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Effects of Pavlovian Conditioning and MIF-I on the Development of Morphine Tolerance in Rats[1]

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LAHOSTE, G. J., R. D. OLSON, G. A. OLSON AND A. J. KASTIN. *Effects of Pavlovian conditioning and MIF-I on the development of morphine tolerance in rats.* PHARMAC. BIOCHEM. BEHAV. 13(6)799–804, 1980.—Thirty male Sprague-Dawley-derived rats were given daily IP injections of morphine (5.0 mg/kg) in the presence of a specific set of environmental cues for eleven consecutive days. Twelve hours after each morphine session, a control injection was given in a different environment. On Day 12 through 14 the environmental cues associated with each session were reversed. On Day 15 environmental cues associated with each session were the same as on Days 1–11. Analgesia was assessed by the tail-flick method 30 minutes after each morphine and control injection. Four independent groups (n=6) received either a lower (0.1 mg/kg) or a higher (5.0 mg/kg) dose of MIF-I either 10 minutes before or immediately after each morphine and control session. A control group received an injection of a diluent vehicle both before and after each session. None of these peptide-treatments significantly affected either acute action of morphine or the development of tolerance across days. Tail-flick latencies from both morphine and control sessions significantly decreased across days. On Day 12, when morphine was administered in the presence of cues not previously associated with its administration, tail-flick latencies were significantly longer than on the previous day. Tail-flick latencies did not change from Day 11 to Day 15 during control sessions. Morphine-session latencies did not change from Day 14 to Day 15, although they did decrease from Day 12 to Day 14. The significant morphine-induced analgesia on Day 15 of the experiment increases a remarkable resistance to the development of tolerance to morphine. The results partially support the hypothesis proposed by Siegel [15–18] that principles of Pavlovian conditioning exert an important influence on the development of tolerance to morphine.

Pavlovian conditioning MIF-I Morphine tolerance

RECENT empirical evidence has revealed the importance of behavioral factors in the development of drug tolerance. For example, drug-induced behavioral effects may become attenuated over the course of repeated administration because the organism learns a behavioral strategy that compensates for drug-induced impairments [5]. The attenuation of effect is termed "behavioral tolerance." Studies of this phenomenon led to the finding that the environmental stimuli which are present when the animal is drugged may acquire properties that later influence the test for tolerance. It was shown, for example, that when experience was acquired in the test environment, even when no response was required, this led to a greater degree of tolerance when the animals were subsequently tested in that environment [1,6]. Although this effect has been referred to as behavioral tolerance, it is clearly different from the phenomenon which the term originally described because tolerance in this case cannot be attributed to acquired behavioral proficiency in coping with drug-induced impairments.

The distinction between these two behavioral phenomena is prominent in a recent hypothesis proposed by Siegel [15] which emphasizes the role of Pavlovian conditioning principles. This analysis of tolerance is based on the suggestion by Pavlov [13] that the administration of a drug constitutes a classical conditioning trial since the pharmacological stimulation (the unconditioned stimulus, UCS) is always preceded by a constellation of cues (the conditioned stimulus, CS) uniquely associated with the injection procedure. After a number of conditioning trials (i.e., drug administrations), the CS complex acquires the capacity to elicit a conditioned response (CR) which may be revealed by presenting the usual predrug cues not followed by the usual pharmacological stimulation but rather by a control. In this paradigm, however, it is a typical finding that the CR is opposite in direction to the usual pharmacological effect of the drug, the UCR [18]. This has led to the conceptualization that the measured effect of a drug is in fact the net result of two opposing forces: the stimulatory effect of the drug on the physiological

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systems of the organism, and the compensatory counteraction of the organism in response to this physiological assault. Therefore, when the CS is presented in the absence of the UCS, only the compensatory physiological response is observed. Siegel has proposed that tolerance to the analgesic effect of opiates is due to the attenuation of the pharmacological action of the drug by a compensatory conditioned response which is elicited with increasingly greater magnitude by the conditioned stimulus as the number of associations between the CS and the UCS increases [15].

Support for the role of conditioning processes in the development of tolerance also comes from several studies which show that various manipulations that are known to affect learning also affect tolerance. Cohen, Keats, Krivoy and Ungar [3] suggest that morphine tolerance may be a form of learning since metabolic inhibitors which impair learning also impair the acquisition of tolerance. Subsequent studies have shown that protein synthesis inhibitors [8] and electroconvulsive shock [9] attenuate the development of both conditioned responses and tolerance. This has led to a series of investigations which have attempted to assess the influence on tolerance of a number of neurohypophyseal principles and related brain chemicals that have been shown to affect learning. Krivoy, Zimmermann and Lande [10] were the first to show that a vasopressin analog facilitated the development of tolerance to morphine in mice. Van Ree and de Wied [20] extended these findings to rats using vasopressin analogs and oxytocin. Schmidt, Holaday, Loh and Way [14], however, were unsuccessful in a recent attempt to replicate those findings. Van Ree and de Wied [20] also reported that the most potent peptide in facilitating the development of tolerance was the C-terminal fragment of oxytocin, prolyl-leucyl-glycinamide, also known as MIF-I (for melanocyte stimulating hormone inhibiting factor-I). This facilitation of tolerance by MIF-I was confirmed in a recent paper which suggests that the neuropeptide exerts its influence on tolerance by depleting levels of melanocyte stimulating hormone (MSH) since MIF-I was effective only when administered one hour before morphine and since administration of α-MSH inhibited the development of tolerance [19]. Studies from another laboratory, however, suggest that MIF-I inhibits rather than facilitates the development of tolerance [2,21].

The present experiment was designed to bring the putative compensatory conditioned response (which is proposed to attenuate the pharmacological action of morphine, thereby producing tolerance) under a greater degree of stimulus control than has previously been demonstrated. This was to be achieved by presenting two distinct conditioned stimuli: one (CS+) was systematically associated with the presentation of the UCS morphine while the other (CS−) was systematically associated with the presentation of the diluent control. The compensatory CR should, therefore, come to be elicited only by the CS+ and not by the CS−. After a sufficient number of associations, the compensatory CR should be observable by presenting the CS+ in the absence of the UCS. Accordingly, the presentation of the UCS morphine in the presence of the CS− should reveal an analgesic response that has not been attenuated by the development of a compensatory hyperalgesic CR. Additionally, the continued presentation of the CS+ in the absence of the UCS should serve to extinguish the compensatory CR and the continued association of the CS− with morphine should result in the acquisition by the CS− of the capacity to elicit a compensatory CR. The acquisition and extinction of the CR could then be oberved, as the original acquisition of the CR was expected to be observed, by switching the substances (i.e., morphine or diluent control) with which the respective CS's had just previously been associated.

In addition to investigating the role of Pavlovian conditioning principles in the development of tolerance to the analgesic effect of morphine, this study was also concerned with the influence of MIF-I on the development of such tolerance and the possible involvement of this neuropeptide in conditioning processes. Administration of MIF-I before morphine allows for evaluation of its subsequent effects on the development of tolerance as well as its acute effects on morphine action, and any interaction with the conditioning factor would suggest that MIF-I affects the acquisition of a classically conditioned response. Administration of MIF-I after assessment of analgesia allows for the evaluation of the effects of daily administration of the neuropeptide on the development of tolerance independent of its acute effects on morphine action, and any interaction with the conditioning factor would imply that MIF-I affects the consolidation or memory component of a conditioning process.

METHOD

Animals

Thirty, experimentally naive, male, Sprague-Dawley-derived rats (obtained from King Laboratories, Oregon, WI, weighed 250–325 g at the beginning of the experiment. The animals were individually housed, maintained on a 12-hour light, 12-hour dark lighting schedule, and given free access to food and water from the time of their arrival in the laboratory (ten days before the beginning of the experiment) throughout the duration of the experiment.

Apparatus

The tail-flick apparatus consisted of a nichrome heating element, a photoelectric unit, and a digital electronic timer. A grooved tray was mounted over the heating element so that a rat's tail could be placed on the tray, covering a small opening, positioned about 4 cm posterior to the base of the tail, through which the heat from the wire radiated. Placed in this position, the rat's tail blocked a photobeam which ran perpendicular to, anterior to, and above the heating element. The electronic timer was activated by turning on the heating element and it was deactivated when the rat flicked its tail, thereby closing the photoelectric circuit and providing an accurate measure of the latency (to the nearest thousandth of a second) to tail-flick. An automatic cut-off time of ten seconds was used to prevent burning of the rat's tail.

In one CS environment, 17.8×17.8×39 cm wooden boxes, topless and painted white, and into which the rats were placed for the duration of a session, were located in a well-lighted, quiet room. The scent of cinnamon was permeated throughout the apparatus by suspending small cheesecloth sachets, filled with ground cinnamon, above each open box.

On the other CS environment, 17.8×12.8×17.8 cm metal baskets with grated tops, and into which the rats were placed for the duration of a session, were located in a darkened, white-noise-filled room. The scent of anise was permeated throughout the apparatus by suspending small cheese-cloth sachets, filled with whole anise seeds, above the grated tops of the baskets.

Drugs

MIF-I (prolyl-leucyl-glycinamide) was dissolved in a diluent solution of 0.9% saline made to 0.01 M with acetic acid. The concentrations of the MIF-I solutions were varied to provide two doses: 5.0 mg/kg and 0.1 mg/kg. All injections were given intraperitoneally (IP) in a volume of 1.0 μl/kg.

Procedure

All animals underwent 15 consecutive days of treatment and testing. On each day, each rat underwent two drug-test sessions. In one session, morphine (5.0 mg/kg) was administered and pain sensitivity was assessed 30 minutes later by the tail-flick method. In the other session, a control substance (1.0 μl/kg diluent) was administered and pain sensitivity was assessed 30 minutes later. For a random half of the subjects, morphine was always given in the morning and the control session was 12 hours later; for the other half, the order of the sessions was reversed. Thus, successive morphine sessions were 24 hours apart.

In addition to the drug (morphine or control) treatment, peptide treatment was given during each session. The 30 animals were randomly assigned to five independent groups (n=6) which differed from each other with respect to the peptide treatment they received (either 0.1 mg/kg MIF-I, 5.0 mg/kg MIF-I, or diluent) and the time that they received it (either 10 minutes predrug or immediately posttest). If an animal was to receive peptide before the drug treatment, then it was given a posttest injection of diluent; if it was to receive peptide posttest, then it was given a predrug injection of diluent. A control group was given diluent both predrug and posttest. The peptide treatment given to each of the five groups can, therefore, be summarized as follows: (1) predrug MIF-I (0.1 mg/kg); (2) posttest MIF-I (0.1 mg/kg); (3) predrug MIF-I (5.0 mg/kg); (4) posttest MIF-I (5.0 mg/kg); and (5) predrug and posttest diluent.

A session proceeded as follows: Each animal was taken in its home cage to one of two testing rooms containing a different CS and given the appropriate predrug injection. The rat was then placed in the CS apparatus for ten minutes. At this time, the animal was removed just long enough to be given the appropriate drug injection (either morphine or diluent, depending on the session) and then immediately returned to the apparatus. Thirty minutes after the injection, the animal was removed and placed on the tail-flick apparatus which was located in the same room. Tail-flick latency was assessed only once each session. After the tail-flick latency was determined, the animal was given the appropriate posttest injection, returned to its home cage, and taken back to the colony room.

On Day 1, half the animals were designated to receive morphine in the presence of one of the CS environments (CS+) and the other half were designated to receive morphine in the other CS environment. These contingencies remained unchanged from Day 1 through Day 11.

On Day 12, the CS-UCS contingencies were reversed and remained this way through Day 14. For this period, then, morphine was administered in the presence of the CS− and the control injection was made in the presence of the CS+. On Day 15, the CS-UCS contingencies were again reversed, returning them to the original contingencies of Day 1.

In addition to the original thirty animals, eighteen male, Sprague-Dawley-derived rats were tested once for morphine analgesia (nine animals in one CS environment, nine in the other) thirty minutes after morphine (5.0 mg/kg, IP) with no maximum cut-off on the tail-flick apparatus. The mean results from these animals are included at the top of Fig. 1 to give some indication to the extent to which imposing a ten-second maximum latency produces a ceiling effect.

RESULTS

The results were analyzed by ANOVA for each of the four parts of the experiment: Days 1–11, Days 11–12, Days 12–14, and Days 14–15. Three-factor mixed-design analyses of variance (peptide treatment-by-days-by-drug-session) showed that tail-flick latencies under the influence of morphine were significantly longer than latencies after control injections during all four phases of the experiment (Days 1–11, $F(1,25)=401.753$, $p<0.01$; Days 11–12, $F(1,25)=109.807$, $p<0.01$; Days 12–14, $F(1,25)=156.139$, $p<0.01$; Days 14–15, $F(1,25)=40.586$, $p<0.01$. However, the ten-second upper limit that was placed on the latencies produced a ceiling effect on the morphine scores (i.e., the distribution of these scores was negatively skewed) rendering analysis of the interaction between drug-session and days invalid. To assess the changes that occurred across days, separate two-factor analyses of variance with repeated measures on days were performed on the data obtained from the control and morphine sessions, respectively.

Latencies under the influence of morphine were significantly changed across days from Day 1 through Day 11, $F(10,250)=6.976$, $p<0.01$, with the morphine score on Day 11 being significantly lower than the morphine score on Day 1 (Duncan's New Multiple Range Test, $p<0.001$). Control latencies, however, also changed significantly across these days, $F(10,250)=10.411$, $p<0.01$, with the Day 11 score being significantly lower than the Day 1 score (Duncan's New Multiple Range Test, $p<0.005$).

On Day 12, when the morphine analgesia was measured in the presence of the CS− for the first time, the morphine latencies were significantly longer than the morphine latencies from Day 11, $F(1,25)=6.312$, $p<0.02$. For the control scores, there was no change from Day 11 to Day 12.

Morphine scores showed a significant decrease from Day 12 to Day 14, $F(2,50)=4.434$, $p<0.02$. Control scores remained unchanged on these days. Both morphine-session and control-session latencies were unchanged from Day 14 to Day 15, when the second reversal of the CS's occurred.

Peptide-treatment was not a significant factor in any analysis of variance. It did not exert a reliable main effect or interaction with either or both of the other factors.

DISCUSSION

The results lend support to the hypothesis that principles of Pavlovian conditioning play an important role in the development of analgesic tolerance to morphine. This support comes from the finding that on Day 12, when morphine-induced analgesia was assessed in the presence of environmental stimuli that had not previously been associated with the administration of morphine, a significantly less tolerant response was observed compared with the morphine-induced analgesic response of the previous day.

However, there were expectations based on Siegel's analysis of tolerance that were not observed in the present experiment. According to this explanation of tolerance, the suddenly restored potency of morphine observed when the

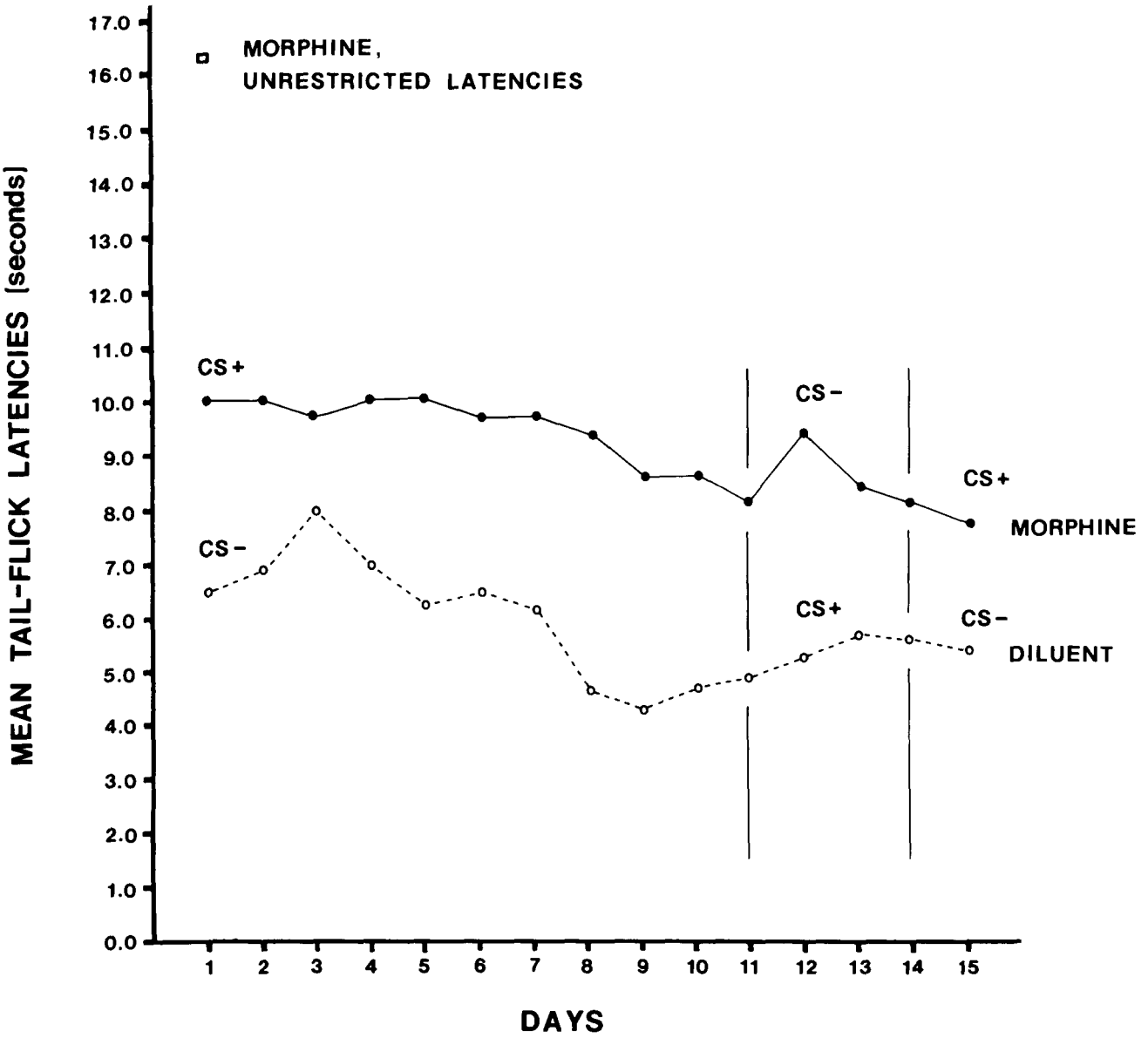

FIG. 1. Mean tail-flick latencies in seconds across days.

CS's were reversed is due to the absence of a conditioned hyperalgesic response. Yet, no such CR was observed when the CS which was assumed to elicit it was presented in the absence of the UCS. It is possible that this lack of hyperalgesia during the control session of Day 12 was due to the fact that control tail-flick scores are normally low. Siegel [15, 16, 18] measured analysis with the hot-plate method in which baseline latencies are considerably longer than those obtained with the tail-flick method. However, the control-session latencies in the present experiment were not unusually short. The putative compensatory CR was also not elicited by the CS on the last day of the experiment even though morphine-induced analgesia had significantly decreased during the previous three days in the presence of this CS. The fact that tolerance to morphine did not lessen again when the CS's were reversed once more on the last day is also inconsistent with the conditioning hypothesis although it could be argued that the CS presented with morphine on this day had already been associated with morphine eleven previous times, and that the period of three days of presenting this CS during the control session was insufficient for extinction of the proposed compensatory CR. It must be remembered, however, that no such CR was observed, rendering the question of its extinction moot.

Although Siegel has not observed practice effects in his experiments [15, 16, 18], such effects were clearly observed in the present experiment. This was evidence by the significant decrease in control-session tail-flick latencies that oc-

curred from Day 1 to Day 11. Although the tail-flick is assumed to be a reflexive response, the response apparently comes under some operant control with repeated exposure since removal of an aversive stimulus (i.e., termination of the heat source) is contingent upon the measured response. Gebhart, Sherman and Mitchell [6] have observed this practice effect but have also found that the decrease in baseline latency does not affect post-morphine changes in reaction time. This effect can, therefore, be controlled by observing postdrug change with respect to predrug baseline. In the present experiment, however, no predrug latency was measured in order that pretest environmental stimuli which were not specific to each CS situation could be minimized. Instead, it was hoped that the morphine-session latencies could be compared directly to the control-session latencies. Because of the unexpected high number of maximum latencies obtained in the morphine sessions, a resulting ceiling effect prevented such comparisons from being made validly. Therefore, it cannot be stated with certainty that tolerance, independent of the decrease in latencies due to the practice effect, did indeed occur during the first eleven days of the experiment.

The above considerations, raise the possibility for other explanations of drug tolerance. For example, if the decreasing response latencies represent improvement in learning the escape task, the increased latencies observed when morphine was given in the presence of the alternate CS can be explained as the dissociation of learning that has been termed "state-dependent learning" [12], although the term is usually reserved for those drug-state effects in operant conditioning procedures. If this were the case, however, one would have expected a comparable decrement of learning during the control session of Day 12. Many narcotic drugs, including morphine, are known to produce discriminable drug states (for review, see [4]). Although state-dependency of operant-type learning does not bear directly on the phenomena that Siegel proposes to account for tolerance, his analysis acknowledges a crucial role for state-dependency of Pavlovian-type learning.

The morphine-induced analgesia observed throughout the course of the present experiment was remarkably resistant to tolerance. After fifteen days of administration of a moderate dose of morphine, the drug still produced analgesic responses that were significantly different from control responses. In four experiments reported by Siegel [15,16] using hot-plate and paw-pressure methods, complete tolerance (i.e., morphine scores no different from control scores) was observed by no later than the fifth injection of the same dose of morphine as was used in the present experiment. The strong resistance to tolerance observed in the present experiment is probably due to the partial reinforcement effect which Siegel [17] has shown to retard the development of tolerance to morphine although not to as great a degree as shown here. In the present experiment, the different CS's were designed to be easily distinguishable from each other. However, by their design, the CS's were more similar to each other than either was to the home environment. Thus, there were many predrug cues, such as removal from the colony room, transportation down a corridor, insertion of a needle, and placement in a small container, which reliably preceded a drug-test session but which were not specific to a particular CS environment. If these non-specific predrug cues were more readily discriminable from the home-cage cues than the specific cues were from each other, then this would present the situation in which a CS (the non-specific cues) is followed by a UCS (morphine) fifty percent of the time. This partial reinforcement effect, which is known to inhibit the acquisition of CR's, may have inhibited the acquisition of the compensatory CR that Siegel proposes to explain tolerance. Thus, partial reinforcement may be of great importance in preventing the development of tolerance to the analgesic effect of morphine.

The present experimental results do not support previous findings that MIF-I influences the development of tolerance or dependence. This may have been due largely to procedural differences. The design of the present experiment required manipulations of conditioning factors that are not involved in traditional studies of tolerance and dependence. In van Ree and de Wied's work [20], in which MIF-I was shown to facilitate the development of dependence on morphine, large daily doses of morphine were given and on the test day, withdrawal was precipitated by injection of naloxone, an opiate antagonist. Two other studies in which MIF-I attenuated the development of tolerance [2] or dependence [21] used implantation of morphine pellets. Since the presentation of temporally discrete environmental stimuli are apparently very important in conditioning procedures and perhaps in the development of tolerance, the pellet-implantation method in which morphine is released continuously, seems to be a significantly different procedure from the method whereby small to moderate doses of a drug are given in separate daily injections. In fact, it has recently been shown that tolerance and cross-tolerance to opiates may be dependent on the method used to induce tolerance [11]. A recent study that is reasonably similar to the present experiment showed that pretreating rats with 1.0 μg/kg of MIF-I one hour before morphine reduced the analgesic potency of morphine on a subsequent test day when compared to animals given the same dose of MIF-I just before morphine [19]. When MIF-I was given just before morphine, however, this did not affect the subsequent potency of morphine as compared to animals pretreated with saline. The failure of MIF-I to influence tolerance development when given ten minutes before morphine in the present experiment is similar to these results. It is also possible that the facilitation of tolerance-development when MIF-I was given one hour before morphine is not due to a specific effect of the neuropeptide but rather due to the fact that this first injection served as a CS, thereby facilitating the acquisition of a compensatory CR.

Thus, the reported results are not easily explained by traditional pharmacological interpretations of morphine tolerance. They imply that conditioning processes apparently influence the development of tolerance to the analgesic effect of morphine. The interspersing of control injections between successive morphine injections severely retarded the development of tolerance to morphine over the course of the fifteen administrations. This suggests that these conditioning factors may be manipulated in such a way as to provide potent analgesia without the rapid development of tolerance, a finding which may have clinical relevance.

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