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# Morphine Preexposure Attenuates the Aversive Properties of Opiates Without Preexposure to the Aversive Properties

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MARTIN, G. M., A. BECHARA AND D. VAN DER KOOY. *Morphine preexposure attenuates the aversive properties of opiates without preexposure to the aversive properties.* PHARMACOL BIOCHEM BEHAV 30(3)687–692, 1988.—Evidence that action on peripheral opiate receptors is necessary to produce aversive effects with morphine, enabled us to determine whether preexposure to these aversive effects is necessary for the later attenuation of morphine's aversive properties. We found that blockade of the aversive effects of morphine with the peripheral antagonist methylnaltrexone during morphine preexposure had no effect on the later attenuated development of conditioned taste aversions to morphine. Moreover, in the same rat morphine preexposure did not affect the development of a place preference to an environment paired with injections of morphine. The results suggest that an effect of central opiate action is able to attenuate the later peripheral aversive, but not the central rewarding, effects of morphine.

Morphine Methylnaltrexone Preexposure effects Conditioned taste aversion Place conditioning

OPIATES can serve as positive reinforcers in self-administration and place conditioning experiments and can serve as positive punishers when they follow consumption of novel flavors [1, 11, 22]. Preexposing animals to morphine reduces the capacity of morphine to produce a conditioned taste aversion (CTA) [10, 11, 13, 24]. Pharmacological tolerance to morphine does not explain this preexposure effect since preexposure to other drugs, which do not produce flavor aversions through the same physiological system as morphine, also attenuates the capacity of morphine to produce a CTA [1, 4, 26]. Moreover, parametric manipulations of morphine preexposure have not supported a pharmacological tolerance explanation for the attenuation of morphine's aversiveness [9]. Associative blocking effects which successfully account for preexposure effects with other drugs [6, 7, 12] do not explain the opiate phenomenon. Preexposure to morphine does not result in an association between environmental cues and morphine, which with other drugs blocks the association between a novel flavor and the drug [11,24].

The absence of a clear mechanism underlying the attenuating effects of morphine preexposure indicates the importance of establishing which feature of the drug is responsible for the inhibitory effects of preexposure. Peripheral opiate receptors are the primary site where opiates act to produce aversive effects, whereas central opiate receptors are the primary site mediating the positive reinforcing effects [1,4]. This anatomical separation of the two opposite opiate motivational effects allows us to determine if preexposure to morphine in the presence of its rewarding effects, yet in the absence of its aversive effects, will still attenuate the later development of taste aversions produced by morphine. We now report that blockade of peripheral opiate aversive effects with the peripheral antagonist methylnaltrexone [8] has no effect on the later attenuation of the development of conditioned morphine taste aversions. Thus, experience with the aversive effects of opiates is not necessary for the attenuation of opiate aversive effects.

## EXPERIMENT 1

Previous evidence has shown that vagotomy abolishes the

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aversive effects of morphine in the CTA paradigm, without affecting its positive reinforcing effects in the place conditioning paradigm, suggesting an independent peripheral mechanism mediating opiate aversive effects [1]. Furthermore, intraperitoneal (IP) administration of a low 0.1 mg/kg dose of naltrexone or 1 mg/kg of its quaternary derivative methylnaltrexone, which does not cross the blood-brain barrier effectively [8], attenuate morphine CTA by local action in the gut, but do not affect morphine's positive reinforcing effects [1,4]. Therefore, we hypothesized that pretreating animals with 1 mg/kg of methylnaltrexone IP during the preexposure phase to morphine should prevent access of morphine to the putative peripheral receptor substrate underlying morphine CTA, yet, morphine could still activate the central receptor substrate subserving rewarding opiate effects.

Tests were made of the ability of morphine or vehicle preexposure to attenuate the later development of opiate aversive and positive reinforcing effects. The same rats were examined for opiate aversive effects using the CTA paradigm and for opiate rewarding effects using the conditioned place preference paradigm. Separate groups of rats were pretreated with methylnaltrexone or saline during morphine preexposure to test the importance of experience with opiate aversive properties in any preexposure effects. The experiment did not include a separate group which tested whether methylnaltrexone itself has any motivational effects in the CTA paradigm, since previous data have shown that methylnaltrexone itself does not produce a CTA [4].

## SUBJECTS

All animals used in these experiments were male Sprague-Dawley rats, obtained from Canadian Breeding Laboratories. Animals were given one week habituation to the laboratory prior to the start of an experiment. Throughout the experiments the animals had access to food ad lib and were allowed 15 min access to tap water per day unless otherwise stated.

## METHOD

### *Preexposure Phase*

Thirty-six rats served as subjects for this experiment. Animals were assigned to one of three group during this phase. Nine animals, the methylnaltrexone-morphine group (MN-M), were injected with 1 mg/kg of methylnaltrexone IP 15 min prior to a subcutaneous (SC) injection of 5 mg/kg of morphine in saline. Nine animals, the vehicle-morphine group (V-M), were injected IP with saline followed 15 min later by a SC injection of 5 mg/kg of morphine. The remaining 18 animals, the vehicle-vehicle (V-V) group, were injected IP with saline followed by 15 min later by a SC injection of saline. Each pair of injections was repeated four times over the course of eight days.

### *Training Phase*

A novel flavor and a novel place were both paired with injections of morphine in all subjects. Tastes were presented before and places were presented after morphine injections. The place conditioning procedure was identical to that previously described [19]. Briefly, all animals were given 5 min exposure to a grey box prior to the start of training. During training animals were exposed to two square boxes that differed in color, texture, and smell: black walls, smoother Plexiglas floors and smell of 2% glacial acetic acid versus white walls, wood chip floors, and smell of wood.

The animals in the MN-M, the V-M, and nine animals from the V-V groups had 0.1% saccharin substituted for tap water and were injected with morphine immediately after saccharin removal. Five animals from each of these groups were placed in white boxes for 30 min after the morphine injections, and the remaining four animals in each group were placed in black boxes after the morphine injections. On alternative days these animals were given tap water and were injected SC with saline prior to being placed in the box opposite to the one they had been placed in on morphine days. This procedure was repeated once (for a total of two drug pairings). The remaining nine animals in the V-V group were treated identically to the other groups with the exception that all SC injections (prior to both boxes) were with the saline vehicle.

### *Test Phase*

All animals were given a 15 min choice between saccharin and tap water on two occasions. Following the flavor tests the animals were tested for their place preferences. Testing was carried out in a large rectangular box with the two training environments on opposite sides, separated by a grey area with a grid floor. Rats were placed in the grey area and the amounts of time (in sec) spent in the two treatment environments over the next 10 min were measured.

## RESULTS AND DISCUSSION

### *Taste Aversion Conditioning*

The saccharin and tap water scores of the four groups during testing were converted to saccharin preference ratios (saccharin/saccharin + tap water). A 4×2 ANOVA (Groups ×Tests) revealed a significant group effect, $F(3,32)=8.85$, $p<0.01$, and no other reliable differences. The group means were collapsed over the two days and are shown on the left side of Fig. 1. Newman-Keuls tests showed that all three groups that had morphine paired with saccharin consumption MN-M/M, V-M/M, and V-V/M) had a saccharin aversion relative to the group that had saccharin paired with an injection of saline (V-V/V) ($p$'s$<0.01$). Newman-Keuls tests also revealed that animals that were preexposed to morphine (MN-M/M and V-M/M) had reliably weaker aversions than the animals that were not preexposed to morphine (V-V/M) ($p$'s$<0.01$). The two preexposure groups (MN-M/M and V-M/M) did not differ reliably ($p>0.05$). These findings indicate that preexposure to morphine reduced the capacity of morphine to produce a flavor aversion. Moreover, the peripheral aversive effects of morphine did not have to be present during the preexposure phase in order to achieve attenuated CTA.

It should be noted that although methylnaltrexone blocks morphine-induced taste aversions ([4], Experiment 2) pretreatment with methylnaltrexone does not unequivocally rule out the possibility that at least some aversive effects of morphine were expressed during preexposure to morphine. If the antagonist methylnaltrexone had a shorter half life than the agonist morphine, then some delayed aversive effects may still have been experienced, although the delay between the taste and aversive effects would have mitigated against conditioning. However, pharmacokinetic as well as pharmacodynamic evidence from morphine [16,17] and naltrexone [5, 18, 27] indicate that the antagonistic effects of

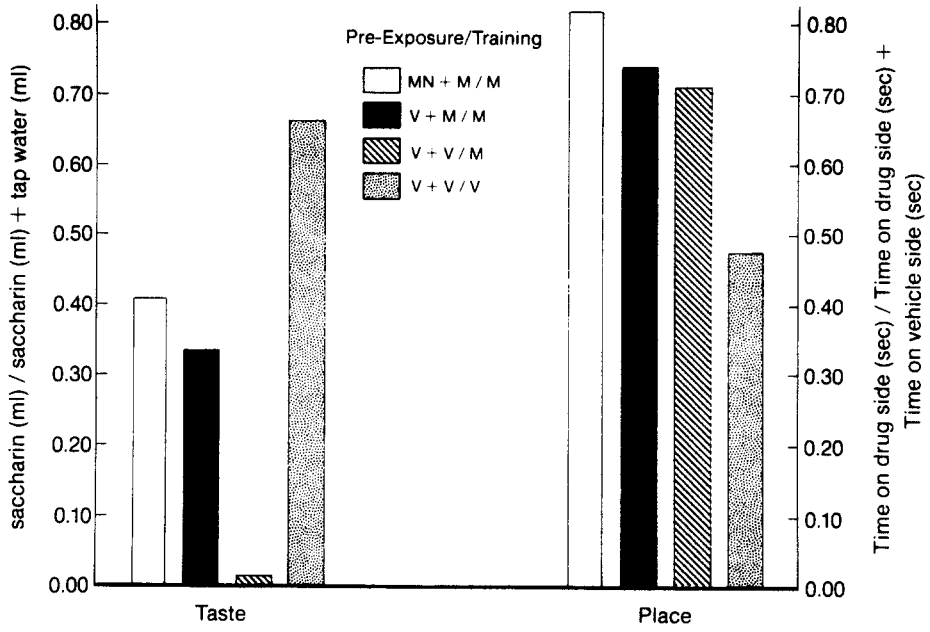


FIG. 1. Left side represents saccharin preference ratios and right side represents place preference ratios. The MN-M/M group was preexposed to morphine in the presence of methylnaltrexone, the V-M/M group was preexposed to morphine in the presence of saline, and the V-V/M group was preexposed to the vehicle for both drugs. All three groups had saccharin consumption and a novel place paired with an injection of morphine (M). The V-V/V group received only injections of the vehicle during preexposure and during place and flavor conditioning.

naltrexone far outlast the agonistic effects of morphine. Although methylnaltrexone and naltrexone do differ in terms of potency and site of action [8], there is no evidence that the two drugs differ significantly in terms of duration of action or elimination kinetics. Thus, it seems unlikely that any aversive effects of morphine escaped methylnaltrexone blockade during the preexposure phase.

*Place Conditioning*

The amount of time spent on the drug side was also converted to a preference ratio (time on drug side/time on drug side plus time on saline side). The V-V/V group did not have a drug side. Consequently their preference ratios represent time on first side they were exposed/time on first side plus time on second side. The preference ratios are shown on the right side of Fig. 1. A one-way ANOVA revealed that the four groups did differ, $F(3,24)=5.19$, $p<0.01$. Newman-Keuls tests revealed that the three drug groups (the MN-M/M, V-M/M, and V-V/M groups) had a preference for the side paired with the drug relative to the V-V/V group ($p$'s$<0.05$). No other differences were reliable, $p$'s$>0.05$. These data indicate that preexposing animals to morphine, with or without methylnaltrexone, did not affect the capacity of morphine to produce place preferences.

## EXPERIMENT 2

We argued on the basis of earlier findings that methylnaltrexone blocked the aversive effects of morphine [4]. However, the dose of morphine, route of administration, and number of drug pairings previously used [4], differed from those used in Experiment 1. Therefore, the present experiment further characterized the methylnaltrexone blockade of morphine's aversive effects, by investigating if 1 mg/kg of methylnaltrexone IP would prevent morphine (at the same dose, route of administration, and number of drug pairings used in Experiment 1) from producing a flavor aversion.

### METHOD

*Training Phase*

Twenty-seven rats served as subjects for this experiment. Saccharin was substituted for tap water once every three days on five occasions after the rats had habituated to the deprivation regime. After saccharin removal, 9 animals, the methylnaltrexone-morphine (MN-M) group, were injected IP with 1.0 mg/kg of methylnaltrexone followed 15 min later by a SC injection of 5 mg/kg of morphine. Another 9 animals, the vehicle-morphine (V-M) group, were injected IP with an equivalent volume of saline followed 15 min later by a SC injection of 5 mg/kg morphine. The remaining 9 animals, the vehicle-vehicle (V-V) group, had saline substituted for both the IP and SC injections.

*Testing Phase*

Animals were given a 15 min choice between saccharin and tap water three days after the fifth set of injections.

### RESULTS AND DISCUSSION

The saccharin and tap water scores were converted to

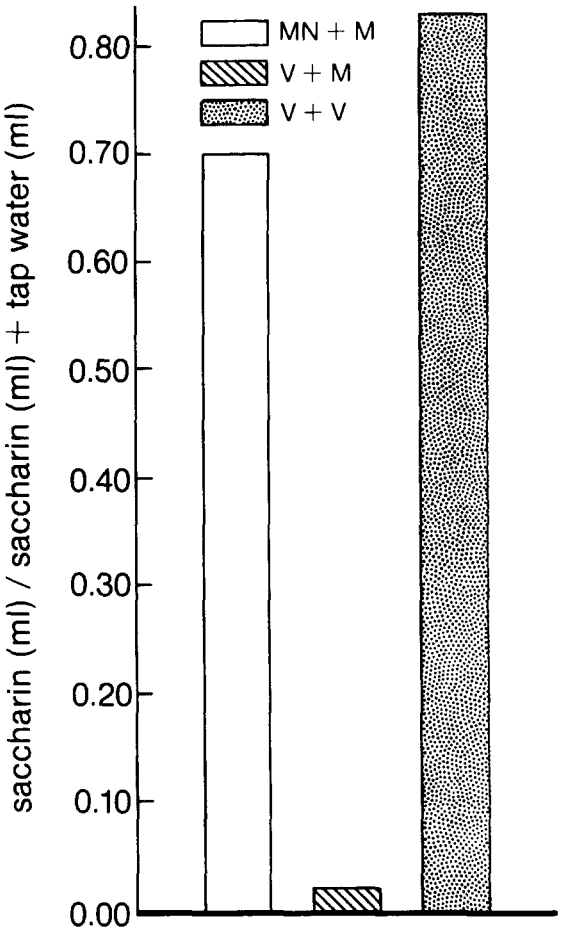


FIG. 2. Saccharin preference ratios for animals that were injected with methylnaltrexone and morphine (MN-M group), morphine and methylnaltrexone vehicle (M-V group), or with both vehicles (V-V group).

saccharin preference ratios (saccharin/saccharin plus tap water) and are presented in Fig. 2. A one-way ANOVA on the saccharin preference scores revealed that the three groups differed reliably, $F(2,24)=24.69$, $p<0.01$. Newman-Keuls tests revealed that both the V-V and MN-M groups has reliably higher saccharin preference ratios than the V-M group ($p$'s$<0.01$). No other differences were significant. These findings indicate that methylnaltrexone prevented the morphine from producing a saccharin aversion.

## GENERAL DISCUSSION

We found that we could reduce morphine's capacity to produce a conditioned taste aversion when we preexposed animals to the effects of morphine in the presence or absence of morphine's peripheral aversive effects. This preexposure did not affect morphine's positive reinforcing effects as measured by conditioned place preferences. We also replicated previous findings [1,4] showing that the central effects of morphine, in the absence of peripheral effects, do not produce conditioned taste aversions.

The preexposure effect obtained with morphine, when methylnaltrexone is present during preexposure, suggests that exposure to the central effects of morphine initiates a process either centrally or peripherally that ameliorates the aversive properties of morphine. At present, no good candidates (either associative or unconditional, which may be produced during morphine preexposure) exist that might explain the source of the attenuation observed. One class of associative explanations of the preexposure effect would suggest that some environmental or drug produced stimulus was associated with the aversive properties of morphine during preexposure and that this association blocked the association between saccharin and the aversive aspects of morphine. This explanation seem unlikely since we demonstrated a block of aversive effects of morphine with methylnaltrexone in Experiment 2. Consequently, associative explanations based on the idea that environmental or drug produced stimuli were associated with aversive consequences of morphine are not supported. Alternatively, environmental cues or some specific effect of morphine could have been associated with the positive reinforcing effects of morphine during preexposure and this could have blocked the formation of an association between a novel flavor and morphine. Evironmental cues are unlikely candidates since it has been shown that conditioned environmental cues do not underly the preexposure effect observed with morphine [11,24]. An association between internal cues is possible given evidence that three specific effects of opiates, the discriminative, the positive reinforcing and the aversive, are processed in parallel by the nervous system [1–3, 15]. Hence, an association between internal cues themselves, the discriminative with the positive reinforcing, during the preexposure phase might interfere with the formation of an association between a novel flavor and the aversive properties of morphine during training. The blocking effect would have to be specific since morphine preexposure does not attenuate the later conditioned place preferences induced by morphine. Moreover, blocking of an association between a flavor and the aversive effects of morphine by means of some stimulus that was associated with the positive reinforcing effects of morphine would be inconsistent with the observation that one does not observe blocking when the unconditional stimuli differ [14,20].

Explanations of the preexposure effect in terms of some unconditional change in the animal's response to morphine would require that some long-lasting change in the animal's processing of the aversive aspects of morphine had occurred. Perhaps a centrally-mediated long-lasting release of peripheral hormones could disrupt vagal processing of the opiate's aversive signal or its association with taste during conditioning. There is no direct evidence or any candidate hormones to support this possibility at present. Finally, preexposure to the central reinforcing properties of morphine might sensitize the central substrates of opiate reward so that during taste aversion training the positive reinforcing effects would overshadow the aversive effects of morphine. A similar explanation has been used to account for the observation that certain psychoactive drugs, such as marijuana, have been used to alleviate sickness during cancer chemotherapy in drug familiar but not drug naive patients [21]. However, there is evidence against a sensitization explanation in our own findings. We have not seen any significant increases in the place preferences produced by morphine when multiple preexposures preceded place preference conditioning. Consequently, it is unlikely that our preexposed animals found the aversive aspects of morphine less severe because of increases in morphine's positive reinforcing aspects.

Our findings are not consistent with the notion that there is a correlation between aversiveness of the noxious agent used during preexposure and the magnitude of the aversion the preexposed drug will subsequently produce [7,20]. During preexposure we eliminated the aversive properties of morphine that produce both place and flavor aversions [1,4], yet morphine still lost its capacity to produce a flavor aversion. A similar reversible removal of the aversive properties of other psychoactive drugs cannot yet be achieved, as the anatomical separation of the substrates for the rewarding and aversive effects of drugs other than opiates is not well advanced. Thus, it is not possible to assess whether exposure to the aversive effects of other psychoactive drugs is necessary for the production of preexposure attenuation effect.

Successful blocking of the formation of conditioned taste aversions with peripheral injections of methylnaltrexone in Experiment 2 indicates that morphine was successfully prevented from stimulating peripheral receptors that are responsible for the formation of taste aversions. These data contradict suggestions that drug novelty alone mediates the formation of taste aversions, because it is clear that morphine will produce place preferences when methylnaltrexone is present [4].

The presence of place preferences in all the groups studied is consistent with the suggestion that preexposing animals to morphine does not readily attenuate the reinforcing effects of opiates [25]. It remains possible that more than five exposures to morphine might have resulted in an attenuation of morphine's capacity to produce a place preference, but 5 preexposures were certainly sufficient to attenuate the subsequent aversive effects of morphine. Alternatively, it can by hypothesized that the preexposure did attenuate the later reinforcing effects of morphine but that observation of this attenuation was masked. By this argument, the continued success in demonstrating place preferences could be attributed to the development of place aversions to the saline paired environment in the animals that had been preexposed to morphine. During the training phase the saline environment would be paired with the putative withdrawal symptoms elicited by the injection procedure [23]. These symptoms could then become associated with the saline environment and could have elicited avoidance, and thus apparent preference for the morphine paired environment on the test day. This explanation of the place preferences observed in our experiments is not compelling in light of recent data which show that the aversive effects of withdrawal only produce place avoidance after repeated high dose morphine exposure and not after a few low dose exposures [3]. Moreover, the fact that morphine produces conditioned place preferences after only one low dose exposure [19] suggests that place conditioning in the present study measured the primary rewarding properties of morphine. The basic finding that opiate preexposure attenuates the aversive but not the positive reinforcing properties of morphine remains a puzzle. In so far as separate anatomical pathways in the nervous system mediate the rewarding and aversive properties of opiates [1, 2, 4], perhaps it should not be surprising that the structural operating characteristics (presence or absence of preexposure attenuation) of the motivational effects are different.

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