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Differential disappearance of tolerance to thermal, hormonal and locomotor effects of morphine in the male rat

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Abstract

Development and disappearance of tolerance to various effects of morphine was studied by comparing the effect of acute morphine at 6 h and at 92 h after cessation of a 5-day regimen with increasing doses of morphine. After the 6-h lag time, tolerance manifested to the thermal, locomotor depressant and hormonal (stimulation of growth hormone and prolactin secretion) effects of morphine. The hypokinetic effect of morphine was replaced by a hyperkinetic effect and increased locomotor activity was evident following the challenge dose of morphine. Tolerance disappeared in different ways during the 92-h lag time. Tolerance persisted (hypothermic and hypokinetic effect) or disappeared considerably (prolactin secretion) during the 92-h withdrawal period. Tolerance to some effects also faded completely, and in contrast, even sensitization to various effects of morphine (growth hormone secretion, hyperthermic effect) could be seen after the 92-h withdrawal period. In addition, the original hypokinetic effect of morphine was replaced by a hyperkinetic effect (i.e., enhanced locomotor activity), which was even stronger after the 92-h lag time. The observed dissociation, which has not been seen to such an extent before, may be due to the differential modulation of the subtypes of μ-opioid receptors or differences in the adaptive mechanisms, e.g. conditioning, in various brain areas. Faster recovery of tolerance to an inhibitory than to a stimulatory effect of morphine during the withdrawal period may partially explain the sensitization to some effects of morphine.

Keywords: Tolerance; Morphine; Thyrotropin; Prolactin; Growth hormone; Locomotor activity; Rectal temperature

1. Introduction

Apart from antinociception, opioids have a number of pharmacological effects, such as actions on respiration, bowel function, secretion of anterior pituitary hormones, core temperature and locomotor activity. The clinical usefulness of opioids is limited by tolerance and dependence. Both of these are thought to result from neuronal adaptations produced by repeated drug exposure but the mechanisms of tolerance and dependence are still poorly understood.

The present understanding of the development and the disappearance of tolerance to the various effects of morphine is based on animal experiments in which various regimens and withdrawal periods have been used. There are only few reports where the development of tolerance to multiple effects of morphine have been studied in the same study using an identical protocol (Fernandez et al., 1977; Mucha et al., 1979; Kuhn and Bartolome, 1985). Tolerance develops easily to the hypothermic (Gunne, 1960) and locomotor depressant actions of morphine (Vasko and Domino, 1978). However, the development of tolerance to the hyperthermic (Mucha et al., 1987) and locomotor stimulant (Vasko and Domino, 1978) actions of morphine generally requires quite intense pretreatment regimens. In fact, only the sensitization to the locomotor stimulant effect of morphine has been found in most of the studies (Babbini and Davis, 1972; Locke and Holtzman, 1986). This kind of behavioral sensitization may be linked to the rewarding property of drugs (Wise and Bozarth, 1987).

The regulation of anterior pituitary hormones has been comprehensively used as an index of the action of

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drugs in the brain (Tuomisto and Männistö, 1985). Tolerance to the effects of opioids on the secretion of anterior pituitary hormones seems to develop at different rates (Kuhn and Bartolome, 1985; Rauhala et al., 1988, 1992; Ling et al., 1989). The stimulatory effect of methadone on growth hormone (GH) and prolactin secretion has been found to be quite resistant to the development of tolerance (Kuhn and Bartolome, 1985). The disappearance of tolerance to the various effects of morphine, however, has rarely been examined in a single study (Cox et al., 1975; Kuhn and Bartolome, 1985; Villar and Bhargava, 1992).

The main aim of this study was to compare in one large study, using a unified protocol, the development and especially the *disappearance* of tolerance to various effects of morphine. To the best of our knowledge, such a comprehensive comparison has not been done before. This study was undertaken to test whether the various effects of morphine could be grouped together according to the equal time course in these events.

2. Materials and methods

2.1. Animals

Male outbred Wistar rats (Han/Kuopio) weighing 210–260 g at the beginning of the treatment were kept one to a cage at +30°C in a quiet, dark room illuminated artificially from 7 a.m. to 7 p.m. throughout the treatment schedule. They were given laboratory pellets and water ad libitum. The warm-room was used to accentuate the hyperthermic effect of morphine (Adler et al., 1988) as well as to maintain comparability with our previous studies (Rauhala et al., 1988,1992; Männistö et al., 1994). The temperature used is a little above the upper limit recommended for rats. However, rats can conform to a wide range of environmental temperatures when the time for adaptation is long enough (Baker et al., 1979).

2.2. Experimental designs

Chronic morphine administration (the 5-day morphine regimen)

Increasing doses of morphine were administered thrice a day i.p. for a total of 5 days according to the following schedule (Männistö et al., 1994): day 1, two doses at 10 mg/kg and one at 15 mg/kg; day 2, two doses at 20 mg/kg and one at 25 mg/kg; day 3, two doses at 30 mg/kg and one at 35 mg/kg; day 4, two doses at 40 mg/kg and one at 45 mg/kg; day 5, two doses at 50 mg/kg (only two doses). The injections were given at 8:00 a.m., 4:00 p.m. and 12:00 p.m. The control animals received equivalent volumes of saline (0.5 ml/100 g body weight). The lag time between the acute experiment and the last pretreatment dose was either 6 or 92 h. When the lag time was 6 h, instead of the last pretreatment dose, the rats were given an acute challenge dose of morphine or a saline injection. The 6-h and 92-h lag times were used to compare the disappearance of tolerance during the abstinence period. The 6-h lag time was selected in order to study the effects of morphine *before* the beginning of the abstinence syndrome and, on the other hand, to be sure that the last pretreatment dose would not have any major effect on hormone levels. The longer lag time was selected in order to let abstinence symptoms and residual morphine disappear and serum prolactin levels return to the normal level (Rauhala et al., 1988).

Measurement of rectal temperature

Because handling of the rats would have seriously disturbed the hormonal responses, rectal temperature was measured within 1 min after decapitation. An electric thermocouple (MD 852, Ellab Instruments, Copenhagen, Denmark) was inserted 3 cm inside the anal sphincter and allowed to stabilize for 30 s.

The basal rectal temperatures were obtained from the rats used in the basal hormonal studies and kept at +30°C. Separate groups of rats were moved from the adaptation room to the cold room (+4°C) in order to induce hypothermia after morphine administration (Männistö et al., 1994). No hormonal analyses were done for these cold-exposed rats.

Measurement of locomotor activity

The drugs were administered 10 min before the beginning of the test period. The rats were placed in a computerized photoelectric box (68 cm × 68 cm × 45 cm) (Kungsbacka Mät- and Reglerteknik AB, Fjäras, Sweden) and the activity was recorded thereafter in 20-min periods for a total of 120 min. The measurements were made between 12:00 a.m. and 4:00 p.m. The same rats were used in both 6-h and 92-h lag time studies.

Basal GH and prolactin levels

The rats were kept in the warm-room (+30°C) throughout the experimental schedule and transferred to the adjacent room (+22°C) just before decapitation. Decapitation was used to avoid handling-induced hormonal changes. The whole trunk blood was collected, the serum was separated by centrifugation and stored at −20°C until hormone assays were run. All the experiments were done between 11:00 a.m. and 3:00 p.m.

2.3. Analytical

Serum GH and prolactin concentrations were determined from duplicate samples (0.1 ml) by specific ra-

dioimmunoassays. The rat GH and prolactin kits were gifts from the National Institutes of Health, USA. The assays were run according to the instructions provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). GH results are expressed in ng/ml of the NIDDK-GH-RP-2 standard which has a biological potency of 2 IU (bovine units)/mg. The prolactin results are expressed in ng/ml of NIDDK-rPRL-RP-2, which has a biological potency of 30 IU/mg as determined in the pigeon local crop sac assay of Nicoll. The intra-assay variation was less than 10% and the interassay variation less than 20%.

Serum GH levels were exceptionally high after acute morphine administration, and even several of the samples diluted 1:10 gave values exceeding our highest standard (96 ng/ml). Therefore, we could not measure any values higher than 960 ng/ml. Although the samples from tolerant rats and their corresponding controls were always treated identically, the accuracy of the high GH values should be considered as tentative.

2.4. Drugs

Morphine hydrochloride was purchased from the Pharmacy of the University of Helsinki, Finland. The dose of morphine refers to base. It was dissolved in a solution of 0.9% NaCl in distilled water and given at a volume of 0.5 ml per 100 g body weight i.p.

2.5. Statistics

The results are expressed as the means ± S.E.M. The Student's t-test was used to determine the differences between two means. With three or more means, analysis of variance was used first. The observed significances were then confirmed with the Newman-Keuls test. Pharmacological Calculations System software was used for statistical analysis (Tallarida and Murray, 1987).

3. Results

All experiments were carried out in rats pretreated for 5 days with either saline (naive rats) or morphine. The succeeding lag time was either 6 or 92 h. In the acute experiment, challenge doses of morphine (5, 15 and 50 mg/kg) were given to all the rats at 30 min, 60 min or 120 min before killing.

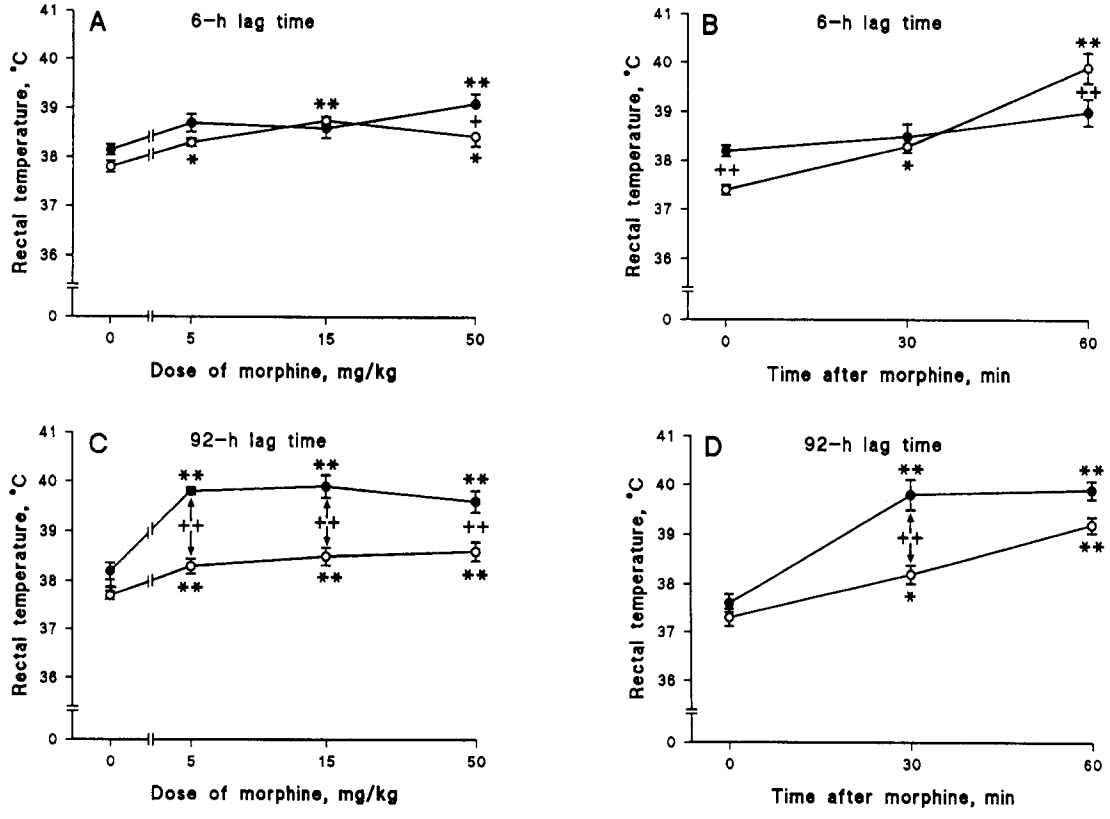

Fig. 1. The effects of various doses of morphine (5, 15 and 50 mg/kg, i.p.) at 30 min (A and C) and the time course of the effect of acute morphine (15 mg/kg, i.p.; B and D) on the rectal temperature of either 5-day saline (open circles)- or morphine (solid circles)-pretreated rats. The acute treatments were given after either a 6-h (A and B) or a 92-h (C and D) lag time. Means ± S.E.M., $n = 6$–14. Statistics: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. corresponding acute saline-challenged rats (value at 0-dose or 0-time); $^{+}P < 0.05$, $^{++}P < 0.01$ between morphine-pretreated rats and corresponding saline-pretreated rats.

3.1. Rectal temperature after the 6-h lag time

After the 6-h lag time, the rectal temperatures were somewhat higher in morphine-pretreated than in naive rats (Fig. 1A,B). In naive rats, all doses of morphine (5–50 mg/kg) increased rectal temperatures (Fig. 1A,B) significantly at 30 min, whereas in the morphine-pretreated rats the highest dose (50 mg/kg) of morphine was needed for this effect (Fig. 1A,B). Acute morphine 15 mg/kg increased rectal temperatures in naive rats but not in the morphine-pretreated rats also at 60 min (Fig. 1B).

In naive rats rectal temperatures decreased during the 30-min cold-exposure following an acute dose of morphine (15 mg/kg) (Table 1). However, the challenge dose of morphine had no hypothermic effect on the morphine-pretreated rats (Table 1).

3.2. Rectal temperature after the 92-h lag time

All doses of morphine (5–50 mg/kg) increased rectal temperatures in both naive and morphine-pretreated rats at 30 min. However, this increase was more pronounced in the morphine-pretreated than in naive rats (Fig. 1C). The time course study showed that the increase occurred more rapidly in the morphine-pretreated group than in the naive rats (Fig. 1D).

During the 30-min cold-exposure, morphine (15 mg/kg) decreased rectal temperatures in naive but not in the morphine-pretreated rats (Table 1).

3.3. Locomotor activity after 6-h and 92-h lag times

Following an acute saline injection, the locomotor activities of both morphine-pretreated and naive rats were the same (Fig. 2A). Morphine, at 15 mg/kg, had a biphasic effect on the locomotor activity of the naive rats with an initial decrease during the first 20 min followed by an increase over that of the naive rats challenged with saline after 80 min (Fig. 2A). The challenge dose of morphine did not decrease locomotor activity in the morphine-pretreated rats compared to saline-challenged rats. On the contrary, a statistically significant stimulation of locomotor activity was seen during the 20–40-min and during the 60–80-min observation periods (Fig. 2A).

The results were similar after the 92-h lag time with one exception. Following the challenge dose of morphine, the observed increase in the locomotor activity of the morphine-pretreated rats was even stronger and lasted for the entire observation period (Fig. 2B).

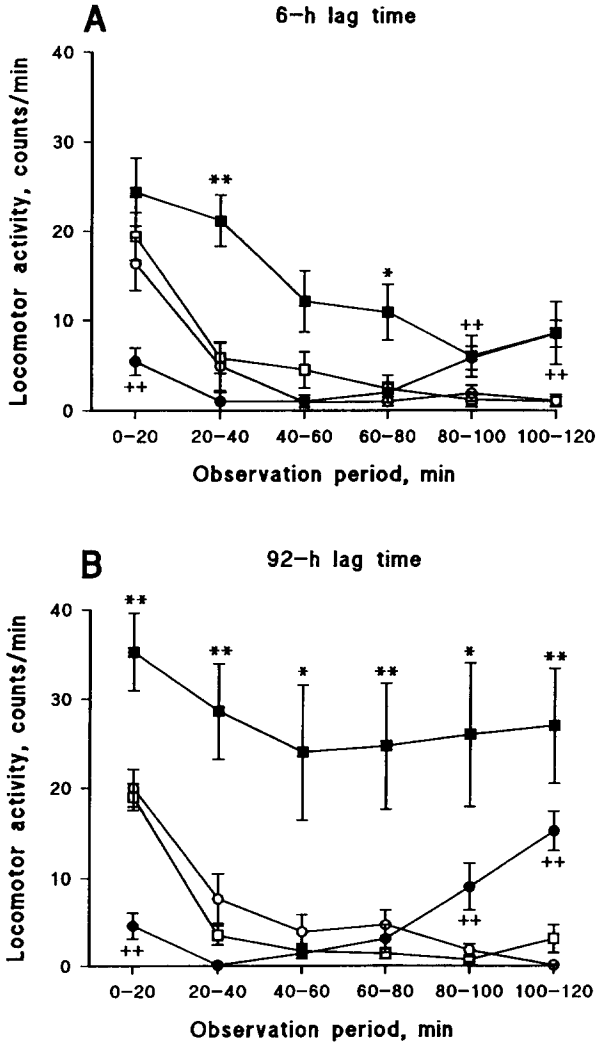

Fig. 2. Time course of the effects of acute saline (open marks) or morphine (solid marks, 15 mg/kg, i.p.) on the locomotor activity of either 5-day saline (circles)- or morphine (squares)-pretreated rats after 6-h (A) or 92-h (B) lag time. Means ± S.E.M., $n = 6–8$. Statistics: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. corresponding saline-challenged and morphine-pretreated rats (open squares); $^{++}P < 0.01$ vs. corresponding saline-challenged and saline-pretreated rats (open circles).

Table 1
The effect of acute saline or morphine (15 mg/kg, i.p.) on the rectal temperature of either 5-day saline- or morphine-pretreated rats during a 30-min cold-exposure at +4°C

Chronic pretreatment	Acute treatment	Rectal temperature (°C)
After 6-h lag time		
Saline	Saline	37.5 ± 0.18
Saline	Morphine	35.8 ± 0.19 [a]
Morphine	Saline	38.5 ± 0.36
Morphine	Morphine	38.3 ± 0.80 [b]
After 92-h lag time		
Saline	Saline	36.6 ± 0.18
Saline	Morphine	35.2 ± 0.19 [a]
Morphine	Saline	37.6 ± 0.34
Morphine	Morphine	37.5 ± 0.30 [b]

The acute treatments were given after a 6-h or a 92-h lag time. Means ± S.E.M. Statistics: [a] $P < 0.01$ vs. corresponding acute saline-challenged rats; [b] $P < 0.01$ between morphine-pretreated rats and corresponding saline-pretreated rats. $n = 6–7$.

3.4. GH secretion after the 6-h lag time

In naive, but not in the morphine-pretreated rats, the challenge doses 5 and 15 mg/kg of morphine increased GH levels at 30 min (Fig. 3A). The stimulatory effect of the 15 mg/kg dose of morphine was also seen in naive but not in the morphine-pretreated rats at 60 min (Fig. 3B). The effect of the highest dose of morphine (50 mg/kg) did not reach statistical significance in naive rats (Fig. 3A,C).

3.5. GH secretion after the 92-h lag time

In naive and now also in the morphine-pretreated rats, the challenge doses 5 and 15 mg/kg of morphine stimulated GH secretion at 30 min after the acute morphine (Fig. 3C). In the time course study, the GH levels were increased maximally at 30 min (Fig. 3D). The stimulatory effect of morphine tended to be enhanced in the morphine-pretreated rats and in the time course study, and a statistical significance between naive and the morphine-pretreated rats was observed at all time intervals (Fig. 3D).

3.6. Prolactin secretion after the 6-h lag time

Morphine at 15 and 50 mg/kg increased prolactin secretion in naive rats but not in the morphine-pretreated rats (Fig. 4A). A similar effect was observed in the time course study both at 30 and 60 min after administration of the 15 mg/kg dose of morphine (Fig. 4B).

3.7. Prolactin secretion after the 92-h lag time

The highest (50 mg/kg) dose of morphine stimulated prolactin secretion in the dose response study (Fig. 4C). In the time course study the recovery of the prolactin secretion-stimulating effect of morphine was seen at 30 min but not at 60 min after the 15-mg/kg challenge dose of morphine (Fig. 4D).

4. Discussion

The key findings of the present study were as follows. Tolerance manifested to the thermal, locomotor

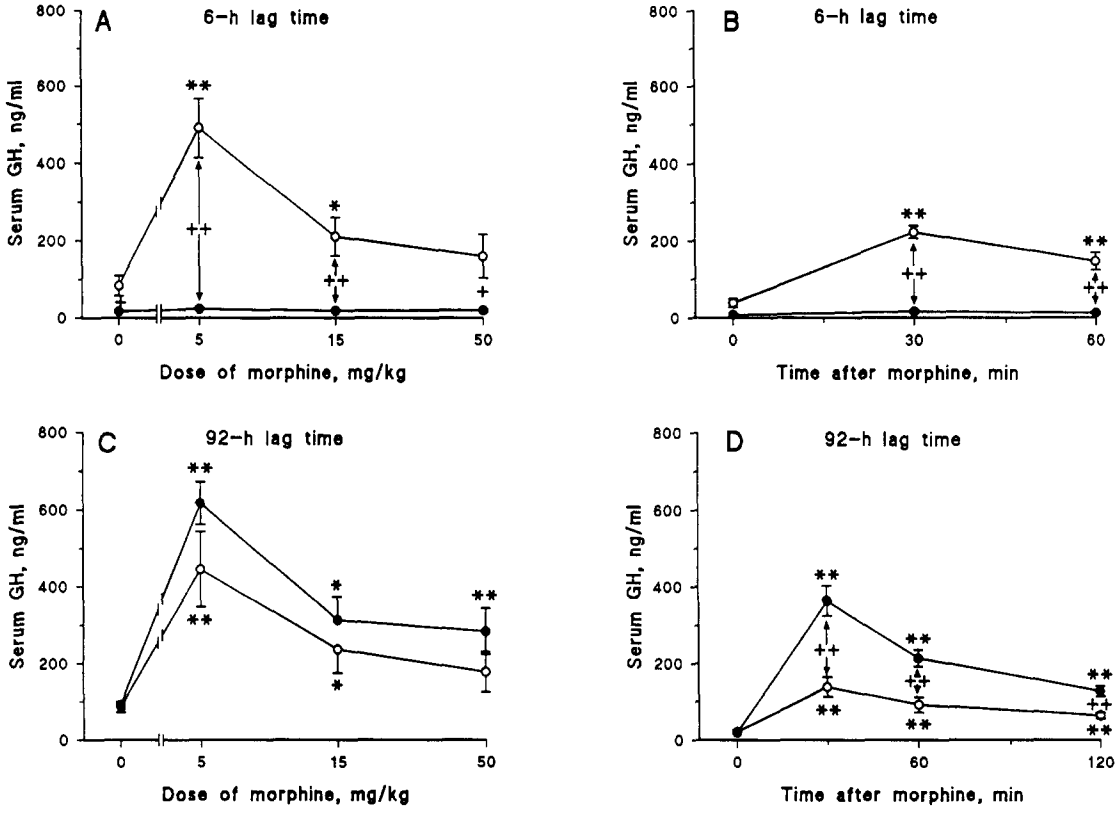

Fig. 3. The effects of various doses of morphine (5, 15 and 50 mg/kg, i.p.) at 30 min (A and C) and the time course of the effects of acute morphine (15 mg/kg, i.p.; B and D) on growth hormone levels of either 5-day saline (open circles)- or morphine (solid circles)-pretreated rats. The acute treatments were given after either a 6-h (A and B) or a 92-h (C and D) lag time. Means ± S.E.M., $n = 6–14$. Statistics: $^{*}P < 0.05$, $^{**}P < 0.01$ vs. corresponding acute saline-challenged rats (value at 0-dose or 0-time); $^{+}P < 0.05$, $^{++}P < 0.01$ between morphine-pretreated rats and corresponding saline-pretreated rats.

depressant and hormonal (GH and prolactin) effects of morphine during a 5-day morphine pretreatment. The hypokinetic effect of morphine was even inverse, and on the contrast, sensitization and increased locomotor activity was evident following the challenge dose of morphine. Tolerance disappeared in different ways during the 92-h lag time. Tolerance persisted (hypothermic and hypokinetic effect) or disappeared considerably (prolactin) during the 92-h withdrawal period. Tolerance to some effects had also faded completely, and in contrast, even sensitization to various effects of morphine (GH secretion, hyperthermic effect) could be seen after the 92-h withdrawal period. In addition, the original hypokinetic effect of morphine was replaced by a hyperkinetic effect (i.e., enhanced locomotor activity), which was even stronger at the 92-h lag time.

Complete tolerance developed to the hypothermic (at +4°C) and a partial tolerance to the hyperthermic (at +30°C) effect of a challenge dose of morphine when administered 6 h after cessation of the 5-day morphine pretreatment. During the 92-h lag time, tolerance to the hypothermic effect was sustained but that to the hyperthermic effect totally disappeared. Even a sensitization to the hyperthermic effect of morphine was seen. The fast disappearance of tolerance to the hyperthermic effect of morphine is in line with a previous report (Villar and Bhargava, 1992). The sensitization to the hyperthermic effect of morphine, after the 92-h lag time, may be due to the disappearance of tolerance to the hyperthermic while tolerance to the hypothermic effect of morphine still persists.

The acute dose of morphine had a biphasic action on locomotor activity in naive rats; decreased activity at the beginning and enhanced activity at the end of the observation period. Chronic administration of morphine produces tolerance to the akinetic effect and a potentiation of the locomotor stimulating effect of acute morphine, a phenomenon called behavioral sensitization (Babbini and Davis, 1972; Vasko and Domino, 1978). This phenomenon has been found to be long lasting (Babbini et al., 1975; Spanagel et al., 1993). After the 92-h lag time, the sensitization seems to be even more pronounced compared to that after the 6-h lag time. In line with the present results, a challenge dose of morphine had no effect on behavior on the first day of withdrawal but increased activity on 3rd–7th days of withdrawal (Acquas and Di Chiara, 1992).

Tolerance to the effect of morphine on GH secre-

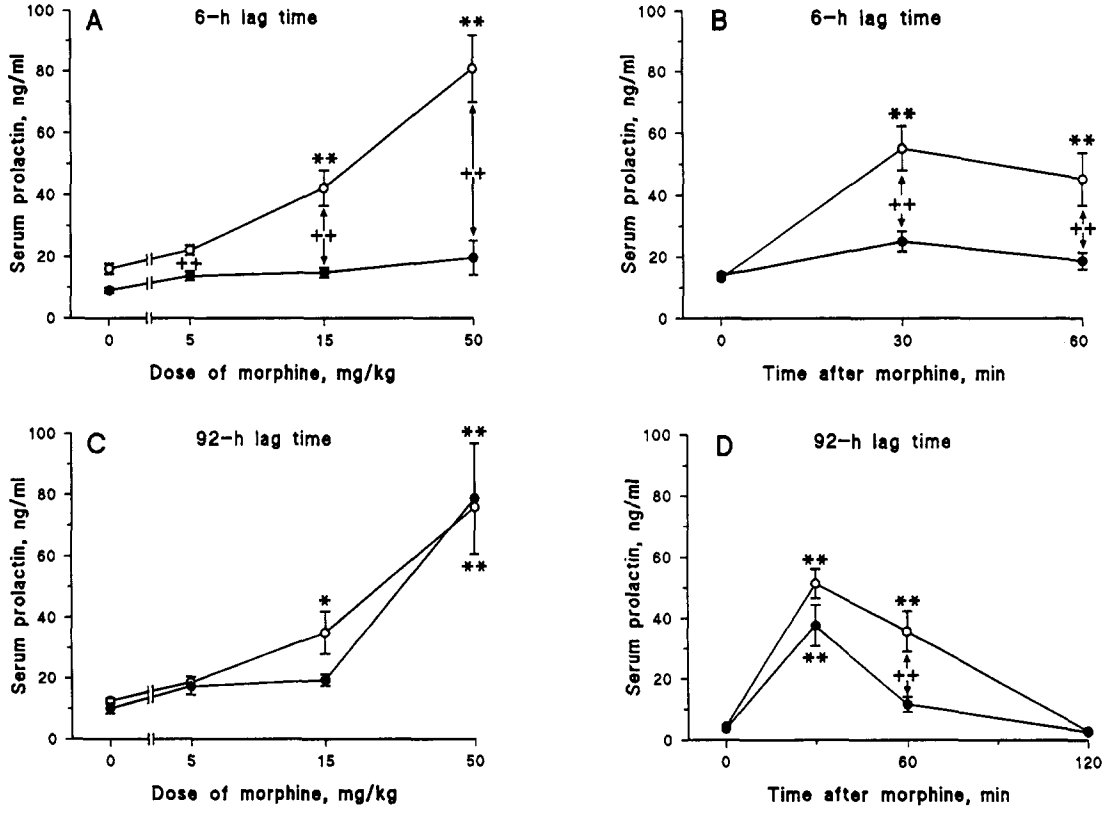

Fig. 4. The effects of various doses of morphine (5, 15 and 50 mg/kg, i.p.) at 30 min (A and C) and time course of the effects of acute morphine (15 mg/kg, i.p.; B and D) on prolactin levels of either 5-day saline (open circles)- or morphine (solid circles)-pretreated rats. The acute treatments were given after either a 6-h (A and B) or a 92-h (C and D) lag time. Means ± S.E.M., $n = 6–14$. Statistics: * $P < 0.05$, ** $P < 0.01$ vs. corresponding acute saline-challenged rats (value at 0-dose or 0-time); $^{++}$ $P < 0.01$ between morphine-pretreated rats and corresponding saline-pretreated rats.

tion does not develop in a short infusion model (Ling et al., 1989) and a rather long administration of methadone is needed before tolerance to a challenge dose of methadone can be seen (Kuhn and Bartolome, 1985). In the present study, acute morphine given after the 6-h lag time, had no effect on GH levels indicating tolerance. This result must be carefully interpreted. After a 6-h lag time, the withdrawal symptoms are not yet fully developed, and a lot of residual morphine from the last pretreatment dose may still be present. This residual morphine may well activate κ-opioid receptors which are known to inhibit GH secretion (Krulich et al., 1986). The lower potency of the dose of 50 mg/kg of morphine compared to that of 5 mg/kg also supports this hypothesis. Consequently, the lack of the effect of the challenge dose may be due to the inhibitory effect of large doses of residual morphine mediated by κ-opioid receptors rather than to a real tolerance. This would also explain the disappearance of tolerance during the 92-h lag time. However, it does not explain the development of sensitization to the GH releasing effect of morphine. Apparently the lag time is a very important factor when evaluating the development of tolerance to GH secretion.

Nearly complete tolerance to the stimulatory effect of all doses of acute morphine on prolactin secretion could be seen after the 6-h lag time, confirming the results of several previous studies (Muraki and Tokunaga, 1978; Deyo et al., 1980; Kuhn and Bartolome, 1985). Recovery of the stimulating effect of morphine was seen after the 92-h lag time at 30 min but not at 60 min which is a clear piece of evidence that only a partial disappearance of tolerance occurs during the withdrawal period.

It is not known why tolerance develops and disappears at different rates for the various effects of morphine. One explanation for this may be the fact that morphine interacts with multiple (μ-, δ- and κ-) opioid receptors and with the various known subtypes of these receptors. Two populations of the μ-opioid receptors has been suggested (Pasternak and Wood, 1986). Morphine has its highest affinity for μ-opioid receptors, 5 times higher for the μ_1- than for the μ_2-subtype, a much smaller affinity for the δ-opioid receptors and only a weak affinity for the κ-opioid receptors (Pasternak, 1993). Tolerance has been reported to develop far more rapidly to the naloxonazine-sensitive (μ_1-opioid receptor-mediated) actions than to the naloxonazine-insensitive (non-μ_1-opioid receptor-mediated) actions of morphine in a short infusion model (Ling et al., 1989). In the present study and in our previous study (Männistö et al., 1994), tolerance to the non-μ_1-opioid receptor-mediated actions (GH secretion, hyperthermia) of morphine disappeared more rapidly, during the 92-h lag time, whereas tolerance to the μ_1-opioid receptor-mediated actions (antinociception, hypothermia, prolactin secretion) did not disappear. Collectively, these results give evidence that tolerance to the μ_1-opioid receptor-mediated actions of morphine should develop faster and disappear more slowly than tolerance to its non-μ_1-opioid receptor-mediated actions.

The mechanisms of tolerance could relate to a reduction in the number of receptors or uncoupling of the receptor from its second messengers and/or changes in parallel antagonistic or facilitatory systems. Although downregulation of μ-opioid receptors has been demonstrated during chronic morphine exposure (Werling et al., 1989), changes in receptor number are not necessary for tolerance to occur (Cox, 1991). Tolerance may manifest itself more easily to the actions of morphine which need large receptor occupation and thus are more sensitive to decreases in the number of functional opioid receptors. The importance of a receptor reserve is supported by the finding that opioids with a low intrinsic efficacy lose their potency far more rapidly than opioids with high intrinsic efficacy (Young et al., 1991; Paronis and Holtzman, 1992). However, it is obvious that tolerance and especially sensitization are complicated phenomena and various kinds of adaptive processes take place (see below).

The sensitization of GH elevation and hyperthermia and the conversion of the initial akinetic effect into hyperlocomotion is of a special interest since the rewarding effect of morphine also shows a similar pattern of changes and may be a crucial factor in the development of drug abuse (Bartoletti et al., 1989; Lett, 1989; Gaiardi et al., 1991). The mechanism of sensitization is not known, but the length of the withdrawal period seems to be an important factor for the appearance of sensitization, especially after a high-dose morphine pretreatment. This is also the case with psychostimulants (Kalivas and Stewart, 1991). The expression of behavioral sensitization, by for example conditioning, has been related to both augmented dopamine release in the nucleus accumbens (Kalivas and Stewart, 1991; Acquas and Di Chiara, 1992; Spanagel et al., 1993) and complex adaptive changes in dopamine neurotransmission within rat striatum (Tjon et al., 1994) during the withdrawal period. Furthermore, slower recovery from tolerance to the inhibitory than to the stimulatory effect of morphine during the withdrawal period could cause the observed sensitization.

The specific conditions under which an animal is exposed to morphine during pretreatment sessions and during acute experiments may contribute to the expression of tolerance and sensitization (Stewart and Badiani, 1993). We have implemented several procedures to deal with these difficulties. To overcome the problem of conditioning, we pretreated the control group with saline using the same injection protocol as used with

morphine. In addition, the present study examined the locomotor effects of a challenge dose of morphine given on two occasions (after 6-h and 92-h lag times) in the same group of animals. Accordingly, some of the sensitization of the locomotor activity could be due to this repeated testing. On the other hand, serum levels of the hormones and rectal temperatures in the warm environment were also measured from the same animal. However, both the morphine pretreatment and the withdrawal period affected these variables in a different way. Thus, the conditioning seems not to explain to a marked extent the variation in the development and disappearance of tolerance or the sensitization to the various effects of morphine.

In conclusion, tolerance manifested itself in all the effects of morphine and the initial hypokinetic effect was replaced by a hyperkinetic effect. However, tolerance to the various effects of morphine disappeared at very different rates. The observed dissociation, which has not been reported to such an extent before, may be due to the differential modulation of the subtypes of μ-opioid receptors or differences in the adaptive mechanisms in various brain areas. Faster recovery of tolerance to an inhibitory than to the stimulatory effect of morphine may also partially explain the sensitization to some effects of morphine observed during the withdrawal period.

Acknowledgements

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