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Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice

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Abstract

The aversive response to naloxone administration observed in human and animal studies suggests the presence of an endogenous opioid tone regulating hedonic state but the class(es) of opioid peptides mediating such opioid hedonic tone is uncertain. We sought to address this question using mice deficient in either beta-endorphin or pro-enkephalin in a naloxone-conditioned place aversion paradigm. Mice received saline in the morning in one chamber and either saline or naloxone (0.1, 1 or 10 mg/kg, s.c.) in the afternoon in another chamber, each day for 3 days. On the test day they were given free access to the testing chambers in the afternoon and the time spent in each chamber was recorded. Whereas wild-type and beta-endorphin-deficient mice exhibited a robust conditioned place aversion to naloxone, pro-enkephalin knockout mice failed to show aversion to naloxone at any dose tested. In contrast, these mice showed a normal conditioned aversion to the kappa opioid receptor agonist, U50,488 (5 mg/kg), and to LiCl (100 mg/kg) indicating that these mice are capable of associative learning. In a separate experiment, pro-enkephalin knockout mice, similar to wild-type and beta-endorphin-deficient mice, demonstrated a significant conditioned place preference to morphine (2.5, 5 and 10 mg/kg s.c.). These data suggest that enkephalins, but not endorphins, may mediate an endogenous opioid component of basal affective state and also indicate that release of neither endogenous enkephalins nor endorphins is critical for the acquisition or expression of the association between contextual cues and the rewarding effect of exogenously administered opiates.

Introduction

Endogenous opioid peptides have been postulated to be key regulators of 'hedonic homeostasis', a term coined to describe maintenance of a balanced affective, emotional or motivational state (Koob & Le Moal, 2001). This is largely based on the observation that, in addition to the well-known rewarding effect of exogenously administered opiates (see Gerrits *et al.*, 2003), blockade of opioid receptors with the general opioid antagonists naloxone and naltrexone is dysphoric in humans (Grevert & Goldstein, 1977; Hollister *et al.*, 1981) and produces robust conditioned aversive responses in animals (Mucha *et al.*, 1985; Bals-Kubik *et al.*, 1989; Parker & Rennie, 1992; Skoubis *et al.*, 2001). Thus, the concept has arisen of an endogenous opioid tone that may be at least partially responsible for modulating the basal (as opposed to drug-induced) hedonic state of the organism. Study of such endogenous regulatory systems may be particularly important as repeated drug administration may alter their set-points, thereby producing hedonic homeostatic dysregulation or allostasis, potentially resulting in a drug-dependent state (Koob & Le Moal, 2001).

Mu opioid receptors are crucial mediators of the rewarding effects of exogenous opiates such as morphine (Negus *et al.*, 1993; Matthes *et al.*, 1996; Piepponen *et al.*, 1997). The mu receptor also appears to mediate the apparent endogenous opioid-mediated basal hedonic tone, as naloxone administration is not aversive in mice lacking this receptor (Skoubis *et al.*, 2001). However, the identities of the opioid peptides involved are less certain, due in large part to the fact that peptides from each of the precursor families (pro-opiomelanocortin, pro-enkephalin and pro-dynorphin) bind to each of the three opioid receptors with low selectivity (Reisine & Pasternak, 1996).

We sought to address this question using mutant mice selectively lacking either beta-endorphin (Rubinstein *et al.*, 1996) or pro-enkephalin (Konig *et al.*, 1996). The rationale was that removal of the endogenous ligand(s) responsible for hedonic tone would render the action of a general opiate receptor antagonist redundant. Both beta-endorphin-deficient and pro-enkephalin knockout mice were examined for their ability to demonstrate a conditioned place aversion (CPA) to naloxone. Conditioned aversive responses to the kappa agonist, U50,488, and LiCl were tested as a positive control for general associative learning deficits. Beta-endorphin-deficient and pro-enkephalin knockout mice were also studied for their ability to acquire a conditioned place preference (CPP) to morphine because it has been proposed that the release of endogenous opioid peptides may also be an important factor in cue-induced reward after repeated drug exposure (see Gerrits *et al.*, 2003).

Materials and methods

Experimental subjects

Adult male beta-endorphin-deficient (Rubinstein *et al.*, 1996; B6.129-*Penk-rs*^{tm1Pig}/J), pro-enkephalin knockout (Konig *et al.*, 1996; B6.129S2-*Pomc1*^{tm1Low}/J) and age-matched (8–10 weeks old

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at start of study) wild-type (C57BL/6) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) or bred in-house from breeders obtained from Jackson Laboratories and housed on a 12/12-h light/dark cycle with food and water available *ad libitum*. Both congenic strains were previously backcrossed at least 10 generations onto a C57BL/6 background by Jackson Laboratories. Animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all procedures were approved by the Institutional Animal Care and Use Committee.

Place conditioning protocol

Details of the conditioning apparatus, providing automated recording of subject location (Coulbourn Instruments, Allentown, PA, USA), were described previously (Skoubis *et al.*, 2001). Briefly, a square arena was divided into three chambers: a neutral start chamber (gray walls and floor) and two conditioning chambers (black and white checkers and black and white cow patterns) that were accessible via the neutral chamber through guillotine doors. The two conditioning chambers were also distinguishable on the basis of odor, i.e. almond or lemon scent (McCormick and Co., Hunt Valley, MD, USA). The conditioning chambers were designed such that animals would generally have no bias for one over the other and the drug-paired chamber was randomized across subjects and treatments in an attempt to normalize any small biases that might occur. The place conditioning protocol was as follows.

Day 1, habituation

Subjects were placed in the start chamber and permitted free access to the entire apparatus for 15 min. The time spent in each of the chambers was recorded to measure any initial bias.

Days 2–4, conditioning

In the morning, animals received an s.c. injection of saline and were confined to the 'vehicle-paired' chamber for 30 min and subsequently returned to their home-cage. Four hours later, animals received an s.c. injection of one of the test drugs or saline and were confined to the 'drug-paired' chamber for 30 min. The drug-paired chamber was randomized across subjects.

Day 5, test

At 24 h after the last drug treatment, animals, in a drug-free state, were placed in the neutral chamber and permitted to freely explore the apparatus with the doors removed for 15 min. The time spent in each chamber was recorded.

Treatment groups

For Experiment 1, beta-endorphin-deficient, pro-enkephalin knockout and wild-type mice were each divided into four treatment groups differing in the dose of naloxone (0, 0.1, 1 and 10 mg/kg s.c.) administered before confinement to the 'drug-paired' chamber. For Experiments 2 and 3, pro-enkephalin knockout and wild-type mice were similarly divided into two treatment groups (U50,488, 5 mg/kg s.c. or vehicle s.c. and LiCl, 100 mg/kg s.c. or vehicle s.c., respectively). For Experiment 4, again each of the three genotypes was similarly divided into four treatment groups based on the dose of morphine (0, 2.5, 5 and 10 mg/kg s.c.) administered before confinement to the drug-paired chamber. Separate groups of animals were used for each experiment.

Statistical analysis

Analysis of initial bias between the two conditioning chambers during the habituation session was analysed by ANOVA. Analysis of potential imbalance in time spent in the future 'drug-paired' chamber during the habituation session across drug treatment and across genotype was by two-way ANOVA. Time spent in the 'drug-paired' chamber on the test day (day 5) was analysed by two-way ANOVA (genotype × drug treatment) followed by Student Neuman Keuls multiple comparison two-tailed posthoc tests ($P < 0.05$ was considered statistically significant).

Drugs

All drugs were dissolved in 0.9% filtered saline and injected in a volume of 10 mL/kg. The quoted doses of naloxone HCl and morphine SO_4 (Medisca Inc., Plattsburgh, NY, USA) are with respect to the salt in each case. The quoted dose of U50,488 CH_4SO_3 (Sigma, St. Louis, MO, USA) is with respect to the base.

Genotyping

Genotyping of mice was performed by standard polymerase chain reaction analysis of DNA obtained from mouse ear biopsies using oligonucleotide primer sequences provided by Jackson Laboratories.

Results

Experiment 1: naloxone-conditioned place aversion

Analysis of habituation data (not shown) revealed no initial bias for either of the conditioning chambers ($F_{240} = 0.70$, $P > 0.05$) and no significant imbalance in the time spent in the future drug-paired chamber across genotype ($F_{2,109} = 2.69$, $P > 0.05$) and treatment ($F_{3,109} = 0.96$, $P > 0.05$) groups.

Naloxone produced a significant place aversion in wild-type and beta-endorphin-deficient mice but had no such effect in pro-enkephalin-knockout animals (Fig. 1). Statistical analysis of time in the 'drug-paired' chamber on the test day, after place conditioning training, revealed a significant overall effect of drug treatment ($F_{3,109} = 18.13$, $P < 0.0001$), a significant effect of genotype ($F_{2,109} = 9.89$, $P < 0.0001$) and a significant treatment–genotype interaction ($F_{6,109} = 6.76$, $P < 0.0001$).

Posthoc analysis showed that wild-type mice treated with the two highest doses (1 and 10 mg/kg) of naloxone spent significantly less time in the drug-paired chamber compared with vehicle-treated animals ($P < 0.002$). Beta-endorphin-deficient mice similarly displayed significant aversion to naloxone at the two highest doses ($P < 0.0002$). The maximal aversive effect of naloxone was greater in the beta-endorphin knockout mice compared with their wild-type counterparts ($P < 0.05$) (Fig. 1).

In direct contrast, naloxone conditioning had no effect on time spent by pro-enkephalin knockout mice in the drug-paired chamber, compared with that spent by vehicle-treated animals, at any dose tested ($P > 0.05$), resulting in a significant difference in the time spent in the drug-paired chamber between pro-enkephalin knockout and both wild-type and beta-endorphin-deficient mice at the 1 and 10 mg/kg doses ($P < 0.05$; Fig. 1). This latter difference was apparent despite the unexpected observation that pro-enkephalin knockout mice in this experiment (but not in Experiments 2–4) exhibited a small but significant bias against spending time in the afternoon 'drug-paired' chamber when both conditioning chambers

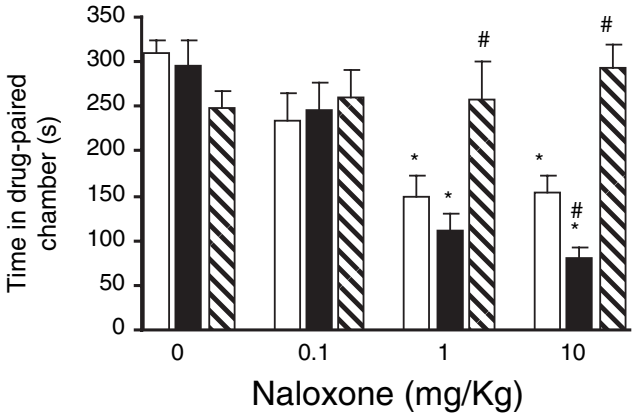

FIG. 1. Naloxone-conditioned place aversion in wild-type and beta-endorphin-deficient but not pro-enkephalin knockout mice. Time spent in the 'drug-paired' chamber during the 15-min test session on the afternoon of day 5 for wild-type (white bars), beta-endorphin-deficient (black bars) and pro-enkephalin knockout (hatched bars) mice previously conditioned to receive vehicle or naloxone in that chamber in the afternoon on days 2–4 at the doses indicated. *Statistical significance (*P*-values given in the Results) relative to vehicle-treated animals of the same genotype. #Significant difference from wild-type for the identical treatment in each case. Wild-type and beta-endorphin-deficient animals developed a significant naloxone place aversion at 1 and 10 mg/kg, whereas pro-enkephalin knockout mice failed to develop place aversion at any dose tested. Beta-endorphin-deficient mice exhibited a significantly stronger place aversion than their wild-type counterparts at the highest naloxone dose. The number of animals in each genotype group at the four doses of naloxone (0, 0.1, 1 and 10 mg/kg, respectively) were as follows: wild-type, 19, 10, 11 and 15; beta-endorphin-deficient, 13, 5, 6 and 12; pro-enkephalin knockout, 10, 7, 7 and 6.

were paired with saline. Thus, mice lacking enkephalin peptides, in contrast to their wild-type and beta-endorphin-deficient counterparts, failed to develop a CPA to naloxone.

Experiment 2: U50,488-conditioned place aversion

Analysis of habituation data (not shown) revealed no initial bias for either of the conditioning chambers ($F_{78} = 0.22$, $P > 0.05$) and no significant imbalance in the time spent in the future drug-paired chamber across genotype ($F_{1,36} = 0.15$, $P > 0.05$) and treatment ($F_{1,36} = 0.41$, $P > 0.05$) groups.

U50,488 (5 mg/kg) produced a significant place aversion in wild-type and pro-enkephalin knockout mice (Fig. 2A). Statistical analysis of time in the 'drug-paired' chamber on the test day, after place conditioning training, revealed a significant overall effect of drug treatment ($F_{1,36} = 7.92$, $P < 0.01$), no significant effect of genotype ($F_{1,36} = 0.97$, $P > 0.05$) and no significant treatment–genotype interaction ($F_{1,36} = 0.01$, $P > 0.05$). Posthoc analysis revealed a significant overall effect of drug treatment ($P < 0.01$).

Experiment 3: LiCl-conditioned place aversion

Analysis of habituation data (not shown) revealed a small but statistically significant initial bias for the cow (326 ± 10 s) over the checker (290 ± 10 s) conditioning chamber in this group of animals ($F_{68} = 2.55$, $P < 0.02$). However, randomization of the LiCl-paired chamber resulted in no significant imbalance in the time spent in the future drug-paired chamber across genotype ($F_{1,31} = 0.03$, $P > 0.05$) and treatment ($F_{1,31} = 0.06$, $P > 0.05$) groups.

LiCl (100 mg/kg) produced a significant place aversion in wild-type and pro-enkephalin knockout mice (Fig. 2B). Statistical analysis

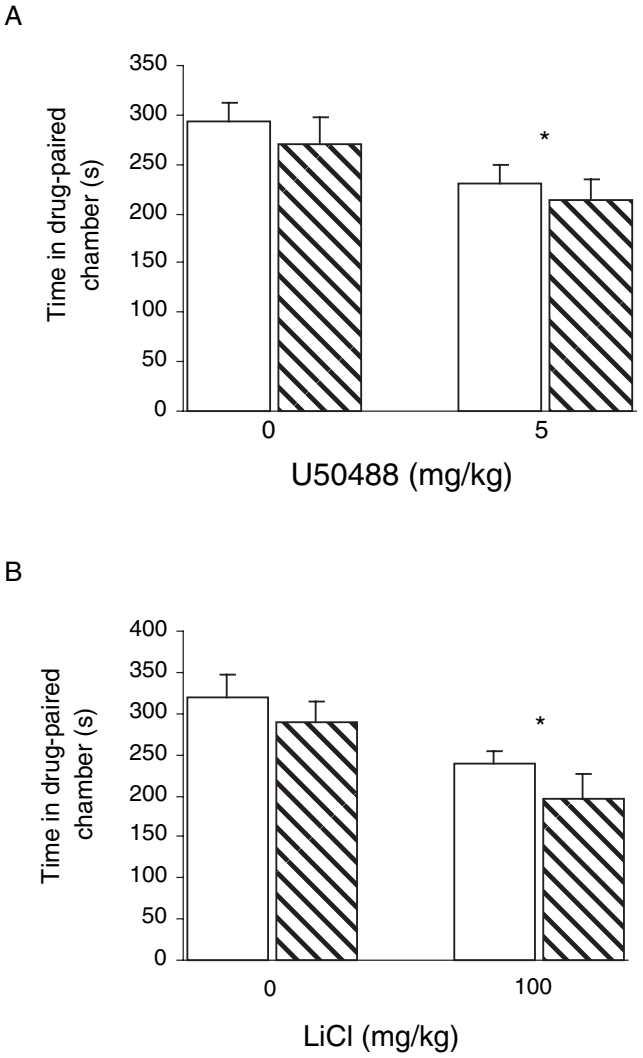

FIG. 2. U50,488- and LiCl-conditioned place aversion in wild-type and pro-enkephalin knockout mice. Time spent in the 'drug-paired' chamber during the 15-min test session on the afternoon of day 5 for wild-type (white bars) and pro-enkephalin knockout (hatched bars) mice previously conditioned to receive (A) vehicle or U50,488 or (B) vehicle or LiCl in the 'drug-paired' chamber in the afternoon on days 2–4 at the doses indicated. *Statistical significance (*P*-values given in the Results) for the overall effect of drug treatment across the genotypes. The aversive effects of U50,488 and LiCl did not differ between the two genotypes. The number of animals in each group were as follows: (A) wild-type vehicle, 12; pro-enkephalin knockout vehicle, 8; wild-type U50,488, 12; pro-enkephalin knockout U50,488, 8; (B) wild-type vehicle, 9; pro-enkephalin knockout vehicle, 8; wild-type LiCl, 10; pro-enkephalin knockout LiCl, 8.

of time in the 'drug-paired' chamber on the test day, after place conditioning training, revealed a significant overall effect of drug treatment ($F_{1,31} = 14.00$, $P < 0.001$), no significant effect of genotype ($F_{1,31} = 2.43$, $P > 0.05$) and no significant treatment–genotype interaction ($F_{1,31} = 0.06$, $P > 0.05$). Posthoc analysis revealed a significant overall effect of drug treatment ($P < 0.001$).

Experiment 4: morphine-conditioned place preference

Analysis of habituation data (not shown) revealed no initial bias for either of the conditioning chambers ($F_{268} = 0.19$, $P > 0.05$) and no significant imbalance in the time spent in the future drug-paired

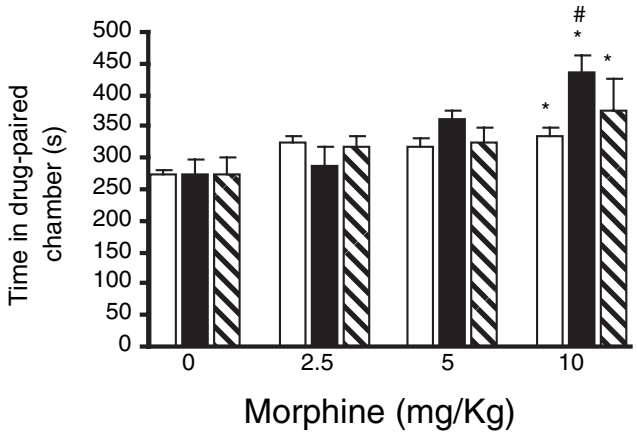

FIG. 3. Morphine-conditioned place preference in wild-type, beta-endorphin-deficient and pro-enkephalin knockout mice. Time spent in the 'drug-paired' chamber during the 15-min test session on the afternoon of day 5 for wild-type (white bars), beta-endorphin-deficient (black bars) and pro-enkephalin knockout (hatched bars) mice previously conditioned to receive vehicle or morphine in that chamber in the afternoon on days 2–4 at the doses indicated. *Statistical significance (*P*-values given in the Results) relative to vehicle-treated animals of the same genotype. #Significant difference from wild-type for the identical treatment. All three genotypes developed a significant place preference to morphine at the highest dose tested. Time spent in the morphine-paired chamber did not differ between pro-enkephalin knockout and wild-type mice at any morphine dose tested but beta-endorphin-deficient mice spent significantly longer in the morphine-paired chamber relative to wild-type at the highest dose tested. The number of animals in each genotype group at the four doses of morphine (0, 2.5, 5 and 10 mg/kg, respectively) are as follows: wild-type, 18, 16, 20 and 19; beta-endorphin-deficient, 8, 6, 8 and 8; pro-enkephalin knockout, 9, 7, 9 and 7.

chamber across genotype ($F_{2,123} = 0.08$, $P > 0.05$) and treatment ($F_{3,123} = 2.10$, $P > 0.05$) groups.

Morphine produced a significant place preference in all three genotypes (Fig. 3). Statistical analysis of time in the 'drug-paired' chamber on the test day, after place conditioning training, revealed a significant overall effect of drug treatment ($F_{3,123} = 17.83$, $P < 0.0001$), no significant overall effect of genotype ($F_{2,123} = 2.12$, $P > 0.05$) but a significant genotype–treatment interaction ($F_{6,123} = 2.61$, $P < 0.02$).

Posthoc analysis revealed that the highest dose of morphine (10 mg/kg) produced a significant place preference in all three genotypes ($P < 0.02$ in each case). The two lower doses were not statistically effective in any of the genotypes. At the 10 mg/kg dose, the time spent in the morphine-paired chamber by the beta-endorphin-deficient mice was significantly greater than that of the wild-type mice ($P < 0.0005$) (Fig. 3).

Discussion

The well-documented CPA induced by repeated naloxone administration indicates the presence of tonic activity in the endogenous opioid component of circuitry regulating basal affective state or 'hedonic homeostasis'. We previously provided evidence that such tonic activity is mediated via mu opioid receptors by demonstrating that naloxone-induced CPA is absent in mu opioid receptor knockout mice (Skoubis *et al.*, 2001). The current data implicate endogenous pro-enkephalin-derived peptides, but not beta-endorphin, as mediators of this basal tone as naloxone failed to induce CPA in pro-enkephalin knockout mice but retained this ability in beta-endorphin-deficient animals. The persistence of aversive responses to the kappa agonist, U50,488, and to LiCl indicates that the absence of pro-enkephalin does not produce a general learning deficit. Further, the exhibition of a morphine CPP by both pro-enkephalin- and beta-endorphin-deficient mice demonstrates that release of neither class of endogenous opiates is essential in the acquisition or expression of associations between environmental cues and the rewarding effect of exogenous opiate administration.

Hypotheses invoking endogenous opioid peptides as mediators of euphoria or positive affect date back to the time of their isolation in the early 1970s (Kosterlitz & Hughes, 1975). Interest in this potential role has resurfaced recently (Koob & Le Moal, 2001) due, in part, to the reconsideration of the role of dopamine in such processes [recent conceptualizations invoking dopamine as a mediator of associative learning processes or of the attribution of incentive salience to conditioned cues (Berridge & Robinson, 1998)]. The involvement of mu receptors in mediating the rewarding effects of exogenous opiates is well established both on the basis of pharmacological (Negus *et al.*, 1993; Piepponen *et al.*, 1997) and genetic (Matthes *et al.*, 1996) manipulations. Similarly, the observation that the general opioid antagonist, naloxone, is aversive whereas the delta antagonist, naltrindole, is not (Shippenberg *et al.*, 1987; Bals-Kubik *et al.*, 1989; De Vries *et al.*, 1995) and the absence of naloxone CPA in mu knockout mice (Skoubis *et al.*, 2001) all point to mu involvement in mediating endogenous opioid tone.

As all three known opioid precursor genes encode peptides with activity at the mu receptor, it is difficult, on the basis of pharmacological data alone, to unequivocally determine which peptides are being blocked by naloxone to produce aversion. To our knowledge, the only previous attempt to address this question utilized lesions of the arcuate nucleus of the hypothalamus in rats, the primary source of endorphin-containing neurons in the rodent brain. This resulted in the reversal of the aversive effect of naloxone and the conclusion that beta-endorphin mediated endogenous opioid tone regulating positive affect (Mucha *et al.*, 1985). This contrasts with the present data showing very clearly that selective genetic removal of pro-enkephalin, but not beta-endorphin, results in loss of the aversive property of naloxone. Species differences notwithstanding, the most likely explanation for this apparent discrepancy lies in the fact that destruction of the entire arcuate nucleus may have disturbed the integrity of several key neuronal circuits in addition to beta-endorphin-containing neurons and would have resulted in loss of other pro-opiomelanocortin-derived non-opioid peptides such as adrenocorticotropin and melanocyte-stimulating hormone. In contrast, the beta-endorphin-deficient mice employed in this study display intact expression of other pro-opiomelanocortin-derived peptides and normal hypothalamic-pituitary-adrenal axis function (Rubinstein *et al.*, 1996). Of course, as with all constitutive knockout studies, we cannot rule out the possibility that developmental adaptations mask an otherwise significant role for beta-endorphin in the naloxone-induced aversive process.

Although Met- and Leu-enkephalin are the most likely mediators of the apparent basal endogenous hedonic tone revealed by naloxone administration, several C-terminal extended forms of Met-enkephalin and other, larger, receptor-active sequences including peptide E, peptide F and BAM 18, potentially derived from the precursor, are also candidates (Evans *et al.*, 1986). Indeed, we have previously demonstrated that Met-enkephalinArgGlyLeu, in addition to Met- and Leu-enkephalin, is released within the pallidum using microdialysis (Maidment *et al.*, 1989). We did not investigate pro-dynorphin knockouts in this study as kappa agonists are themselves aversive (Mucha & Herz, 1985; Mucha *et al.*, 1985; Skoubis *et al.*, 2001) and thus any action of naloxone at the kappa receptor to block an endogenous dynorphin peptide tone would be predicted to alleviate rather than produce aversive effects. It must be noted, however, that

the pro-dynorphin precursor also encodes several copies of Leu-enkephalin (Evans *et al.*, 1986) that, by acting at mu receptors, could potentially contribute to a positive affective tone. The complete absence of aversion in the pro-enkephalin knockout mice would suggest otherwise, however. The potential role of endomorphins (Zadina *et al.*, 1997) in this process cannot be ruled out until such time as the precursor for these potential mu-selective endogenous ligands is identified and null mutants produced.

We can be confident that naloxone is acting within the brain to exert its aversive effect as methylated analogs of this compound, which do not readily penetrate the blood–brain barrier, do not produce CPA when administered peripherally (Hand *et al.*, 1988), whereas intracerebroventricular injections of naloxone are effective (Bals-Kubik *et al.*, 1989). The site of action of naloxone within the brain is less certain. Although the current study made no attempt to identify the brain regions involved, previous studies have implicated the ventral tegmental area and nucleus accumbens as potential sites of action for naloxone and have implicated reductions in dopaminergic activity as a mediator of the effect of naloxone (Shippenberg & Herz, 1988; Shippenberg & Bals-Kubik, 1995). However, recent studies in our laboratory indicate that naloxone may act downstream of dopamine systems to produce aversion (Narayanan *et al.*, 2004) and implicate the ventral pallidum as a potential site of action (Skoubis & Maidment, 2003), an area receiving a dense enkephalinergic input from the nucleus accumbens (Napier *et al.*, 1983; Zahm *et al.*, 1985) and possessing an abundance of mu opioid receptors, located both pre- and postsynaptically (Mansour *et al.*, 1988, 1995; Olive *et al.*, 1997). Other potential sites of action for the aversive action of naloxone include the amygdala (Stinus *et al.*, 1990) and the basal nucleus of the stria terminalis (Carr *et al.*, 1998).

An alternative explanation that we considered for the lack of an aversive response to naloxone in pro-enkephalin knockout mice was that these mutants may be inherently incapable of developing the conditioned Pavlovian associations required for place conditioning. This was shown not to be the case as pro-enkephalin knockout mice did not differ from wild-type mice in exhibiting CPA to both the kappa agonist, U50,488, and to LiCl, both of which have previously been shown to produce CPA in rodents (Shippenberg *et al.*, 1988; Skoubis *et al.*, 2001). Thus, a general deficit in associative learning cannot be responsible for the lack of CPA in these mice. It is noteworthy that the magnitude of the maximally aversive response to naloxone was greater than that of both LiCl and U50,488, emphasizing the power of the discriminative stimulus properties of naloxone. The specific nature of the internal cues elicited by blockade of endogenous enkephalin action at the mu receptor producing the conditioned avoidance behavior in the mouse is unknown. Therefore, our interpretation that such behavior reflects 'negative affect' and the inference that endogenous enkephalins are mediating 'basal positive affect' or 'hedonic tone' is necessarily somewhat vague and is based on the known euphoric effects of exogenous opiates and on reports of the dysphoric effects of naloxone in humans. It is quite possible, however, that the conditioned aversive effect of naloxone in the rodent is secondary to enkephalin involvement in other behavioral systems, e.g. basal ganglia-mediated motor control or hypothalamic-pituitary-adrenal axis response to stress, that, when blocked, results in an aversive stimulus. Future studies employing site-specific conditional knockouts may aid in examining such possibilities.

The morphine CPP experiment was conducted to examine the proposed role of endogenous enkephalin and endorphin release in morphine reward processes. We have shown previously, using microdialysis, that acute or repeated morphine or heroin administration results in a transient increase in enkephalin release in the pallidum of rats (Olive *et al.*, 1995; Olive & Maidment, 1998). It has been speculated that exposure to environmental cues previously associated with opiate administration also induces such release and that this may mediate conditioned euphoria or craving (see Gerrits *et al.*, 2003). In support of this idea, an increase in nucleus accumbens enkephalin release has been reported in rats during exposure to an environment previously paired with morphine administration (Nieto *et al.*, 2002). The present data indicate that such release of neither enkephalins nor endorphins is an absolute requirement for either the acquisition or expression of morphine CPP. However, we cannot rule out the possibility that each peptide system compensates for the absence of the other in these genetically manipulated mice or that additional compensatory systems may come into effect as a result of the absence of these peptides during development. Indeed, the increased magnitude of the CPP to the highest dose of morphine in the beta-endorphin-deficient mice may reflect subtle modulatory roles for these peptides in morphine reward. The enhanced CPA to the highest dose of naloxone observed in these mice may similarly reflect a secondary, compensatory role for the endorphin system that is activated in the wild-type only when the primary enkephalin-mediated hedonic system is perturbed by naloxone.

Although our data suggest that these endogenous opioid peptides are not required for expression of morphine reward in the context of pavlovian conditioning, recent evidence suggests that enkephalins and endorphins are important components of processes driving the organism to work for natural reinforcement. Hayward *et al.* (2002) showed that mice deficient in either or both groups of peptides were less prepared to work for food on a progressive ratio instrumental task. Interestingly, this was not the case in a food-deprived state, leading the authors to conclude that both of these opioid peptides are involved in the hedonics of feeding. This is distinct from our finding that pro-enkephalin-derived peptides, rather than beta-endorphin, are the primary mediators of basal hedonic tone and may reflect differential involvement of these groups of peptides in basal vs. stimulus-induced hedonia.

In summary, the absence of naloxone CPA in pro-enkephalin knockout mice and the persistence of such behavior in beta-endorphin-deficient mice indicate that endogenous enkephalins, but not endorphins, may mediate a 'basal hedonic tone' in mice. The persistence of morphine CPP in both opioid-deficient genotypes studied indicates that endogenous release of these peptides is not essential in the acquisition, recall or expression of conditioned associations between the rewarding effect of exogenous opiates and the context of their administration.

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Abbreviations

CPA, conditioned place aversion; CPP, conditioned place preference.

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