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Peripheral Receptors Mediate the Aversive Conditioning Effects of Morphine in the Rat

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BECHARA, A., K. A. ZITO AND D. VAN DER KOOY. *Peripheral receptors mediate the aversive conditioning effects of morphine in the rat.* PHARMACOL BIOCHEM BEHAV **28**(2) 219–225, 1987.—Previous evidence has shown that morphine produces positive reinforcing effects (as measured in the place conditioning paradigm) through an action in the central nervous system (CNS). The aversive conditioning effects of morphine (as measured in the place and taste conditioning paradigms) were produced when drug action was restricted to peripheral sites, particularly in the gut region. We now demonstrate that most of the aversive conditioning effects of morphine (using place and taste conditioning paradigms) are receptor mediated effects exerted through an action on peripheral opiate receptors. The conditioned taste aversions induced by intraperitoneal (IP) morphine (15 mg/kg) but not amphetamine (1 mg/kg) were attenuated by low IP doses of opiate antagonists (0.1 mg/kg of naltrexone or 1 mg/kg of the peripherally acting antagonist methynaltrexone (MN)). Morphine-, but not amphetamine-induced conditioned taste aversions were also attenuated in animals whose small sensory neurons, bearing the majority of primary afferent opiate receptors, were destroyed by neonatal treatments with capsaicin. In the place conditioning paradigm, the aversive conditioning effects produced by low IP administrations of morphine were blocked by opiate antagonists. Intraperitoneal pretreatments with 1 mg/kg of the quaternary opiate antagonist MN (which does not cross the blood-brain barrier effectively) were shown to block the conditioned place aversions produced by low IP doses of morphine (0.05 mg/kg), but not the place aversions produced by lithium chloride (75 mg/kg IP), or by high doses of naloxone (10 mg/kg SC). These results demonstrate that the aversive conditioning effects of morphine are primarily mediated through an action on peripheral opiate receptors. Further results showing that MN pretreatments (1 mg/kg IP) as well as neonatal capsaicin treatments did not attenuate the positive reinforcing effects of morphine (10 mg/kg SC), as shown in the place conditioning paradigm, provide support to the notion that the neuronal systems mediating the aversive effects of opiates are independent of the systems mediating the positive reinforcing effects of opiates.

Morphine Methylnaltrexone Naltrexone Capsaicin Opiate aversions Conditioned taste aversion Place conditioning

THE positive reinforcing as well as the aversive properties of opiates have been demonstrated in the rat. Rats prefer an environment that has been previously paired with morphine [2, 13, 28]. In contrast, experimental animals avoid novel tastes that have been paired previously with morphine given at similar doses and over the same routes of administration [5,25]. Furthermore, under certain experimental conditions animals were shown to avoid a place that had been paired previously with low intraperitoneal doses of morphine [2].

Recent evidence suggests that separate neural substrates mediate these opposite motivational effects of morphine in the rat [2]. The positive reinforcing effects of morphine are produced through an action in the brain, and the aversive conditioning effects are mediated through drug action in the periphery, especially in the gut region [2]. Although evidence exists for an anatomical specificity of morphine positive reinforcing and aversive effects [2], the pharmacological specificity of these motivational effects to the opiate receptor has not been fully clarified. Several lines of evidence showed that the positive reinforcing effects of opiates are receptor mediated [13, 21, 27]. However, questions have been raised concerning the non-specificity of opiate aversive conditioning effects [12], although there are reports that opiate antagonists attenuate the aversive effects of morphine in the conditioned taste aversion paradigm [10,25]. The demonstration that the aversive conditioning effects of morphine are produced through drug action in the periphery [2] now allows more exacting tests of receptor specificity through manipulations of peripheral opiate receptors. Therefore, the present experiments focus on the pharmacological specificity of morphine aversive conditioning effects to peripheral opiate receptors. The aversive conditioning effects of morphine were studied after the blockade of peripheral opiate receptors with opiate antagonists or after the destruction of

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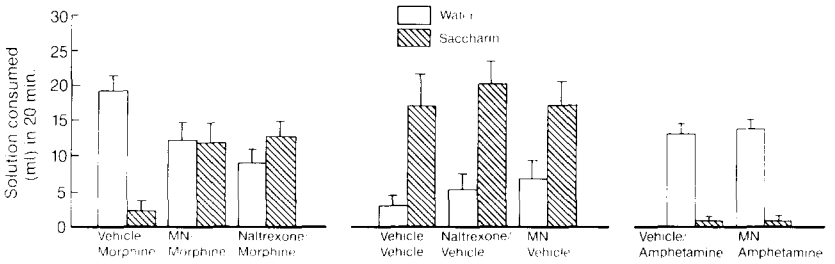

FIG. 1. The effects of methylnaltrexone (1 mg/kg IP) or naltrexone (0.1 mg/kg IP) pretreatment on morphine-induced (15 mg/kg IP) or amphetamine-induced (1 mg/kg IP) conditioned taste aversions. Bars represent means±SEM of the amount of liquid consumed during a 20 min, drug free, two-bottle test session for (n=6–8) rats.

small sensory neurons, bearing the majority of primary afferent opiate receptors, with neonatal capsaicin treatments.

EXPERIMENT 1

We first sought to confirm that the high dose opiate aversion produced by morphine in the conditioned taste aversion (CTA) paradigm was receptor mediated. The aversive effects of morphine in the CTA paradigm are attenuated by fairly high doses of opiate antagonists [10,25]. However, it is known that high doses of opiate antagonists can themselves produce aversions [2,13]. Vagotomy abolishes the aversive effects of morphine in the CTA paradigm [2], suggesting a peripheral mechanism in the mediation of morphine aversive conditioning effects. Thus, we hypothesized that much lower doses of opiate antagonists applied locally in the gut (as well as peripherally acting antagonists) might be more effective blockers of opiate receptor-mediated aversive conditioning effects. Indeed, a low dose, intraperitoneal (IP) administration of 0.1 mg/kg of naltrexone or 1 mg/kg of its quaternary derivative methylnaltrexone (MN), which does not cross the blood-brain barrier effectively [4,22], has been shown to produce local effects by blocking opiate receptors in the gut, without exerting significant effects on brain opiate receptors [2]. Therefore, we predicted that pretreating animals with 1 mg/kg of MN or 0.1 mg/kg of naltrexone IP should attenuate the normal aversion acquired to saccharin taste when paired with 15 mg/kg of morphine (IP).

Subjects

All animals used in these experiments were adult male Wistar rats (Charles River) weighing between 300–350 grams. Subjects were housed individually in suspended grey wire cages in a room kept at a temperature of 22°C and lit between 0900 to 2100 hr. Purina rat chow was available ad lib throughout the experiments. Water was also continuously available except during CTA training and testing.

Method

CTA procedures and doses were similar to those described elsewhere [2,25]. Briefly, eight groups of rats (n=6–8) were initially trained to consume water on a limited access regime of 20 min a day for five training days. On the following experimental days, one group of animals (vehicle/morphine) received IP vehicle pretreatments each day, followed immediately by a 15 mg/kg injection of morphine (IP) when saccharin (0.1%) was present or saline vehicle injection when water was present in the home cage. Treatment on experimental days alternated between each of these procedures for a total of six days. All injections were made immediately following the 20 min fluid access period each day. On the seventh day a two bottle choice test was given to each rat by simultaneously presenting both saccharin and water for 20 minutes. The amount consumed of both liquids was recorded for each rat. The two bottle choice method has been shown to be the most sensitive and reliable of the various CTA testing methods [6]. The side of the cage where the saccharin tube was placed during conditioning and testing as well as the order of saccharin/water presentation were counterbalanced within each group but remained consistent throughout the experiment for each rat.

Two other groups (MN/morphine and naltrexone/morphine) received identical treatment to the vehicle/morphine group, except that vehicle pretreatments each day were replaced by 1 mg/kg of MN or by 0.1 mg/kg of naltrexone, respectively. In order to avoid contamination by the motivational effects of the opiate antagonist itself [2], the antagonist was paired with both the saccharin and water tastes in all the CTA experiments. One control group (vehicle/vehicle) received daily vehicle pretreatments (IP) immediately followed by a vehicle injection (IP) when either saccharin or water was present. Additional control groups investigated whether pairing naltrexone or MN with both tastes in morphine naive rats, has any effects on the normal preference of animals for a saccharin taste. Therefore, these additional control groups received identical treatment to the vehicle/vehicle control group, except that saline vehicle pretreatments were replaced by 1 mg/kg of MN (MN/vehicle) or 0.1 mg/kg of naltrexone (naltrexone/vehicle). Finally, in order to assess the possibility that the antagonists may non-specifically interfere with the learning mechanisms underlying the acquisition of all CTA's, we tested the effects of MN pretreatments on the CTA induced by a non-opiate drug (1 mg/kg of amphetamine IP). For this purpose, two groups (vehicle/amphetamine and MN/amphetamine) received daily vehicle or MN (1 mg/kg IP) pretreatments, followed by amphetamine injections (1 mg/kg IP) when saccharin was present or vehicle injections when water was present.

Results and Discussion

Morphine induced a distinct CTA in saline pretreated animals (vehicle/morphine), but not in rats pretreated with either MN or naltrexone (MN/morphine and naltrexone/morphine groups, respectively) (Fig. 1). Furthermore, pretreatments with the antagonists did not have any effects on the normal preferences for a saccharin taste nor on the mechanisms underlying the learning of conditioned taste

aversions with non-opiate drugs (i.e., amphetamine) (Fig. 1).

An analysis of variance comparing vehicle/morphine, MN/morphine and naltrexone/morphine groups revealed a significant interaction between fluid intake and drug pretreatment, $F(2,28)=5.53$, $p<0.05$. The amount of saccharin versus water consumed was dependent upon the pretreatment drug. That is, vehicle/morphine animals consumed significantly more water than saccharin, $t(6)=4.5$, $p<0.05$, whereas this was not true for either the naltrexone/morphine, $t(7)=0.4$, $p>0.05$, or MN/morphine groups, $t(7)=0.03$, $p>0.05$. An analysis of variance comparing the vehicle/vehicle, MN/vehicle, and naltrexone/vehicle groups revealed a significant effect of saccharin versus water intake, $F(2,21)=15.2$, $p<0.05$. A significant interaction between liquid consumed and drug pretreatment was not observed, $F(2,21)=1.5$, $p>0.05$. Thus, pretreatment with MN or naltrexone did not affect the normal preference for a saccharin taste observed in drug free animals (vehicle/vehicle). Furthermore, MN did not block amphetamine CTA. An analysis of variance comparing the vehicle/amphetamine and MN/amphetamine groups revealed a significant effect of saccharin versus water intake, $F(1,14)=442$, $p<0.05$, indicating an aversion to a saccharin taste paired with amphetamine. A significant interaction between liquid consumed and drug pretreatment was not observed, $F(1,14)=0.28$, $p<0.05$, thus showing that MN did not have any effects on the taste aversions induced by amphetamine. In sum, these results suggest that the blockade of morphine-induced CTA by MN or naltrexone pretreatment was due to the specific antagonistic effects of these drugs against morphine acting on peripheral opiate receptors rather than to any non-specific effects of the antagonists themselves.

The results, however, did not demonstrate a complete blockade of morphine CTA. Rats receiving morphine injections following pretreatment with either MN or naltrexone did not display the normal preference to saccharin taste observed in vehicle-injected animals. These findings raise the possibility that a minor component of morphine aversive conditioning effects might be attributable to a non-receptor mediated mechanism. However, it is also possible that the failure to observe complete blockade of morphine aversive effects was related to incomplete antagonism of all peripheral opiate receptors, or receptor subtypes.

EXPERIMENT 2

Low dose (0.05 mg/kg) IP administration of morphine produces conditioned place aversions in the place conditioning paradigm [2]. These effects were attributed to local effects on opiate receptors in the gut [2]. Vagotomy blocks the aversive conditioning effects produced by the administration of low IP doses of morphine (without affecting the positive reinforcing effects produced by higher doses), suggesting a peripheral mechanism in the mediation of opiate aversions [2]. Based on this evidence for peripheral involvement in opiate aversions [2], we predicted that pretreating animals with MN, which does not cross the blood-brain barrier effectively [4,22], should block the conditioned place aversions induced by low IP doses of morphine. Experiment 2 tested this prediction. In order to assess the possibility that MN may non-specifically block the mechanisms underlying the learning of all conditioned place aversions, we tested the effects of MN pretreatments on the place aversions produced by other known aversive drugs (lithium chloride (LiCl) and naloxone). High doses of naloxone were hypothesized to produce place aversions due to an action in the brain [2]. Therefore, we predicted that MN pretreatments should not block the aversive effects of naloxone in the place conditioning paradigm.

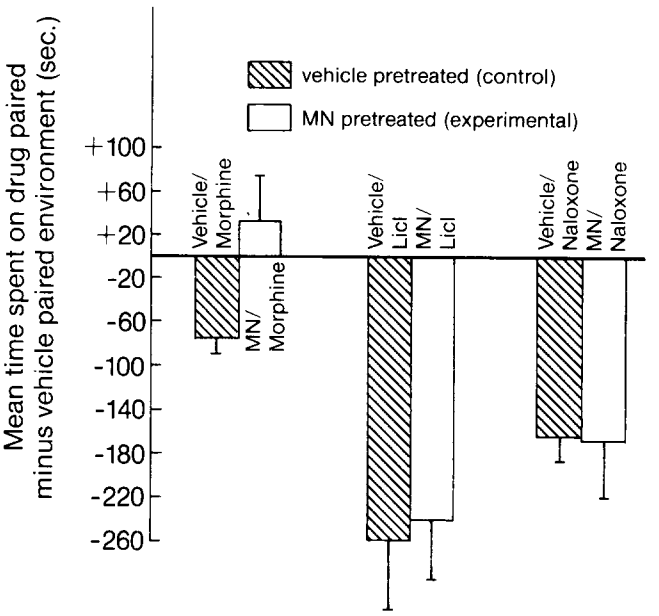

FIG. 2. The effects of methylnaltrexone (1 mg/kg IP) pretreatment on low dose morphine (0.05 mg/kg IP) place aversions. Bars represent means±SEM of time spent on drug side minus time spent on non-drug side during a 10 min, drug free, place conditioning test session for (n=8) rats.

Method

Six groups of animals (n=8) were used in this experiment. Place conditioning procedures were identical to those described previously [2,13]. Briefly, conditioning took place in two boxes which differed in colour, texture and smell. One box had black walls and a black Plexiglas floor which was wiped with a 2% vinegar solution just prior to placing each rat inside. The other box had white walls and a wood chip floor which gave off a slight smell of wood. After handling the animals for six consecutive days, place conditioning was achieved by administering low IP doses of morphine (0.05 mg/kg), LiCl (75 mg/kg IP) or naloxone (10 mg/kg SC) one day and a vehicle injection on the next, for a total of eight days (i.e., 4 drug pairings). Following drug injections, each rat was immediately placed into one box and on alternate days, when injected with vehicle, it was placed in the other box. Each pairing lasted 30 min. The order of drug and vehicle presentation and the choice of environment paired with drug injection were counterbalanced within each group.

Control animals received IP vehicle pretreatments prior to place conditioning with morphine (0.05 mg/kg IP), LiCl (75 mg/kg IP) or naloxone (10 mg/kg SC). The experimental animals received identical handling and conditioning, except that the vehicle pretreatments were replaced by IP pretreatments with 1 mg/kg of MN. In order to avoid any motivational effects of the antagonist drug itself, MN pretreatments were paired with both environments.

On the ninth day, each rat was placed into a larger rectangular test box, which consisted of environments

exactly the same as the two conditioning boxes at each end separated by a smaller grey area (neutral zone). The time each rat spent in each end of the test box was recorded over at 10 min period. Evidence exists that this unbiased method of running place conditioning is the most reliable [14,23].

Results and Discussion

MN significantly blocked the place aversions produced by 0.05 mg/kg of morphine IP but not the aversions produced by 75 mg/kg of LiCl IP or 10 mg/kg of naloxone SC (Fig. 2). An analysis of variance on the data from both control (vehicle/morphine) and experimental (MN/morphine) groups revealed a significant interaction of group with time spent in drug versus saline paired environments, $F(1,14)=4.9$, $p<0.05$. Vehicle/morphine rats spent significantly more time on the vehicle paired side, $t(7)=4.2$, $p<0.05$, whereas MN/morphine animals showed no preferences for either side, $t(7)=0.5$, $p>0.05$. On the other hand, MN did not block the place aversions produced by 75 mg/kg of LiCl IP. An analysis of variance on the data comparing the vehicle/LiCl (control) and MN/LiCl (experimental) groups revealed a significant effect of time spent in drug versus saline paired environments, $F(1,14)=80.1$, $p<0.05$. That is, animals spent considerably more time in vehicle paired environment than in drug paired environment. However, no significant interaction between time spent in each environment and drug pretreatment was observed, $F(1,14)=0.06$, $p>0.05$. This indicates that MN pretreatments did not block LiCl place aversions. Similar results were seen in naloxone treated animals. An analysis of variance on the data comparing control (vehicle/naloxone) and experimental (MN/naloxone) groups revealed a significant effect of time spent in drug versus saline paired environments, $F(1,14)=29.8$, $p<0.05$, but not a significant interaction between time spent in each environment and drug pretreatment, $F(1,14)=0.005$, $p>0.05$.

These results suggest that the place aversions produced by the IP administrations of low doses of morphine (0.05 mg/kg) are receptor mediated effects. MN specifically blocked the aversive conditioning effects produced by low IP doses of morphine by antagonizing the activity of morphine on peripheral opiate receptors. The blockade by MN of morphine aversive conditioning effects cannot be attributed to a non-specific interference of the drug with mechanisms underlying the learning of all place aversions. Thus, the attenuation of morphine's aversive effects by peripherally acting antagonists is not limited to conditioned taste aversions, but is also seen in the conditioned place aversion paradigm.

EXPERIMENT 3

In light of the evidence that the positive reinforcing effects of opiates are due to central mechanisms [2, 21, 27], we tested whether the blockade of peripheral opiate receptors with MN has any effects on the positive reinforcing aspects of opiates in the place conditioning paradigm. Previous evidence has shown that vagotomy blocks the aversive but not the positive reinforcing effects of morphine, thus suggesting that the neural substrates mediating the positive reinforcing effects of opiates are independent of the neural substrates mediating the aversive effects of opiates [2]. Therefore, we predicted that MN should block morphine's aversive conditioning effects (Experiments 1 and 2) but not morphine's positive reinforcing effects. Experiment 3 tested the latter prediction.

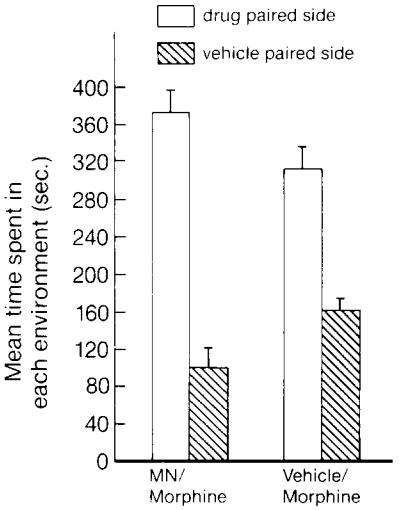

FIG. 3. The effects of methylnaltrexone (1 mg/kg IP) pretreatment on morphine (10 mg/kg SC) place preferences. Bars represent means±SEM of time spent in drug and in saline paired environments for (n=8) rats.

Method

Two groups of animals (n=8) served as subjects. Employing the place conditioning procedure as described earlier, the two groups underwent place conditioning with 10 mg/kg morphine (SC). Control animals received IP vehicle pretreatments prior to place conditioning with morphine. The experimental animals received identical treatment and conditioning except that the vehicle pretreatments were replaced by IP pretreatments with 1 mg/kg of MN. In order to avoid the motivational effects of the antagonist drug itself [2], MN pretreatments were paired with both environments.

Results and Discussion

As predicted, MN did not block morphine place preferences (Fig. 3). An analysis of variance on the data from both control and experimental groups revealed a significant effect of time spent in vehicle versus drug paired environment, $F(1,14)=94.2$, $p<0.05$. That is, animals spent considerably more time in drug paired environment as opposed to the vehicle paired environment. However, the analysis also revealed a significant interaction of group with time spent in saline versus morphine paired environment, $F(1,14)=5.5$, $p<0.05$. MN pretreatments did not block, but actually potentiated slightly the morphine place preferences. The slight potentiation of morphine place preferences could be the result of blocking peripheral morphine aversive effects with MN.

The present results, demonstrating that MN blocked the aversive conditioning, but not the positive reinforcing effects of morphine, further support previous findings [2] that the neural substrates mediating the aversive effects of opiates are separate from those mediating the positive reinforcing effects.

EXPERIMENTS 4 AND 5

These experiments were designed to test further the role of peripheral opiate mechanisms in the aversive conditioning effects of opiates by removing the putative peripheral sub-

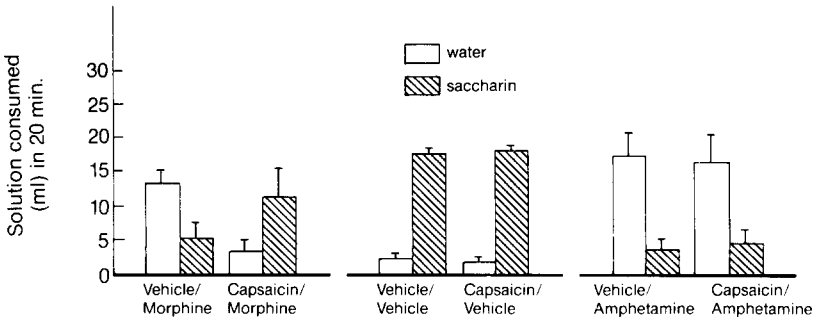

FIG. 4. The effects of neonatal capsaicin treatment on morphine-induced (15 mg/kg IP) conditioned taste aversions. Bars represent means±SEM of the amount of liquid consumed during a 20 min, drug free, two-bottle test session for (n=6–8) rats.

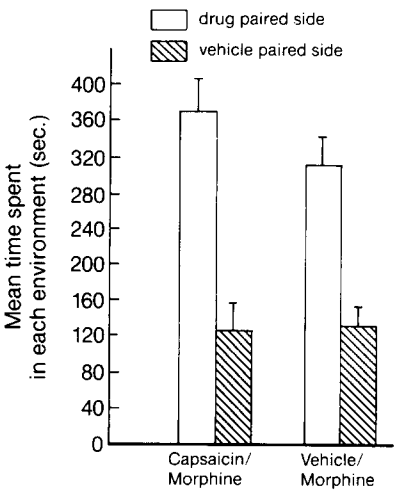

FIG. 5. The effects of neonatal capsaicin treatment on morphine (10 mg/kg SC) place preferences. Bars represent means±SEM of time spent in drug and in saline paired environments for (n=6–8) rats.

strate in a manner different from pharmacological antagonism of the receptors. Adult rats treated neonatally with systemic capsaicin injections were tested for the aversive effects of morphine or amphetamine in the CTA paradigm. Neonatal systemic capsaicin treatment destroys a subpopulation of primary sensory neurons with small ganglionic cell bodies [8, 15, 17]. These are the same small sensory neurons which apparently bear opiate receptors [17, 18, 24]. It was predicted that the destruction of the primary sensory neurons bearing opiate receptors, by means of neonatal systemic capsaicin treatment, should abolish the aversive conditioning effects of morphine without affecting their central positive reinforcing effects. Experiments 4 and 5 tested these predictions.

Method

Experimental animals received neonatal treatment with capsaicin. The destruction of small sensory neurons was achieved by a single subcutaneous injection of 50 μl capsaicin (50 mg/kg) given in a saline solution containing 10% ethanol and 10% tween 80 at two days of age. Vehicle-treated littermates served as controls. These treatments are identical to those used previously and characterized in our lab [16,26].

In Experiment 4, CTA procedures were similar to those described earlier (Expeirment 1). Six groups of animals (n=6–8) were used in this experiment. In one experimental group (capsaicin/morphine) and one control group (vehicle/morphine), saccharin taste was paired with IP injections of 15 mg/kg of morphine. Two other control groups were used to test whether neonatal capsaicin treatments have any effects on CTA's induced by non-opiate drugs. Therefore, in these two control groups (capsaicin/amphetamine and vehicle/amphetamine), saccharin taste was paired with IP injections of 1 mg/kg of amphetamine. Finally, additional control groups (capsaicin/vehicle and vehicle/vehicle) tested whether neonatal capsaicin treatment has any effects on the normal preference for a saccharin taste.

In Experiment 5, two groups of animals (n=6–8) that were used in the above experiment (capsaicin/morphine and vehicle/morphine) served as subjects to test for the positive reinforcing properities of morphine using the same place conditioning procedures described earlier (Experiment 2). The two groups underwent place conditioning with 10 mg/kg of morphine (SC), 30 days following the conclusion of their testing in the taste conditioning paradigm.

Results and Discussion

Neonatal capsaicin treatment significantly blocked the CTA induced by 15 mg/kg of morphine IP but not the CTA induced by 1 mg/kg of amphetamine IP (Fig. 4). Furthermore, neonatal treatment with capsaicin did not have any effects on morphine-induced place preferences (Fig. 5).

Indeed, an analysis of variance on the data from Experiment 4 using both control (vehicle/morphine) and experimental (capsaicin/morphine) groups revealed a significant interaction of group with amount of fluid consumed, $F(1,12)=5.61$, $p<0.05$. The amount of saccharin versus water consumed was dependent upon the neonatal treatment with capsaicin or vehicle. That is, vehicle/morphine animals consumed significantly more water than saccharin, $t(7)=2.2$, $p<0.05$, whereas the opposite was true for capsaicin/morphine animals, $t(7)=1.9$, $p<0.05$. These results demonstrate a blockade of morphine induced CTA by the neonatal treatment with capsaicin. On the other hand, an analysis of variance on the data from the amphetamine CTA experiment comparing the vehicle/amphetamine and capsaicin/amphetamine groups, revealed a significant effect of saccharin versus water intake, $F(1,9)=15.2$, $p<0.05$. This shows a significant aversion to a saccharin taste paired with amphetamine. However, a significant interaction between liq-

uid consumed and neonatal treatment with capsaicin or vehicle was not observed, $F(1,9)=0.21$, $p>0.05$, thus indicating that neonatal treatment with capsaicin did not affect the CTA induced by amphetamine. Furthermore, an analysis of variance comparing the vehicle/vehicle and capsaicin/vehicle groups, revealed a significant effect of saccharin versus water intake, $F(1,14)=231$, $p<0.05$. A significant interaction between liquid consumed and neonatal capsaicin or vehicle treatment was not observed, $F(1,14)=0.18$, $p>0.05$, indicating that neonatal capsaicin treatment did not affect the normal preference for a saccharin taste.

These results further support a role for peripheral sensory mechanisms in the aversive conditioning effects of opiates. Experiment 4 suggests that the blockade of morphine-induced CTA was a specific effect on the aversivness of opiates, rather than any non-specific interference of neonatal capsaicin treatment with taste or with the learning mechanisms underlying the acquistion of conditioned taste aversions. However, as in Experiment 1, the results did not show a complete blockade of morphine CTA. Neonatally capsaicin treated animals injected with morphine (capsaicin/morphine) did not show quite as large a preference for saccharin taste as similar animals treated neonatally with capsaicin but injected with vehicle (capsaicin/vehicle). Again, these findings raise the possibility that a minor component of opiate aversions are non-receptor mediated. Alternatively, the failure to observe complete blockade of morphine induced taste aversions may have been related to incomplete destruction of all peripheral opiate receptor bearing neurons.

Experiment 5 revealed that neonatal treatment with capsaicin did not block the positive reinforcing effects of morphine (Fig. 5). An analysis of variance on the data from the morphine place preference experiment using both control (vehicle/morphine) and experimental (capsaicin/morphine) groups revealed a significant effect of time spent on drug versus saline paired evnironment, $F(1,10)=48.2$, $p<0.05$. In other words, the animals spent considerably more time on drug paired side than on vehicle paired side. However, the analysis did not reveal a signficant interaction of group with time spent in saline versus drug paired environment, $F(1,10)=0.1$, $p>0.05$. This suggests that the capsaicin-induced destruction of peripheral opiate receptor bearing neurons did not have any effects on the positive reinforcing effects of morphine.

GENERAL DISCUSSION

These experiments reveal that morphine produces aversive conditioning effects specifically through an opiate receptor-mediated mechanism. Furthermore, peripheral neural substrates mediate the aversive conditioning effects of morphine, and these are separate from the neural systems mediating the positive reinforcing effects of morphine.

The conditioned taste aversion experiments demonstrated that the major portion of the aversive effects of even high doses of morphine are blocked by low IP doses of opiate antagonists (including a peripherally acting antagonist) or by the destruction of peripheral opiate receptor bearing neurons with neonatal capsaicin treatment. Furthermore, the place conditioning experiment demonstrated that the aversive effects of low IP doses of morphine in a non-taste paradigm were also blocked by low IP doses of the peripherally acting antagonist methylnaltrexone. These results strongly suggest that opioid aversions are mediated through an action on peripheral opiate receptors. Separate opiate receptors, most likely restricted to the central nervous system [2, 21, 27], mediate the positive reinforcing effects of morphine.

Although the major portion of the conditioned taste aversions induced by morphine were blocked by opiate antagonists and neonatal capsaicin treatments, the lack of a complete blockade of these aversive conditioning effects may provide support for other studies that suggest some non-specific aversive conditioning effects of opiates [1,12]. However, we cannot rule out the possibility that the incomplete blockades of morphine CTA were due to incomplete antagonist effects of naltrexone or methylnaltrexone on peripheral opiate receptors or to incomplete destruction of all peripheral opiate receptors with capsaicin. Nevertheless, the transmission of all the aversive information must be via the vagus nerve, since subdiaphragmatic vagotomy completely eliminated morphine induced CTA's [2].

Given our evidence that the substrates of morphine's aversive conditioning and positive reinforcing effects are anatomically distinct, it remains to be explained why the blockade of peripheral opiate receptors with MN potentiated the positive reinforcing effects of morphine. Presumably, the reward from the positive reinforcing effects of morphine acting in the brain is mildly attenuated by the aversive conditioning effects of morphine acting on peripheral opiate receptors. The removal of the aversive conditioning stimulus acting on peripheral opiate receptors may allow a slight potentiation of the reward from central positive reinforcing effects. However, this explanation does not account for the failure to potentiate morphine's central positive reinforcing effects as a result of the destruction with neonatal capsaicin of the peripheral substrate of morphine's aversive effects.

It is unclear what the aversive conditioning effects of opiates seen in rodents correspond to in humans. Aversive effects in humans can range from nausea to dysphoria to psychotomimetic effects [20]. Although the present study suggests that most opiate aversive effects are mediated peripherally, there is also one aversive effect resulting from high doses of naloxone that is not blocked by vagotomy [2] or by methylnaltrexone pretreatment (the present results). This aversive effect is anatomically distinct from the primary, peripherally mediated opiate aversions and may correspond to a behaviorally distinct effect in humans.

In conclusion, the aversive effects of morphine as shown in the CTA or place conditioning paradigms, were demonstrated to be mediated through an action on peripheral opiate specific receptors. Despite the existence of at least three different subtypes of opioid receptors in the periphery (mu, kappa and delta) [7, 9, 11, 29], it is not known which of these different receptor subtypes mediates the aversive conditioning effects. The present study employed primarily morphine, which is known to act preferentially on the mu receptor, although it does not do so exclusively [19]. Therefore, in a companion paper we have studied the receptor subtype specificity of peripherally mediated opioid aversive conditioning effects, and demonstrate that the activation of peripheral kappa receptors is aversive [3].

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