EFFECTS OF MORPHINE ON THE SERUM PROLACTIN LEVELS OF MORPHINE-TOLERANT AND NONTOLERANT MALE RATS AND ON THE IN VITRO RELEASE OF PITUITARY PROLACTIN

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Abstract—Morphine increased the serum prolactin (PRL) levels of male rats in a dose response manner. This effect was abolished by naloxone and apomorphine, but was not affected by diphenhydramine. The increase in the serum PRL levels by haloperidol was abolished by apomorphine, but not by naloxone. Repeated administrations of increasing doses of morphine attenuated the response of serum PRL to morphine. Naloxone did not alter the serum PRL levels of morphine-tolerant rats, while it precipitated full withdrawal signs on these rats. Although neither haloperidol nor morphine increased the release of PRL from the isolated anterior pituitary, haloperidol, but not morphine, reversed the inhibition by dopamine of the in vitro release of pituitary PRL. These results indicate that tolerance develops regarding the effect of morphine with a resulting increase in the serum PRL levels, abstinence precipitated by naloxone has no effect on the serum PRL levels, the mechanism of morphine involved in increase in the serum PRL is different from that of haloperidol as the effect of haloperidol is not antagonized by naloxone and morphine does not antagonize the effect of dopamine which inhibits the release of PRL from the anterior pituitary in vitro.

Narcotic analgesics such as morphine or methadone increase the prolactin (PRL) levels in blood in man and male rats (1-7), and this effect was antagonized by naloxone (5-7). Such observations suggest that the PRL effect of morphine is mediated through the opiate receptor. However it has not been elucidated whether tolerance develops regarding the effect of morphine on serum PRL levels.

Other workers have suggested that dopamine inhibits the PRL release by inhibiting the release of PIF (Prolactin inhibiting factor) from the median eminence and by acting directly on the pituitary (8-10). Haloperidol, which may exert effects by blocking dopamine receptors, stimulated the release of PRL (1, 11), while apomorphine, a specific dopamine receptor stimulant, inhibited the release (12). In vitro experiments revealed that the inhibitory effect of dopamine and apomorphine on the release of pituitary PRL was blocked by haloperidol (9, 13).

Many neurotransmitters inclusive of dopamine play a role in the action of opiates. Acute administration of narcotics results in an inhibition of dopamine receptor activity while chronic administration results in an increased response to dopamine agonists (14). The fact that apomorphine inhibits the release of PRL by narcotics may be evidence of the dopamine blocking action of narcotic drugs (1, 2). However unlike the direct action of
haloperidol on the dopamine receptors, the narcotic action was considered to be indirect (15). Recently it was suggested that morphine-induced stimulation of PRL secretion would include a histamine related step (5).

In the present work we examined the effect of morphine and naloxone on the serum PRL levels in both non-tolerant and morphine-tolerant male rats and attempted to determine whether morphine increases the release of PRL from the pituitary by direct blockade of the dopamine receptor at the pituitary, such as in the case with haloperidol. Effects of diphenhydramine on the increase in the serum PRL levels by morphine were also investigated.

MATERIALS AND METHODS

Twelve-week old male Wistar rats were housed in an air conditioned room (22°C) under controlled lighting (lights on 0600 to 1800 hr). After s.c. administration of drugs, the animals were decapitated usually between 0900 to 1200 hr, and the blood from the abdominal trunk was collected. Serum samples were stored frozen at −80°C until the assay of PRL. All drugs were dissolved in physiological saline solution in a volume of 5 ml/kg and given s.c. To make rats tolerant to morphine, the animals were given morphine (20 mg/kg, s.c.) twice daily for 5 and 20 days, or in another experiment, were treated chronically with morphine by injecting increasing doses of the narcotic twice daily: the starting dose of 20 mg/kg was increased by 20 mg/kg every 6 injections until a dose of 100 mg/kg and the administration of morphine (100 mg/kg, twice daily) was reached. Such was continued until the challenge with saline, morphine or naloxone. For non-tolerant controls the rats were given saline (5 mg/kg, s.c.) twice daily for the same period. In the case of tolerant rats and their controls, challenge with drugs was carried out 18 hr after the last injection of morphine or saline.

Although every effort was taken to avoid possible stress to the animals during the experiments, the basal PRL levels of control rats given saline fluctuated considerably from experiment to experiment due to unknown cause. Therefore each experiment included saline controls of its own.

For the in vitro study, the animals were decapitated and the anterior lobe of the pituitary was isolated and divided into two fragments by a sagittal midline cut. Each hemipituitary was placed in a flask containing 1 ml medium 199 (Difco) at pH 7.2. Incubation was carried out in a shaker at 70 cycles/min at 37°C under an atmosphere of 95% O₂: 5% CO₂. After a preincubation period of 30 min, the media were discarded and replaced with that containing drugs. The incubation was then continued for a further 1 hr. At the end of incubation, the medium was separated from the anterior pituitary half and the pituitary was homogenized in 3 ml of phosphosaline (0.01 M PO₄, 0.15 M NaCl, 0.1% NaN₃, pH 7.6). The incubation medium and the pituitary homogenate were stored frozen at −20°C until ready for assay of PRL.

PRL was determined by the double antibody radioimmunoassay method using kits of the Rat Pituitary Hormone Distribution Program, NIAMDD, NIH, following the procedure provided with the kits. PRL was expressed in terms of Rat PRL RP-1. The minimal detectable level of PRL was 30 pg/tube (0.3 ng/ml as serum PRL levels). The intraassay
coefficient of variation for PRL was 13%. All samples from each experiment were measured within a single assay. Data were evaluated statistically using Student's and Welch's t-tests.

RESULTS

Effects of morphine, naloxone, haloperidol, apomorphine and diphenhydramine on serum PRL levels in male rats

Effects of 5, 10, 20 and 50 mg/kg doses of morphine on serum PRL concentrations in rats are shown in Table 1. Morphine at the dose of 5 mg/kg produced a significant rise in the PRL levels at 30 min. The PRL levels of rats given morphine at doses of 20 and 50 mg/kg were significantly higher than those of 5 mg/kg. Therefore, it appears that the effect of morphine on serum PRL levels is dose-related. When rats were given 20 mg/kg of morphine, the serum PRL concentration was elevated at 15, 30 and 60 min but was not significantly different from controls after 120 min (Table 2). With a larger dose of morphine (100 mg/kg) the serum PRL concentration was elevated at 2 hr and returned to the control level within 4 hr. One mg/kg of naloxone did not produce any significant changes in serum PRL levels but blocked completely the increase in serum PRL by morphine at a low ratio of antagonist : agonist (1:20) (Table 3, experiment 1). The PRL release induced by morphine was abolished by apomorphine but not by diphenhydramine, although diphenhydramine lowered the basal levels of PRL slightly (Table 3, experiments 2 and 3). Table 4 shows that

| Treatment       | Dose (mg/kg) | Serum PRL (ng/ml) (mean ± S.E., N=6) |
|-----------------|--------------|-------------------------------------|
| Saline          | —            | 43±8                                |
| Morphine HCl    | 5            | 109±21*                             |
| Morphine HCl    | 10           | 166±29*                             |
| Morphine HCl    | 20           | 218±23*                             |
| Morphine HCl    | 50           | 287±53*                             |

Rats were given saline (5 ml/kg) or morphine and sacrificed 30 min later.
* P<0.025 compared to saline-treated controls.

| Time after morphine (min) | Serum PRL (mean ± S.E.(N)) (ng/ml) | Morphotine HCl (20 mg/kg) | Morphotine HCl (100 mg/kg) |
|---------------------------|------------------------------------|---------------------------|----------------------------|
| 0*                        | 59±13(6)                           | 62±7(6)                   |
| 15                        | 152±17(6)**                        | —                         |
| 30                        | 174±16(6)**                        | —                         |
| 60                        | 145±18(6)**                        | —                         |
| 120                       | 69±16(6)                           | 194±13(6)**               |
| 240                       | —                                  | 52±5(4)                   |

* Rats given saline (5 ml/kg) 30 min prior to sacrifice were used as 0 min.
** P<0.005 vs. 0 min.
the increase in serum PRL levels caused by haloperidol was blocked by apomorphine but not by naloxone.

Effects of chronic administration of morphine on the serum PRL concentration

Repeated prior administration of the same dose of morphine for 5 and 20 days did not affect the basal PRL levels of rats sacrificed 30 min after saline nor the increase in the serum PRL levels by morphine (Table 5). Since the stimulatory effect on the serum PRL secretion by morphine was not decreased by pretreatment with the constant, a rather low dose of morphine, we made the rats tolerant to morphine by injecting the increasing doses of morphine up to 100 mg/kg in the next experiment (Table 6). On the day of sacrifice, body weights of the morphine-tolerant rats and their saline controls (non-tolerant rats) were 277±3 g and 323±2 g (mean±S.E., N=30) respectively. The basal PRL levels (30 min

### Table 3. Effects of naloxone, apomorphine and diphenhydramine on morphine-induced increase in serum PRL levels*

| Treatments                  | Serum PRL (mg/ml) | P         |
|-----------------------------|-------------------|-----------|
|                            | (mean±S.E. (N))   |           |
| **Experiment 1**            |                   |           |
| Saline (5 ml/kg)            | 37±6 (17)         |           |
| Morphine HCl (20 mg/kg)     | 201±38 (6)        | <0.001 vs. saline. |
| Naloxone HCl (1 mg/kg)      | 20±2 (6)          |           |
| Morphine HCl (20 mg/kg) +   | 27±4 (6)          | <0.005 vs. morphine. |
| Naloxone HCl (1 mg/kg)      |                   |           |
| **Experiment 2**            |                   |           |
| Saline (5 ml/kg)            | 38±14 (6)         |           |
| Morphine HCl (20 mg/kg)     | 231±35 (6)        | <0.001 vs. saline. |
| Apomorphine HCl (20 mg/kg)  | 18±2 (6)          |           |
| Morphine HCl (20 mg/kg) +   | 19±2 (6)          | <0.001 vs. morphine. |
| Apomorphine HCl (20 mg/kg)  |                   |           |
| **Experiment 3**            |                   |           |
| Saline (5 ml/kg)            | 53±9 (5)          |           |
| Morphine HCl (20 mg/kg)     | 144±14 (5)        | <0.001 vs. saline. |
| Diphenhydramine HCl (10 mg/kg) | 21±4 (5)    | <0.025 vs. saline. |
| Diphenhydramine HCl (20 mg/kg) | 21±4 (5)    | <0.025 vs. saline. |
| Morphine HCl (20 mg/kg) +   | 176±19 (5)        | <0.001 vs. saline. |
| Diphenhydramine (10 mg/kg)  |                   |           |
| Morphine HCl (20 mg/kg) +   | 128±9 (5)         | <0.005 vs. saline. |
| Diphenhydramine (20 mg/kg)  |                   |           |

* Rats were sacrificed 30 min after administration of drugs.

### Table 4. Effect of apomorphine and naloxone on haloperidol-induced increase of serum PRL.*

| Treatments                  | Serum PRL (mg/ml) | P         |
|-----------------------------|-------------------|-----------|
|                            | (mean±S.E., N=6)  |           |
| Saline                      | 55±4              |           |
| Haloperidol (10 mg/kg)      | 153±24             | <0.025 vs. saline |
| Haloperidol (10 mg/kg)      | 81±3               | <0.025 vs. haloperidol |
| Apomorphine HCl (20 mg/kg)  |                   |           |
| Haloperidol (10 mg/kg) +    | 235±38             |           |
| Naloxone HCl (1 mg/kg)      |                   |           |

* Rats were sacrificed 30 min after injection of drugs.
after saline) of morphine-tolerant rats did not differ from those of non-tolerant rats. Morphine produced a lesser increase in PRL in the tolerant than the non-tolerant rats (Table 6). This fact shows that tolerance developed with the effect of morphine on the serum PRL levels. Although naloxone precipitated withdrawal signs such as diarrhea or body shakes in the tolerant rats, it did not affect the serum PRL levels of the tolerant rats either when administered alone or in combination with morphine.

**Effects of morphine, haloperidol and dopamine on the release of PRL from the isolated pituitary**

Morphine and haloperidol did not increase the release of PRL from the isolated anterior pituitary (Table 7, experiments 1 and 2). The PRL content of the incubated pituitary halves was not changed by morphine (Table 7, experiment 1). Fifty \( \mu \)M dopamine decreased the release of PRL significantly and this effect of dopamine was reversed by 50 \( \mu \)M haloperidol but not by 50 \( \mu \)M morphine (Table 7, experiment 2). Even the higher concentrations of morphine did not antagonize the inhibitory effect of dopamine (Table 7,
DISCUSSION

In the present study we confirmed the previous reports (2-7) that morphine increased the PRL levels in blood and this effect of morphine was antagonized by naloxone (5-7). Morphine increased the serum PRL levels in a dose dependent manner and the duration of action was similar to that seen in experiments of other workers (4). Our in vitro study showed that morphine does not act directly on the pituitary gland to release PRL nor does it block the inhibitory effect of dopamine on the release of PRL. The anterior pituitary apparently has a low level of specific opiate binding (16) and such may be evidence against the direct effect of morphine on the anterior pituitary.

It is considered that the dopaminergic tuberoinfundibular system plays an important role in the inhibition of PRL secretion (17). Dopamine and apomorphine inhibit the release of PRL from the pituitary by a direct action and by stimulating the release of hypothalamic PIF. The inhibition was antagonized by haloperidol (9-13). Although both morphine and haloperidol produce an increase in the serum PRL levels, and such can be prevented with apomorphine, the mechanism by which morphine increases the serum PRL levels is not identical to that seen with haloperidol, as morphine had no direct or dopamine-blocking effect on the pituitary, and the effect of haloperidol was not antagonized by naloxone. Most probably morphine increases the serum PRL levels by inhibiting the release of PIF or by promoting the release of PRF (Prolactin releasing factor) from the
hypothalamus, however, blockade of the action of PIF on the pituitary by morphine may be feasible.

Although it has been suggested that morphine-induced stimulation of PRL secretion would include a histamine-related step (5), our procedures using diphenhydramine did not result in an inhibition of the stimulatory effect of morphine on the serum PRL levels. The fact that Rivier et al. (5) used estrogen-progesterone pretreated male rats may explain the discrepancy.

Bruni et al. (7) reported that naloxone at a dose of 0.2 mg/kg reduced the serum PRL levels, however there was no significant reduction of serum PRL by 5 mg/kg of naloxone in the present study.

Tolerance to the PRL effect of morphine was demonstrated only in the rats treated with a higher dose (100 mg/kg) of morphine, and repeated administration of a low dose of morphine may not be sufficient to make rats tolerant to the PRL effect of morphine. Although the body weight of the morphine-tolerant rat was less than that of the nontolerant animals, reduction in the PRL increase by morphine in the tolerant rats cannot be attributed to the reduced body weight or malnutrition caused by chronic morphinization, because the weight loss caused by restricted feeding does not decrease the serum PRL levels (18).

Since it is suggested that chronic administration of narcotic analgesics results in an increased response of the dopamine receptors to dopamine agonists (15) and the tubero-infundibular dopamine hyperactivity tends to lower the serum PRL levels (17), it may be that the degree of the PRL response to morphine in the tolerant rat decreases with dopamine related hyperactivity.

Although it was reported that 3 days' withdrawal from chronic morphine treatment resulted in lower circulating levels of serum PRL in male rats (19), we did not observe a significant decrease in the serum PRL levels of the abstinent rats. The reason for the discrepancy is not clear, however, it should be pointed out that the rats were made abstinent by the injection of naloxone and not by the abrupt withdrawal of morphine.

In conclusion, our results show that the mechanism by which morphine increases the serum PRL levels is different from that of haloperidol, tolerance develops regarding the stimulatory effect of morphine on the serum PRL levels and that naloxone-induced abstinence does not change the serum PRL levels.

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