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BRIEF COMMUNICATION

Morphine as a Cue in Associative Tolerance to Morphine's Analgesic Effects

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CEPEDA-BENITO, A. AND S. T. TIFFANY. *Morphine as a cue in associative tolerance to morphine's analgesic effects.* PHARMACOL BIOCHEM BEHAV **46**(1) 149–152, 1993.—This study examined the extent to which low doses of morphine paired explicitly with high doses would gain associative control over tolerance development in rats. Tolerance development was assessed by evaluating dose-response curves to the analgesic effects of morphine on the tail-flick test. The results indicated that tolerance development was not influenced by the pairing of low doses with high doses.

Conditioning Tolerance Morphine Tail-flick Dose–response curves Interoceptive cues

IT is widely accepted that tolerance to morphine can readily come under the associative control of exteroceptive stimuli that are paired with morphine administration (1,9). For example, research from our laboratory (2,17,18,19) established that distinctive environments reliably paired with high doses of morphine can become conditioned stimuli (CSs) that elicit robust and long-lasting associative tolerance effects.

There is also evidence that interoceptive stimuli may serve as effective CSs in the development of associative tolerance. It has been demonstrated that magnetic fields can act as CSs for the acquisition of associative tolerance to morphine's analgesic effects (12). Others have shown that pharmacologically generated cues can subserve associative tolerance (10,13). Greeley et al. (10) reported that a low dose of ethanol that reliably preceded a high dose of ethanol could become a CS for the production of associative tolerance to ethanol's hypothermic effect. Findings such as these lend some support to the suggestion that, in the absence of exteroceptive stimuli predictive of drug delivery, initial interoceptive effects of a drug dose may provide adequate cues for the development of associative tolerance to the drug (13,20).

Although morphine has been shown to be a highly effective discriminative stimulus in the control of operant responding (5), there has been no research examining the effectiveness of morphine as a pharmacological cue in the development of associative morphine tolerance. This experiment was designed to determine whether a low dose of morphine could acquire associative control over the development of tolerance to a high dose of morphine. The design of the study was similar to that of Greeley et al. (10) with two major additions. First, tolerance magnitude was assessed by shifts in dose-response curves. This conforms to pharmacological definitions of drug tolerance (7,8,11) and provides a sensitive and precise index of tolerance magnitude not available through the use of single test doses (17,19). Second, the effects of two different levels of cuing doses were evaluated to explore a wider range of conditions for the potential generation of pharmacological cuing effects.

METHOD

Subjects

Subjects were 315 male Holtzman rats, 100–104 days old on their tolerance testing day. Animals were housed individually in cages located in a colony room (average temperature of 22°C) under a 12 L : 12 D cycle with lights turned on at 0630 h. Animals were given ad lib access to food and water throughout the experiment.

Drugs and Analgesia Assessment

The two low doses of morphine sulfate (expressed as the salt) used as cues for the delivery of a 20-mg/kg dose of morphine were 1.25 and 2.5 mg/kg. Tolerance test doses were 1.25, 2.5, 5, 10, 20, and 40 mg/kg. All morphine was dissolved in saline such that the salinity of the solution was isotonic with physiological saline. Solutions of morphine and saline were injected IP in a volume of 1.25 ml/kg. Analgesia was

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assessed by the tail-flick method, and tolerance was indexed as the shift to the right of the dose-response curve of drug-naive animals. The tail-flick test measured the latency for the rat to remove its tail from a hot beam of light. The intensity of the light was set so that nondrugged animals flicked at approximately 3.5 s and a 15-s cutoff was used to prevent damage to the rat's tail. An assessment consisted of the average of three consecutive trials. The procedures for tail-flick testing were the same as those described in Tiffany et al. (18). During exposure to the tail-flick, the animal was held gently by the experimenter with its tail placed in a grooved acrylic plate. All tail-flick exposures were conducted in a hallway adjacent to the colony room.

Habituation, Tolerance Development Phase, and Testing

To habituate animals, all subjects were weighed once daily for 3 days, then weighed twice daily for 3 additional days, followed by 8 days of weighing and saline injections twice each day. During the last 8 days of habituation, animals were also given daily exposure to mock tail-flick procedures. These consisted of placing each animal on the tail-flick device and activating the lamp for three consecutive 15-s trials with the animal's tail placed to the side of the light beam.

During conditioning, subjects were randomly assigned to eight groups. Animals in the explicitly paired (EP) groups (EP-1.25 and EP-2.5) received the low dose of morphine (either 1.25 or 2.5 mg/kg) exactly 60 min before the high dose (20 mg/kg). Animals in the explicitly unpaired (EU) groups (EU-1.25 and EU-2.5) received the low and high doses on separate days. Four additional control groups consisted of a group that received a saline injection 60 min before the 20-mg/kg morphine dose (EP-sal) and three groups for which both of the paired injections were saline (SCs). The EP and SC groups received 14 conditioning trials randomly distributed over a 28-day period. The average intertrial interval (ITI) was 48 h (range of 24–84 h). The EU groups received the 20-mg/kg morphine dose following the same schedule used for EP animals. However, the interval between the low and high doses for the EU groups varied randomly with an average of 28 h (range of 16–66 h). To reduce the salience of the handling and injection procedures [see (2,6)], all groups also received 28 additional saline injections throughout the tolerance development phase. These injections were randomly distributed with two restrictions: a) Animals did not receive more than three injections in the same day; and b) the interval between morphine and extra saline injections was at least 3 h. Animals in all groups also received a daily, mock tail-flick exposure at a time randomized across days, with the exception that this never occurred between two paired injections. All procedures took place between 0700 and 1800 h.

Tolerance Test Session

The tolerance test session took place on the second day after the last conditioning trial. Each animal received a pair of injections separated by exactly 60 min and was tested on the tail-flick 30 min after the second injection. The first injection of morphine was the same dose as the low dose each rat had received during conditioning. For animals in the EP-sal group, the first injection was of saline. For animals in the three SC groups, the first of the paired injections consisted of saline or 1.25 or 2.5 mg/kg morphine. The second injection given to all animals consisted of one of four doses of morphine for the construction of dose-response curves.

Data Analysis

Multiple regression analyses (4) were performed on the tail-flick data to compare the log dose-response curves of particular groups and group combinations using regression procedures described in Tiffany et al. (19). Parallelism of dose-response curves was evaluated by examination of the interaction of variables representing group condition comparisons and dose level. The α level for all tests was set at 0.05.

RESULTS

The average tail-flick latencies for each test dose of the treatment groups are shown in Fig. 1. The straight lines for each condition represent the best-fitting line calculated with tail-flick latency regressed on log dose of morphine. Table 1 summarizes the statistical analysis for the group comparisons that were conducted. There were no differences between the explicitly paired and unpaired animals at either of the two low-dose conditions. On the other hand, the dose-response curves of the groups that received morphine during conditioning were shifted to the right of saline control animals (see Fig. 1; Table 1). Neither of the two low doses of morphine that were injected 60 min before injecting each test dose had an impact on the analgesia assessment conducted 30 min after injecting the test dose, that is, there were no differences between the three SC groups (see Table 1). All dose-response curves were parallel with the exception of the dose-response curve yielded by the combined data of animals that received the cuing dose of 2.5 mg/kg morphine during conditioning and test day and the curve of SC animals that received the cuing dose of 2.5 mg/kg morphine on test day [i.e., the interaction between the group and dose level variables for this comparison was statistically significant; EP-2.5 and EU-2.5 vs. SC-2.5, $sR^2 = 0.025, F(1, 116) = 6.05, p < 0.05$].

DISCUSSION

All groups exposed to the high dose across conditioning developed tolerance to morphine that was unaffected by the explicit pairing of a low dose with the high dose, that is, there were no significant differences among the dose-response curves of any animals that had been repeatedly exposed to 20 mg/kg morphine during conditioning. Thus, these data provided no evidence that a dose of 1.25 or 2.5 mg/kg morphine repeatedly paired with a high dose of morphine acquired associative control over tolerance. The failure to find such evidence cannot be attributed to inadequate power or an insensitivity of the assessment procedure as we were able to detect tolerance in all groups that were exposed to morphine during the conditioning phase. Moreover, the absence of a drug-cuing effect is not likely due to an insensitivity of the tail-flick assay for demonstrating conditioned tolerance. Several studies from our laboratory clearly established that the tail-flick assay provides a robust assessment of associative tolerance phenomena (2,17,18,19). It is also unlikely that the levels of the cuing doses were too low to produce salient pharmacological effects. Previous research from our laboratory has shown consistently that both 1.25 and 2.5 mg/kg morphine produce significant analgesia on the tail-flick assay. In addition, studies of the efficacy of morphine as a discrimina-

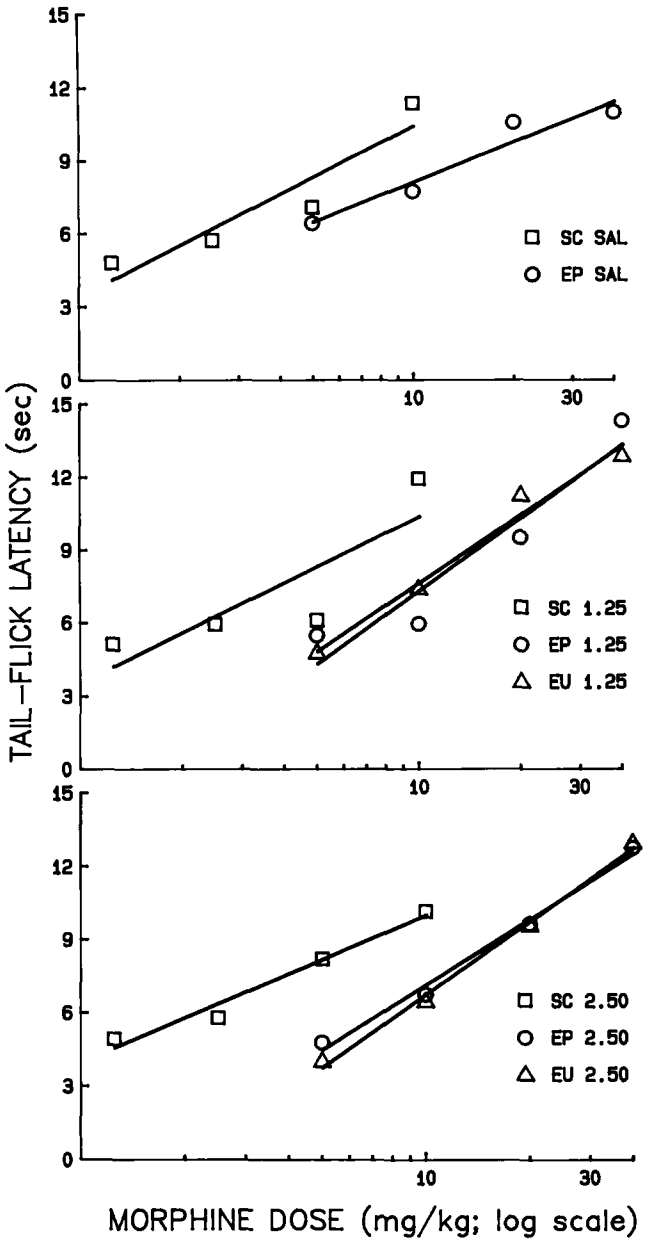

FIG. 1. Mean tail-flick latency on the test session as a function of morphine dose for each of the treatment conditions. The straight lines represent the best-fitting lines for each condition with tail-flick latency regressed on a log scale of morphine dose. Each data point represents the average of a minimum of nine subjects. SC, saline control; EP, explicitly paired; EU, explicitly unpaired; SAL, saline; 1.25, 1.25 mg/kg morphine sulfate; 2.5, 2.5 mg/kg morphine sulfate.

tive stimulus indicate that doses as low as 1 mg/kg can acquire discriminative control over operant responding (15). Overall, our data suggest that the potential associative value of pharmacological stimuli within this tolerance induction paradigm is relatively weak.

It is likely that the tolerance obtained in this study was a classically conditioned form of tolerance. Handling and injection cues that necessarily accompany morphine administration are capable of supporting associative tolerance even with extensive nonreinforced exposure to injection and handling cues prior to conditioning (2,19). Other research from our laboratory (18) indicates that the morphine-exposure procedures used in this study (i.e., a 20-mg/kg dose administered at an average interdose interval of 24 h) are not conducive to the development of nonassociative tolerance. Further, a 30-day retention test conducted on animals from this study revealed essentially the same pattern of results described for the immediate test. Long-term retention of tolerance is characteristic of associative, not nonassociative, forms of tolerance (17). To the extent that handling and injection cues were supporting conditioned tolerance in this study, it is possible that these stimuli overshadowed any associative influence of pharmacological stimuli.

There are only two examples in the animal learning literature of the same unconditioned stimulus (US) functioning as both the CS and US within a classical conditioning paradigm. One is the study by Greeley et al. (10) and the other used paraorbital electrical stimulation as both the CS and US for the classical conditioning of the rabbit's nictitating membrane response (14). While both these experiments indicated that animals might be capable of forming an association between the same US-US pairing, our results suggest that this effect might be specific to the nature of the US used and the conditioned response (CR) that is measured. Moreover, other dimensions such as the intensity of cuing US, the US-US interstimuli presentation, and the ITI may affect the strength of the US-US association. Therefore, although our results produced no evidence that a low and a high dose of morphine can function, respectively, as the CS and US for the development of associative tolerance to that drug, further research needs to be done to study this issue.

Finally, it should be noted that rats might be able to use the initial effects of a high dose of morphine as interoceptive CSs announcing the peak effects of that same dose. If so, explicitly unpairing a low dose of morphine with a high dose may not prevent EU animals from developing a form of morphine tolerance that is associatively supported by the drug's own interoceptive stimuli [cf. (10)]. Therefore, with these procedures there are two sets of cues that might support associative tolerance, either handling and injection stimuli or the initial interoceptive effects of the drug. The specific contribu-

TABLE 1
BETWEEN-GROUP COMPARISONS

Groups Compared	Statistical Analysis
EP-1.25 vs. EU-1.25	$sR^2 = 0.002, F(1, 74) = 0.37$
EP-2.5 vs. EU-2.5	$sR^2 = 0.001, F(1, 77) = 0.17$
EP-sal vs. SC-sal	$sR^2 = 0.047, F(1, 75) = 5.92$*
EP-1.25 & EU-1.25 vs. SC-1.25	$sR^2 = 0.094, F(1, 114) = 23.78$†
EP-1.25 & EU-2.5 vs. SC-2.5	$sR^2 = 0.130, F(1, 117) = 30.39$†
SC-1.25 vs. SC-2.5	$sR^2 = 0.000, F(1, 77) = 0.03$
SC-1.25 & SC-2.5 vs. SC-sal	$sR^2 = 0.001, F(1, 177) = 0.02$

sR^2 = increase in the square of the multiple correlation coefficient added by the variable group condition. Groups connected by "&" indicate that the data were combined for the two groups.
*$p < 0.05$.
†$p < 0.01$.

tion of injection stimuli to the tolerance obtained within this paradigm could be estimated by determining the extent to which multiple, nonreinforced exposures to the injection ritual over a retention interval extinguishes tolerance (3). If tolerance is not affected by these extinction manipulations, then it may be that interoceptive cues are responsible for the tolerance phenomenon.

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