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Lack of Crosstolerance Between Morphine and Morphine-6-glucuronide as Revealed by Locomotor Activity

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GRUNG, M., S. SKURTVEIT, Å. RIPEL AND J. MORLAND. *Lack of crosstolerance between morphine and morphine-6-glucuronide as revealed by locomotor activity.* PHARMACOL BIOCHEM BEHAV **66**(1) 205–210, 2000.—Morphine-6β-glucuronide is a major metabolite of morphine. We wanted to examine whether the effects related to opiate CNS stimulation could be mediated by different receptors for morphine and M6G by studying the development of crosstolerance between these two drugs. The effect studied was locomotor activity in C57BL/6JBom mice. We observed a dose-dependent development of tolerance to daily injections of morphine, with 20 μmol/kg giving the most rapid development of tolerance, apparent already on the second day of treatment. This was also observed for the same dose of M6G. Crosstolerance to M6G was measured both after 1 day pretreatment and 7 days pretreatment with morphine 20 μmol/kg, while the crosstolerance to morphine was tested only after 1 day pretreatment with M6G (20 μmol/kg). Lack of crosstolerance towards M6G after 1 day of morphine pretreatment was observed, whereas crosstolerance to M6G was observed after 7 days of exposure to morphine pretreatment. Crosstolerance after M6G pretreatment to morphine was observed. It was concluded that the main part of the effect caused by M6G was mediated through a specific M6G receptor. © 2000 Elsevier Science Inc.

Opiates Morphine Morhpine-6-glucuronide Locomotor activity Crosstolerance Tolerance

MORPHINE elimination in humans is primarily due to conjugation with glucuronic acid in the liver to form morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). After acute, but in particulate, during chronic morphine administration, both glucuronide metabolites accumulate to reach higher plasma concentrations than the parent drug (27). M6G has been reported to be a more potent analgesic than morphine itself (22), while M3G appears to exert very limited pharmacological activities (3,20,29). Mice show no detectable amounts of M6G in plasma after morphine administration (7,12), and thus represent a suitable species for investigating morphine and M6G actions separately.

The question has been raised as to whether morphine and M6G act through the same receptors in exerting main actions like analgesia and effects related to reinforcement that are probably related to certain aspects in the development of morphine dependence. Although many studies indicate that morphine and M6G cause antinociception through the same receptor systems (1,22), other studies have pointed at different primary sites of action for morphine and M6G. Thus, antisense mapping of the opiate receptors has demonstrated that morphine and M6G might act through different opiate receptors (19,21,24–26).

Tolerance to opioid antinociception develops rapidly, and can readily be demonstrated within 12–24 h of morphine administration (31). Tolerance to opioids shows considerable specificity, in the sense that the development of tolerance to one opioid is not necessarily accompanied by tolerance to others (32). In general, the existence of crosstolerance between two opioids is taken as evidence that both drugs are acting via the same type of receptor. In a study by Rossi *et al.* (23), lack of analgetic crosstolerance between morphine on one hand, and M6G, heroin and 6-monoacetylmorphine on the other was reported. This was interpreted as a demonstration of different receptors being responsible for the analgesia produced by morphine and M6G.

Few studies have approached the receptors involved in behavioral effects related to morphine and M6G reinforcement.

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We have previously demonstrated that M6G, but not M3G, increased locomotor activity in mice when given systemically (10,16). The locomotor activity was mediated via opioid receptors, because the activation was abolished by pretreatment with naltrexone. Increased locomotor activity has been taken as a measure of central nervous stimulation, and there may be a correlation between motor activation and reward (15). Our previous study (10) indicated a partial difference between morphine and M6G with respect to opioid receptors involved in the CNS stimulatory actions. We wanted to examine further whether effects related to opiate CNS stimulation could be mediated by different receptors for morphine and M6G. Uchihashi *et al.* (30) suggested that the δ-receptor played a more important role in M6G-induced hyperlocomotion than in morphine-induced hyperlocomotion. This could have implications for the proposed use of M6G as an analgesic (18), and to characterize the relative importance of parent drug and metabolite for the reinforcing effects of morphine.

Tolerance has been demonstrated to develop for the CNS stimulating effect of morphine under certain experimental conditions. The objective of the present study was to investigate if there was a lack of crosstolerance or whether crosstolerance was present between morphine and M6G when the effect studied was locomotor activity. To do this, we first had to evaluate the importance of morphine dose for tolerance development to the locomotor activity.

METHOD

Animals

C57BL/6J-Bom adult, drug-naive, male mice (18–25 g body weight at testing) from Bomholt, Denmark, were used for the experiments. The animals were housed eight per cage in the vivaria at a room temperature of 22 ± 1°C, on a 12 L:12D schedule with the light period from 0700 to 1900 h, for at least 5 days prior to experiments. They had free access to food and water throughout the acclimatization period. The day before experiments, the animals were transported gently to the laboratory and housed in home cages under the same conditions as described for the vivaria.

The experimental protocol of this study was approved by the Norwegian Review Committee for the use of Animal Subjects.

Materials

Morphine hydrochloride (mol. wt. 375.9) was purchased from Norsk Medisinaldepot (Oslo, Norway), and morphine-6-β-D-glucuronide dihydrate (mol. wt. 497.5) from Ultrafine Chemicals (Manchester, UK). The drugs were dissolved in 0.9% saline. All solutions were used within a week. Saline (0.9%) were used for control injections.

Treatment

Tolerance development was studied for different doses of morphine (10, 20, 30, and 40 μmol/kg) and M6G (20 μmol/kg). The injection of drug or saline was given in total volumes of 0.1 ml per 10 g mouse as one daily injection (SC) at the same time each day. The tolerance development was monitored for periods up to 7 days.

The study of crosstolerance between morphine and M6G (20 μmol/kg) was performed on the second or eighth day after morphine dose of 20 μmol/kg, and on the second day after M6G dose of 20 μmol/kg. Equimolar doses of morphine and M6G were tested in the crosstolerance design.

Locomotor Activity

Locomotor activity was tested individually in chambers of a Digiscan optical animal activity monitoring system (Omnitech Electronics Inc., Columbus, OH). The cage size was 20 × 20 cm with infrared beam spacing of 2.5 cm.

Each animal was individually habituated to an activity chamber for 90 min before injections given in the home cage. After the injection the mice were placed back into the same activity chamber, and the locomotor activity was measured for 3 h.

Each animal's score was expressed as either activity counts per 5-min period, or as accumulated activity counts for the period after drug administration. Twelve different activities were registered as described previously (10). We here report only one of the behaviors, namely total distance traveled (cm). Many of the registered activities were highly correlated, and represented probably only different presentations of the same basic phenomenon. Total distance was found to be a representative behavior for locomotor activity after opiate administration as discussed previously (10).

Data Analysis

Statistical analyses were performed with Minitab statistical software. Significance of differences of medians in the tolerance studies was assessed by the use of the Mann–Whitney test, while significance of differences of means in the crosstolerance studies was assessed by the use of Student's *t*-test. The level of statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

Tolerance Development After Daily Administration of Morphine and M6G

The locomotor activity was measured for 3 h each day. Figure 1 shows the dose–response relationship as the sum of the total distance traveled during 3 h for the first and the seventh day of administration after various doses of morphine. The figure shows that the dose–response curves after repeated treatment of morphine are different from naive mice in the sense that tolerance developed to repeated administration of 20 and 30 μmol/kg morphine.

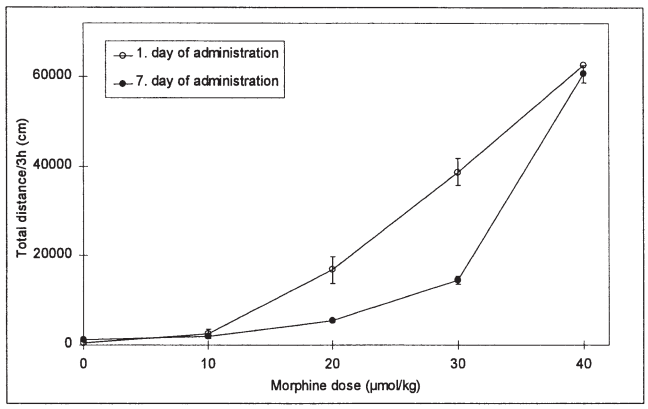

FIG. 1. Dose–response relationship as the sum of the distance traveled during the 3 h for the first and the seventh day of morphine administration. Each point represents the mean activity count ± SEM for 3-h period.

In separate control experiments we found that mice habituated to locomotor activity cages and pretreated with saline for 7 days and then administered morphine, were not different from naive mice administered morphine (data not shown).

The time course of total distance traveled during predrug habituation and during the 3-h test period after 10, 20, 30, and 40 μmol/kg morphine and 20 μmol/kg M6G on each treatment day is illustrated in Figs. 2a–e, respectively. Saline con-

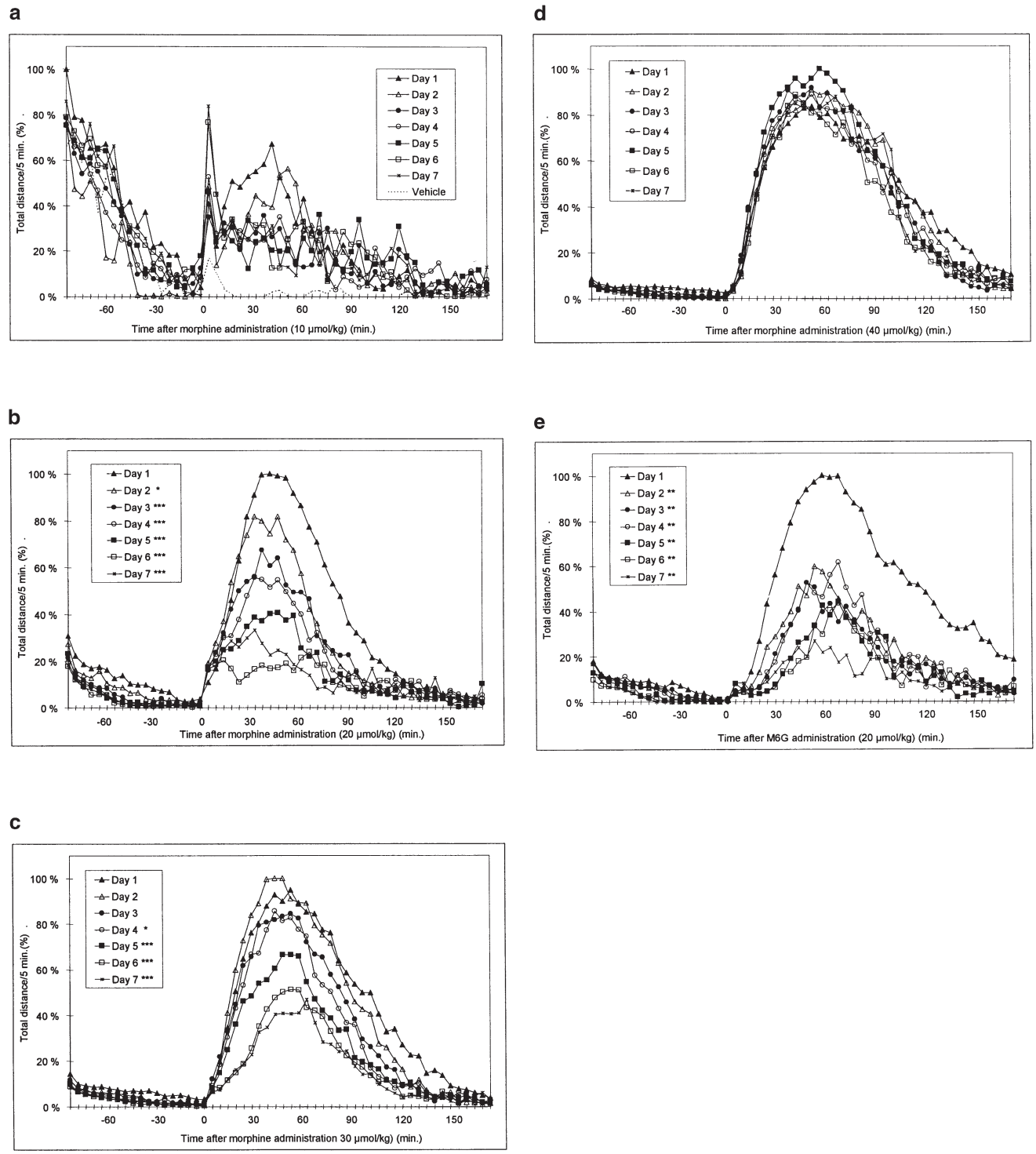

FIG. 2. Time course changes in total distance traveled per 5-min period during 7 days of morphine administration (a) 10 μmol/kg ($n = 16$) and saline controls ($n = 8$), (b) 20 μmol/kg ($n = 18$), (c) 30 μmol/kg ($n = 20$), (d) 40 μmol/kg ($n = 9$), and (e) M6G 20 μmol/kg ($n = 9$). The habituation period (90 min) as well as the locomotor activity during 3 h after morphine administration are shown. Each point represents the mean activity in percent of maximal activity observed for a 5-min interval of that group. Significant differences between the sum of total distance traveled after drug administration between the first day administration and later administrations are indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

trols are shown in Fig. 2a, together with the lowest dose of morphine. The activity increased after the injection of all the doses, and reached a maximum after approximately 45 min. We observed a dose-dependent development of tolerance to daily injections of morphine, as some of the doses of morphine employed gave rise to a tolerance development. During the 90-min predrug habituation, the activity of the mice in all the experiments were reduced to a very low and constant level (Figs. 2a–e). We noted a small, but not significant, reduction in the activity during this predrug habituation period after repeated treatment.

On the first day, the response after a dose of 10 μmol/kg morphine (Fig. 2a) was significantly higher than saline controls, but lower than the initial activities in the habituation period. However, after 7 days, the activity in these two groups was not significantly different. After this dose of morphine, the sum of locomotor activity during the 3-h response was decreased to 70–80% of the first day after a 7-day administration, but the decrease was not statistically significant.

Twenty μmol/kg gave a more pronounced locomotor activity (Figs. 1 and 2b). Two hours after injection, however, the activity decreased to the saline level. During repeated daily treatment, mice developed a significant tolerance to 20 μmol/kg morphine already present on the second day of administration. The total distance traveled was reduced to 80% of the first day. However, we observed variability between groups of mice. One of the groups ($n = 9$) developed a significant decrease (to 40% of first day) in locomotor activity on the second day of administration. The two other groups ($n = 8$ and 10) developed significant tolerance 1 day later, on the third day (to 61% total distance of first day). There was a further decrease in the locomotor activity when the treatment of these 18 mice was continued for 7 days (to 20% of first day) (Fig. 2b).

Thirty μmol/kg (Fig. 2c) gave a significantly reduced activity after 4 days treatment. On the seventh day, the activity was about 35% of the first day.

The increased activity level after a dose of 40 μmol/kg morphine lasted for almost 3 h. We observed little or no changes in the response from day 1 to day 7. At no time during 7 days of morphine administration with 40 μmol/kg (Fig. 2d) did we observed a reduction in total distance traveled compared to the first day.

The time curve after 20 μmol/kg M6G administration (Fig. 2e) was similar to those observed for morphine, but the effect tended to last longer. The onset of tolerance was clearly observed on the second day, and the additional reduction from day 2 to 7 was small.

Crosstolerance Between Morphine and M6G

Crosstolerance to M6G was first measured after 1 day pretreatment with morphine (20 μmol/kg), while the crosstolerance to morphine was tested only after 1 day pretreatment with M6G (20 μmol/kg). The accumulated activities during 3 h for naive mice and mice pretreated for 1 day with either morphine or M6G are presented in Fig. 3. The locomotor activity responses (total distance) after 20 μmol/kg morphine and M6G were quite similar in naive mice. The decrease in locomotor activity after the second morphine administration was considerable ($p < 0.001$). Lack of crosstolerance towards M6G after morphine pretreatment was observed, as the mice administered M6G displayed the same response as drug naive mice (96%). Tolerance to M6G also developed to a significant degree upon the second administration ($p < 0.01$), 57% of the first day. Crosstolerance after M6G pretreatment to morphine was noted as we observed a significant ($p < 0.05$) decrease in locomotor activity (77% of naive morphine).

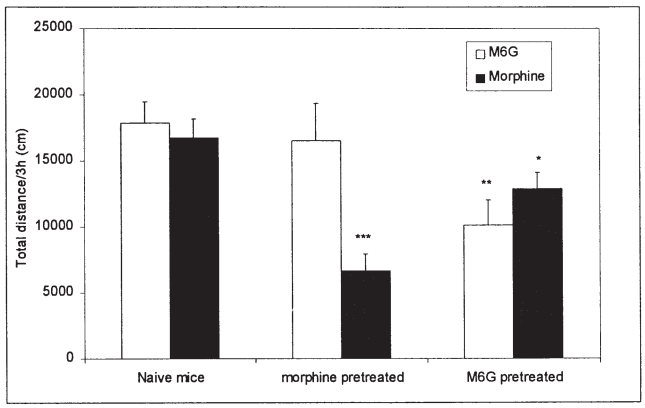

FIG. 3. Total distance traveled for naive mice and mice pretreated for 1 day with either morphine or M6G (20 μmol/kg). Each bar represents the mean ± SEM. The number of mice in the groups were: naive mice: > 20, morphine pretreated: 9–10, M6G pretreated: 12–18. Significant differences between pretreated mice and naive mice are indicated as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

We also wanted to study how prolonged morphine treatment would influence the crosstolerance towards M6G. The results observed after 7 days exposure to morphine pretreatment (20 μmol/kg) are shown in Fig. 4. Morphine pretreatment reduced the acute response in locomotor activity to 28% of the first day for both morphine and M6G demonstrating complete crosstolerance between the drugs.

DISCUSSION

We wanted to examine if there were any differences between morphine and M6G actions on the receptor level with respect to the locomotor activity by studying crosstolerance between the drugs. According to our knowledge, this has not been investigated previously.

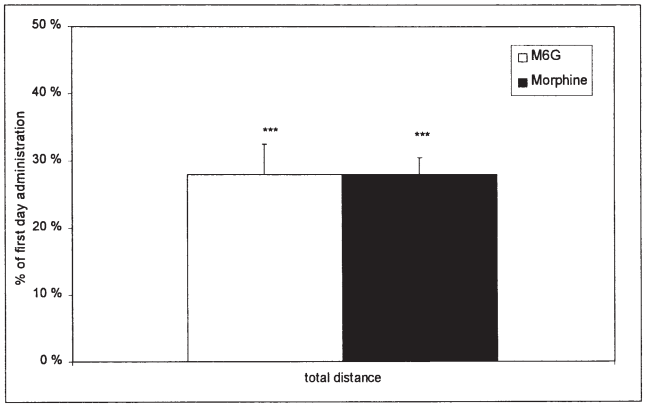

FIG. 4. Locomotor activity in mice pretreated for 7 days day with morphine (20 μmol/kg) in percent activity of the first day. Each bar represents the mean ± SEM. The number of mice in the groups were: morphine 8 and M6G 9. Significant differences between pretreated mice and naive mice are indicated as $***p < 0.001$.

In our study, we first observed a dose-dependent tolerance development after morphine treatment. Tolerance was not observed at the higher doses employed (40 μmol/kg). When the dose was reduced to 30 μmol/kg, a significant reduction in the activity was observed on the fourth day of administration. A dose of 20 μmol/kg gave rise to tolerance already on the second or third day of administration. Observation of tolerance after 10 μmol/kg was difficult to measure because the activity after this dose was relatively low, and a reduction of the activity to saline level was noted after 8 days administration. M6G (20 μmol/kg) gave a significant reduction in locomotor activity on the second day.

A large number of studies have been performed on tolerance to morphine. The majority of these studies were focused on the analgesic action of the opiates, and different species were employed. The degree of tolerance has been reported to depend both on the dosage levels and the interdose intervals (28).

Both tolerance and sensitization to the locomotor activity after repeated morphine administration has been reported (2,6,8,11,13,14,17). Kuribara et al. (13) reported that 26 μmol/kg (10 mg/kg) of morphine administered once daily produced significant sensitization on the third day of administration. On the other hand, Olivero and Castellano (17) observed a tolerance development to locomotor activity and analgesia after 2 days of repeated morphine administration twice a day (5,10, and 20 mg/kg). They reported that the tolerance development was different for various mice strains, and was most pronounced in the C57BL/6J strain for locomotor activity. To our knowledge, no studies have been preformed on tolerance to M6G and locomotor activity.

We examined the tolerance development to different doses of morphine to select the best dose for testing crosstolerance to M6G. According to Rossi et al. (23), it is important to utilize low doses to enhance receptor selectivity. The ability to distinguish between two drugs is in large part dependent on the dosing paradigm, i.e., low doses given only once a day greatly enhances the receptor selectivity of the tolerance paradigm permitting the detection of differences not typically observed in paradigms utilizing high drug doses (23). Therefore, 20 μmol/kg morphine seemed to represent the most suitable dose in our experimental design. In addition, 20 μmol/kg morphine gave rise to a rapid development of tolerance already after one or two injections of morphine. Tolerance to morphine after a single SC injection was also observed by Frigeni et al. (9).

Our crosstolerance studies had two different time paradigms. Crosstolerance was studied both after 1 day pretreatment and after 7 days pretreatment when maximal tolerance appeared to have developed. The group of mice that was tested for crosstolerance vs. M6G the second day, developed tolerance to 20 μmol/kg morphine already after one administration. Our results showed that when mice pretreated with morphine for 1 day were given M6G the next day, there was no crosstolerance between the two drugs. The morphine treated animals displayed the same locomotor activity response to M6G as the naive mice given M6G. The M6G tolerant mice, pretreated with M6G for 1 day, showed crosstolerance to morphine. The activity after morphine was, on the other hand, significantly higher than that observed for mice administered morphine on the second day of daily morphine administration. When the morphine pretreatment period was 7 days, we observed full crosstolerance between morphine and M6G.

Frances et al. (8) showed that both morphine and M6G administration induced tolerance to antinociception. The doses employed were high, 50–100 mg/kg (130–270 μmol/kg) twice daily. They observed crosstolerance between the two drugs at these drug levels. We employed much lower doses when testing for crosstolerance, and this may explain the difference between the observations.

In line with our results, Rossi et al. (23) demonstrated a lack of crosstolerance between morphine and M6G after single daily doses of morphine (5 mg/kg, SC = 13 μmol/kg) measuring antinociception by the tail-flick assay. The lack of crosstolerance also applied to heroin and 6-monoacetylmorphine while codeine was crosstolerant to morphine. Rossi et al. (23) pointed out that using relatively low doses given only once a day greatly enhances the receptor selectivity of the tolerance paradigm, permitting the detection of differences not typically observed in paradigms utilizing high drug doses, such as pelleting.

Binding studies of morphine and M6G show that affinity and efficacy to the μ-receptor of the two drugs are comparable (5,20,22). Detailed binding studies of M6G have revealed evidence for receptor heterogeneity, i.e., one lower affinity component corresponding to the traditional μ-receptor, and in addition, the presence of another component of low abundance with very high affinity. This high-affinity M6G-site has been shown by Brown et al. (4) to be selectively competed by 3-methoxynaltrexone. In vivo, 3-methoxynaltrexone selectively antagonized the analgesic action of M6G without interfering with morphine analgesia. Furthermore, antisense mapping studies of MOR-1 have provided strong evidence for distinct receptors (21,24–26). Our observation that after short-term influence of morphine demonstrating no crosstolerance to M6G also implies that the two drugs act on different receptors with regard to the locomotor activity.

A model to explain our observations of crosstolerance to the induction of locomotion could be the following: morphine mainly acts via the μ receptor, and only a small part of morphine's action is mediated via the M6G receptor. Pretreatment with morphine for only 1 day would, therefore, demonstrate the response mediated via the μ receptor. If we assume that a part of the effect caused by M6G was mediated through a specific M6G receptor, this could explain the lack of crosstolerance to M6G. When the pretreatment period with morphine was extended to 7 days, the M6G receptor could eventually have been affected by the repeated administration of morphine, and would explain our observation that extension of the pretreatment period lead to crosstolerance. M6G action, on the other hand, is partly mediated via both μ and M6G receptors. Pretreatment with M6G, which acts both on μ and M6G receptors would affect the response mediated via both receptors, and consequently be followed by partly crosstolerance to morphine.

Locomotor activity is enhanced after M6G-administration, which shows that also the M6G receptor possibly is linked to CNS stimulation and probably to euphoria. Some studies have reported that heroin in some species acts through the M6G receptor (23). Further work with the M6G receptor linked to heroin is important as the results could have wide implications for the knowledge concerning abuse of this drug.

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