count may be due to the small gross size of their testes. The low serum testosterone levels and reduced sperm counts show that morphinization depresses the function of the testes. We found epithelial atrophy of seminal vesicles similar to that reported by Cicero et al. (9), although changes in the amount of secretory materials in the cytoplasm of the epithelia were not observed. The reduced weight of the male accessory sex organs can be interpreted as being the result of lowered serum testosterone levels, but the absence of the atrophy of testes suggests that the inhibitory effect of morphine on the male reproductive system is weak.

The low serum LH levels in morphinized rats cannot be attributed to the depletion of LH in the pituitary by morphine, since the pituitary LH content was in the control range. Although neither retention nor depletion of hypothalamic LHRH or pituitary LH was found, a plausible explanation of the low serum LH levels of morphinized rats would be that morphine inhibits the release of pituitary LH by inhibiting the release of hypothalamic LHRH or by acting directly on the pituitary. In vitro perfusion studies of the effect of morphine on LH secretion of pituitary may clarify this problem.

A possible explanation of the low copulation rate of morphine-dependent male rats is that morphine inhibits the male sexual behavior by depressing the central nervous activity, although this effect may be modified by tolerance, dependence, or low circulating testosterone in the morphine-dependent rats. The low gestation rate of morphine-dependent rats may be the integrated result of the low sperm count or oligospermia and the low copulation rate.

The high FSH levels of morphinized rats may be explained by the interruption of negative feedback, if any, through the inhibition of spermatogenesis by morphine. Further work is necessary before the FSH changes caused by morphine can be evaluated as the regulation of the serum FSH levels is not fully understood (7). During the preparation of this manuscript, Cicero et al. (10) reported that while serum levels of LH were not detectable in the male rats treated with morphine for 15 days, the serum FSH levels were not altered. This report lends support to our finding that prolonged morphine treatment decreases serum LH levels of male rats.
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