High-Efficacy 5-Hydroxytryptamine 1A Receptor Activation Counteracts Opioid Hyperalldynia and Affective Conditioning

Francis C. Colpaert, Kristof Deseure, Luis Stinus, and Hugo Adriaensen

Centre de Recherche Pierre Fabre, Castres, France (F.C.C.); Laboratory of Anesthesiology, University of Antwerp, Antwerp, Belgium (K.D., H.A.); and Centre National de la Recherche Scientifique, Université Victor Ségalen Bordeaux II, Bordeaux, France (L.S.)

Received September 2, 2005; accepted October 26, 2005

ABSTRACT

Pain may become intractable as tolerance develops to opioids and the opioids, paradoxically, induce pain. We examined the hypothesis that the analgesia produced by the novel analgesic and high-efficacy 5-hydroxytryptamine (5-HT)1A receptor agonist (3-chloro-4-fluoro-phenyl)-[4-fluoro-4-[[5-(methyl-pyridin-2-ylmethyl)-amino][methyl]piperidin-1-yl]methanone, fumaric acid salt (F 13640) may counteract opioid-induced pain. In studies of the somatosensory quality of pain in infraorbital nerve-injured rats, morphine infusion (5 mg/day) by means of osmotic pumps initially caused analgesia (i.e., decreased the behavioral response to von Frey filament stimulation), followed by hyperalldynia and analgesic tolerance. Infusion of F 13640 (0.63 mg/day) prevented the development of opioid hyperalldynia and reversed opioid hyperalldynia once established. In studies of the affective/motivational quality of pain, F 13640 both prevented and reversed the conditioned place aversion induced by naloxone (0.04 mg/kg i.p.) in morphine-infused rats; F 13640 also prevented and reversed the conditioned place preference induced by morphine injections (7.5 mg/kg i.p.). The data confirm that opioids produce bidirectional hypo- and proalgesic actions, and offer initial evidence that high-efficacy 5-HT1A receptor activation counteracts both the sensory and the affective/motivational qualities of opioid-induced pain. The data also indicate that F 13640 may be effective with opioid-resistant pain. It further is suggested that opioid addiction may represent self-therapy of opioid-induced pathological pain.

Although opioids produce powerful pain relief, pain may become intractable as tolerance develops to opioid analgesia; it has been proposed that this is because opioids may, paradoxically, induce pain. Indeed, a concept of signal transduction in central pain-processing systems (Colpaert, 1996) specifies that any input to such systems causes not a single effect but two dual effects that are bidirectional, or opposite in sign. Thus, morphine eventually causes not only analgesia as a “first order” effect but also a “second order” hyperalgesia that outlasts opioid receptor activation for some time. Upon chronic opioid exposure, the second order pain, or sensitization to nociceptive input, grows and neutralizes the first order analgesia. Considerable evidence now indicates that tolerance to opioid analgesia results from opioid-induced pain (“opiod pain”; here used to refer to hyperalgesia, alldynia, and hyperalldynia; Colpaert, 1996; Mao, 2002; Ossipov et al., 2003).

Opioid pain is comprehensive, encompassing both the “physiological” (e.g., nociceptive) and the more recently identified (Scholz and Woolf, 2002) “pathological” (e.g., neuropathic) types of pain. Indeed, opioids not only produce hyperalgesia (i.e., an enhanced response to nociceptive stimulation; Colpaert, 1996) but also neuropathic-like alldynia (i.e., pain behaviors in response to innocuous stimuli; Ossipov et al., 2003). In human, opioid pain occurs after the intraoperative use of opioids (Guignard et al., 2000; Luginbühl et al., 2003) as well as in methadone maintenance patients (Jamison et al., 2000; Rosenblum et al., 2003). In human, opioid pain occurs after the intraoperative use of opioids (Guignard et al., 2000; Luginbühl et al., 2003) as well as in methadone maintenance patients (Jamison et al., 2000; Rosenblum et al., 2003).

The transduction concept further implies that nociceptive stimulation produces not only first order pain but also second order analgesia, and we recently discovered high-efficacy 5-HT1A receptor activation as a molecular mechanism that produces such effects and affords remarkable pain relief (Colpaert et al., 2002; Deseure et al., 2003; Wu et al., 2003). The methylamino-pyridine F 13640 is a high-affinity, selective, and, most importantly, very high-efficacy ligand at 5-HT1A receptors. The agent possesses nanomolar affinity for rat and...
human 5-HT_{1A} receptors, whereas at 1000-fold higher concentrations it does not interact with a host of other target proteins. With 5-HT_{1A} receptors, F 13640 stimulates [35S]guanosine 5'-O-(3-thio)triphosphate binding in Cos-7 and C6-glial cells by 93 and 75%, respectively (5-HT = 100%), and its in vivo effects are reversed by the selective 5-HT_{1A} antagonist WAY 100635 (Colpaert et al., 2002). F 13640 induces effects that inversely mirror those of opioids, where morphine causes analgesia followed by hyperalgesia, F 13640 causes hyperalgesia followed by analgesia. As treatment is repeated/continued and where morphine causes incremental pain at the expense of analgesia (i.e., apparent tolerance), F 13640 causes inverse tolerance; its analgesic effect grows at the expense of hyperalgesia (Colpaert et al., 2002; Deseure et al., 2003). Furthermore, much as opioid pain and analgesic tolerance persist after the discontinuation of opioid treatment (Colpaert, 1996), the discontinuation of F 13640 treatment produces persistent pain relief (Deseure et al., 2003; Wu et al., 2003).

Here, we hypothesized that high-efficacy 5-HT_{1A} receptor activation may prevent and reverse opioid pain. This was studied in rats that developed mechanical allodynia upon chronic constriction injury of the infraorbital nerve (IoN-CCI). Although opioids may alleviate neuropathic as well as nociceptive pain, in morphine-infused IoN-CCI-lesioned rats, the initially marked analgesia decays within 2 weeks (Deseure et al., 2003), thus setting an opportunity to study the actions of pain at the expense of analgesia (i.e., apparent tolerance), F 13640 causes inverse tolerance; its analgesic effect grows at the expense of hyperalgesia (Colpaert et al., 2002; Deseure et al., 2003). Furthermore, much as opioid pain and analgesic tolerance persist after the discontinuation of opioid treatment (Colpaert, 1996), the discontinuation of F 13640 treatment produces persistent pain relief (Deseure et al., 2003; Wu et al., 2003).

Materials and Methods

Subjects

Male Sprague-Dawley rats, weighing 220 to 240 g on arrival, were obtained from Charles River (Brussels, Belgium) and Charles River (Lyon, France) for the IoN-CCI and place-conditioning studies, respectively. The animals were housed by four in Makrolon cages located in colony rooms (temperature 21.5 ± 1°C; relative humidity 50 ± 10%). They had food and water available ad libitum and were kept under a reversed 12:12 h dark/light cycle (lights on at 8:30 PM). Animals were treated and cared for according to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

IoN-CCI Injury

Study Design. Rats on day 0 underwent a unilateral (left side) constriction injury of the infraorbital nerve and were allowed to develop allodynia for 24 days. On day 24, the animals were implanted subcutaneously with two osmotic pumps. Both pumps were replaced 2 weeks later and again another 2 weeks later (i.e., on days 38 and 52). Seven groups (n = 12/group) differed according to the treatments that were so delivered during these three 2-week periods. One group (the all-saline control group) received saline via the two pumps throughout the study; the other six (experimental) groups received different treatments as specified in the horizontal bars in Fig. 1, A to F. von Frey filament assessments of mechanical allodynia were conducted 1 day before the lesioning, on day 24 (a Friday, on which the first set of pumps was implanted) as well as on Monday, Wednesday, and Friday during the six after weeks. On these occasions, body weight was also determined (data not shown).

Injury. As detailed previously (Deseure et al., 2003), rats were anesthetized with pentobarbital (60 mg/kg i.p.) and atropine (0.1 mg/kg i.p.). Then, they were mounted in a stereotaxic frame and had the infraorbital part of the infraorbital nerve exposed unilaterally and dissected free. Two chronic catgut ligatures (5-0, Ethicon; Johnson & Johnson, Brussels, Belgium) were loosely tied around the nerve, 2 mm apart. The scalp incision then was closed using polyester sutures (4-0, Ethicon).

Osmotic pump implantation was carried out as described previously (Deseure et al., 2003). In brief, rats were placed for 3 to 4 min in an induction cage under 4% (v/v) isoflurane, shaved, and then placed under a mask delivering 2.5% (v/v) isoflurane. The pump (nominal pump rate 5 μl/h; ALZET model 2ML2; Alza Corp., Palo Alto, CA) was inserted through a transversal incision in the skin of the lower part of the back, its aperture directed toward the head. For pump replacement, a new incision was made approximately 1 cm from the previous incision.

von Frey Assessments. As described previously (Deseure et al., 2003), a graded series of five von Frey filaments (pressure aesthesiometer, Stoelting Co., Chicago, IL) were applied in ascending order, requiring a force to bend the hairs that ranged from 0.015 to 2.150 g. The filaments were applied ipsilaterally to the injured nerve within the territory of the nerve, near the vibrissae pad’s center, on the hairy skin surrounding the mystacial vibrissae. In a blind manner, responses were scored 0 to 4 (Deseure et al., 2003) and averaged to yield a single value per rat and per time point. The response was observed to belong to one of the following categories: score 0, no response; score 1, detection, the rat turns the head toward the von Frey filament, and the filament is then explored; score 2, withdrawal reaction, the rat turns the head slowly away or pulls it briskly backward when the stimulation is applied, sometimes a single face wipe occurs ipsilaterally; score 3, escape/attack, the rat avoids further contact with the filament, either by moving its body away from the filament to assume a crouching position against the cage wall, or by attacking the filament, making grabbing and biting movements; and score 4, asymmetric face grooming, the rat displays an uninterrupted series of at least three face-wash strokes directed toward the stimulated facial area.

Treatments. F 13640, synthesized in-house, and morphine HCl (Belgapio, Louvain la Neuve, Belgium) were infused by osmotic pumps at 0.63 and 5 mg/day, respectively; these doses correspond with the highest concentration at which either agent is water-soluble and stable (Colpaert et al., 2002) and exerts analgesia in the IoN-CCI model (Deseure et al., 2003). Note that this morphine dose (∼17 mg/kg/day) also induces somatic dependence (Colpaert et al., 2002).

Analyses. As in previous studies (Deseure et al., 2003) postoperative changes of baseline values before drug treatment were analyzed by means of repeated measures ANOVA with time (i.e., rats were tested 1 day before and 24 days after IoN surgery) as within-subjects factor and group (rats were divided into seven experimental groups) as between-subjects factor. No baseline differences were found between the different groups. Changes in the all-saline group during the entire day 27 to 66 treatment period were analyzed by means of repeated measures ANOVA with time (i.e., rats were tested...
Data obtained during each of the three 2-week treatment periods were analyzed by means of repeated measures ANOVA with time (i.e., rats were tested during each period on six different time points) as within-subjects factor and group (i.e., rats were divided into seven experimental groups) as between-subjects factor. These analyses were followed by a unifactorial ANOVA per time point and Student-Newman-Keuls pairwise multiple comparison tests.

Fig. 1. Effects of serial infusions of morphine (5 mg/day), F 13640 (0.63 mg/day), saline, and associations thereof, on nerve lesion-induced alldynia. Rats received a chronic constriction injury of the infraorbital nerve, and 3 weeks were allowed for alldynia to develop. On postinjury day 24, the animals were randomly assigned to one of seven groups (n = 12/group). One of these groups, the all-saline control group, was implanted subcutaneously on day 24 with two 2-week osmotic pumps releasing saline; the two pumps were replaced by another two saline-releasing pumps on day 38 and again on day 52. The other six (experimental) groups were similarly implanted, and reimplanted, with two pumps that released different treatments as specified in the horizontal bars in A to F. Data points are the mean (± S.E.M.) response score to von Frey filament stimulation (scores varied from 0 to 4) as observed at specified times after surgery. Different panels indicate data obtained with the corresponding experimental group as well as those from the all-saline controls that occur in each panel (black triangles). Symbols (asterisks and minuscules) refer to differences revealed by post hoc analyses that were conducted for every (27–66) day postinjury. Asterisks refer to differences compared with the all-saline groups, minuscules (a–f) to differences compared with other experimental groups (A–F); one, two, and three symbols indicate p < 0.05, p < 0.01, and p < 0.001, respectively. In any given panel, statistical significance is reported for the comparison with preceding, not with following panels.
Place-Conditioning Studies

Place-conditioning studies were conducted in material conditions that were similar to those used in the IoN-CCI studies.

Conditioning Apparatus. CPA and CPP were evaluated in a three-compartment apparatus (Imetronic, Pessac, France) that consisted of three rectangular compartments (40 × 33 × 35 cm, length × width × height) spaced at 120° angles, each compartment being accessible from a triangular central zone. The compartments differed in terms of wall coloring and floor texture and were equipped with infrared photocells that determined the animal's position at all times (Caille et al., 1999). The apparatus was situated in a sound-attenuating room with white masking noise (75 dB) and illuminated by a 15-W red light located 1.5 m above the apparatus.

Conditioned Place Aversion. Rat were implanted (day 0) with a first set of two osmotic pumps, one of which released morphine; the second pump released saline in one (control) group and F 13640 in another group. On day 4, rats received an injection of saline; immediately thereafter, their spontaneous (baseline) preference was determined by having them freely explore the apparatus for 20 min. Animals demonstrating strong spontaneous aversion (less than 17% of the session time, i.e., <200 s) or preference (more than 50%; >600 s) for any compartment, were discarded. For each remaining rat, the two compartments with the most similar time allotments were chosen for conditioning. One of them was randomly chosen to be paired to naloxone and the other to saline; the remaining compartment was either the most or the least preferred of the three. Importantly, after the compartment assignments were completed, there were no significant differences between the times spent in the compartments that were to be paired with naloxone and saline, thus avoiding any preference/aversion bias before conditioning.

Conditioning consisted of three pairings of saline and naloxone with the respective compartment allotments. On days 5, 10, and 12, rats received a saline injection immediately before being confined to their preselected saline-paired compartment for 20 min; on days 6, 11, and 13, rats received an injection of 0.04 mg/kg naloxone immediately before confinement in the naloxone-paired compartment for 20 min.

Tests of the acquisition (on day 14) and maintenance (day 28) of CPA consisted of having the animals freely explore the entire apparatus for 20 min immediately after saline injection. On day 14, after the acquisition test, the first set of pumps in both groups was replaced by a second set, one of which released saline; the second pump released either F 13640 or saline.

Conditioned Place Preference. Animals were implanted (day 0) with one pump that released either saline or F 13640; in animals having been infused with saline during CPP acquisition, this pump was replaced (on day 14, after the CPP acquisition test) with a pump that released either saline or F 13640. Baseline preference was determined on day 4, 15 min after saline injection, and animals were selected and compartments were allotted as described above.

Conditioning consisted of three pairings of saline and morphine with the respective compartment allotments. On days 5, 7, and 11, rats received a saline injection 15 min before being confined to their preselected saline-paired compartment for 30 min; on days 6, 8, and 12, rats received an injection of 7.5 mg/kg morphine 15 min before their 30-min confinement in the morphine-paired compartment. Tests of the acquisition (day 14) and maintenance (day 28) of CPP consisted of having the animals freely explore the entire apparatus for 20 min, 15 min after an injection of either saline or 7.5 mg/kg morphine.

Treatments. Pump-delivered F 13640 and morphine doses were identical to those in the IoN-CCI studies. The choices of doses of naloxone HCl (0.04 mg/kg; Sigma, Paris, France) and morphine HCl (7.5 mg/kg; Cooper, Mulhouse, France), of the injection-session intervals, and of the duration of CPA/CPP sessions were based on preliminary experiments that determined optimal conditions and, with naloxone, ensured adequate CPA in the absence of any overtly observable signs of dependence (e.g., diarrhea, wet dog shakes, and abnormal posture). Doses refer to the free base weight; injections were given intraperitoneally (i.p.) in a 1 mg/100 g body weight volume.

Analyses. Data analyses were carried out in a similar manner as for the IoN-CCI studies. Specifically, times spent in the naloxone or morphine compartments were analyzed by means of repeated measures ANOVA with time (i.e., rats were tested before and after conditioning) as within-subjects factor and group (i.e., pump and/or injection treatments) as between-subjects factor. These analyses were followed with post hoc comparisons according to Student-Newman-Keuls method.

Results

IoN-CCI Injury Studies

Twenty-four days after injury, the response score to von Frey filament stimulation had increased to 3.2 ± 0.13 from a baseline of 1.5 ± 0.074 (n = 48; F(1,77) = 1969.4; p < 0.001). During the day 27 to 66 period, the score in all-saline controls decreased slightly but significantly (F(1,17,170) = 3.66; p < 0.001; by 0.24 units).

There were significant differences between the treatment groups (Fig. 1, A–F) in each of the three 2-week treatment periods. Doses refer to the free base weight; injections were given intraperitoneally (i.p.) in a 1 mg/100 g body weight volume.
sia in the third treatment period after the discontinuation of F 13640 treatment (Fig. 1, D and E, right) is likely because of the persistent action of F 13640 on both nerve lesion- and morphine-induced allodynia.

**Place Conditioning**

**Conditioned Place Aversion.** Two-way repeated-measures ANOVA of acquisition data indicated a significant effect of group \([F(1,78) = 10.0; p < 0.01]\), test day [i.e., preconditioning and acquisition tests; \(F(1,78) = 23.5; p < 0.001\)], and group \(	imes\) day interaction \([F(1,78) = 6.7; p < 0.05]\). Post hoc analyses using the Student-Newman-Keuls pairwise multiple comparison tests indicated that significant CPA to naloxone developed in (control) rats infused with morphine and saline (MS), but not \((p > 0.05)\) in animals that were infused with F 13640 in addition to morphine (MF; Fig. 2A). In addition, during the acquisition test, the latter animals spent significantly more time in the naloxone-associated compartment than controls.

Analysis of CPA maintenance data indicated a significant effect of treatment \([F(3,40) = 5.7; p < 0.01]\), test day [i.e., baseline, acquisition, and maintenance tests; \(F(2,80) = 11.4; p < 0.001\)], and treatment \(	imes\) day interaction \([F(6,80) = 2.4; p < 0.05]\). The outcomes of pertinent post hoc analyses are provided in Fig. 2B. Most importantly, after significant CPA had developed in rats that had been infused with morphine and saline during acquisition, this aversion was maintained if these animals were infused with saline for another 2 weeks (MS-SS), but not \((p > 0.05)\) if they were infused with F 13640 (MF-SF). In addition, during the maintenance test, the latter animals spent significantly more time in the naloxone-associated compartment than controls. Animals that had received both morphine and F 13640 at the time of conditioning and that had then failed to develop CPA, also failed to demonstrate \((p > 0.05)\) CPA during the maintenance test, regardless of whether they did (MF-SF) or did not (MF-SS) continue to receive F 13640 at that time.

**Conditioned Place Preference.** ANOVA of acquisition data indicated a significant effect of group \([F(3,99) = 4.8; p < 0.01]\), test day \([F(1,99) = 31.8; p < 0.001]\), and group \(	imes\) day interaction \([F(3,99) = 4.0; p < 0.01]\). Post hoc analysis (Fig. 3A) indicated that the conditioning effectively made saline-infused (control; S) rats to develop CPP for the morphine-associated compartment; this CPP was significant when tested for upon saline injections (s; group Ss) and was even larger when tested for upon morphine injection (group Sm). In contrast, no significant CPP developed \((p > 0.05)\) in animals that were infused with F 13640, regardless of whether they were tested after an injection of saline (Fs) or morphine (Fm). In addition, when tested after morphine injection, saline-infused rats (Sm) spent more time in the morphine-associated compartment than F 13640-infused animals (Fm).

ANOVA of CPP maintenance data indicated a significant effect of group \([F(3,46) = 4.5; p < 0.01]\), test day [i.e., preconditioning and acquisition tests; \(F(1,46) = 28.1; p < 0.001\)], and group \(	imes\) day interaction \([F(3,46) = 3.7; p < 0.05]\). Post hoc analysis (Fig. 3B) indicated that in (control) animals being infused with saline both during and after conditioning, CPP persisted 2 weeks after the conditioning; again, this CPP was larger when tested after the injection of morphine (S-Sm) compared with that of saline (S-Ss). In contrast, animals in which CPP had developed but in which its maintenance was tested whereas F 13640 was infused no longer demonstrated CPP \((p > 0.05)\) when tested after a saline injection (S-Fs); when tested after a morphine injection (S-Fm), such animals then demonstrated a CPP that was modest and smaller than that found in similarly tested (S-Sm) control animals.

**Discussion**

The findings indicate that the selective, high-efficacy 5-HT\(_{1A}\) agonist F 13640 significantly prevents both opioid hyperalldyony and analgesic tolerance in rats, demonstrating allodynia after infraorbital nerve injury. F 13640 also reverses opioid hyperalldyony and re-enables opioid analgesia. Conditioning studies show that F 13640 prevents and reverses the aversive affective/motivational quality of opioid

![Fig. 2. Effects of F 13640 on conditioned place aversion produced by opioid withdrawal. A, acquisition: rats were implanted with two 2-week osmotic pumps releasing morphine (5 mg/day) and saline (MS; n in parentheses) or the same morphine dose and F 13640 (0.63 mg/day; MF); using a three-compartment apparatus, i.p. injections of saline and (0.04 mg/kg) naloxone were associated with the placement of the rat in the saline- and the naloxone compartment, respectively. Data (mean ± 1 S.E.M.) represent the time (in seconds) spent in the naloxone-associated compartment during test sessions that took place before (white columns) or after (gray columns) conditioning had been administered. B, maintenance: rats received MS or MF pumps and underwent conditioning as described in A; thereafter, the two pumps were replaced by two 2-week pumps (SS or SF), and conditioning effects again were tested 14 days later (black columns). , p < 0.05; , p < 0.01; and , p < 0.001.](https://jpet.aspetjournals.org/doi/abs/10.1124/jpet.17-9207)
The data support the concept attributing these actions to a desensitization to nociceptive stimulation. The concept (Colpaert, 1996) suggests that opioid pain and analgesic tolerance involve a single process of sensitization to nociceptive input and that this sensitization is counteracted by nociceptive stimulation. These F 13640 actions persisted upon discontinuation of treatment. This is consistent with the concept attributing these actions to a desensitization to nociceptive input that should outlast the (5-HT\textsubscript{1A}) receptor activation much in the same manner as opioid tolerance outlasts (\mu-opioid) receptor activation. In support of this, the intrinsic analgesic action of F 13640 persisted upon treatment discontinuation and at a time that plasma levels have become undetectable (Wu et al., 2003).

That effects of F 13640 mimic those of nociceptive stimulation is evidenced by data that, not unlike the local injection of such noxious chemicals as formalin and carrageenin, the compound lowers the threshold for mechanical stimulation of the hindpaws to produce vocalization (Colpaert et al., 2002) and induces the expression of c-Fos protein in spinal cord dorsal horn neurons (Buritova et al., 2003). To our knowledge, there is no direct data to determine whether, like F 13640, nociceptive stimulation can counteract opioid pain; however, considerable evidence indicates that such stimulation does counteract the development of tolerance to opioid analgesia (Colpaert, 1996).

Both the sensory and affective/motivational qualities of pain are regulated by ascending and descending neuromodulatory systems (Hunt and Mantyh, 2001). The affective/motivational quality is evidenced by data that pain sustains conditioned preference/aversion and that pain relief reverses such conditioning (Colpaert et al., 1982, 2001; Johansen et al., 2001). Conditioned preference/aversion can offer a highly specific assessment of pain; in rats with adjuvant arthritis, the conditioned taste preference for an opioid is selectively associated with persistent pain, not with the physical dependence and intrinsic reward that opioids may also produce (Colpaert et al., 2001).

CPA studies were conducted in conditions that generate opioid pain. In these conditions, with the sensory quality of pain, F 13640 itself produces analgesia, and does so even while tolerance otherwise develops or has developed to morphine, and hence prevents and reverses the sensory quality of opioid pain. Thus, the CPA studies determined whether because of the analgesia that it produces, F 13640 can similarly counteract the affective/motivational qualities of morphine’s action. The data indicate that F 13640 prevents and reverses opioid CPA, suggesting that this CPA results from opioid pain.

Acute opioid injections also produce a conditioned place/taste preference that is presumably mediated by intrinsic reward and dependence (Shippenberg, 1993; Hutcheson et al., 2001). However, the concept cited above implies that opioid pain and tolerance begin to progressively develop at withdrawal; F 13640 similarly prevents and reverses morphine-induced CPP. The data suggest that 5-HT\textsubscript{1A} activation counteracts opioid pain.

As described previously (Deseure et al., 2003), morphine infusion produced analgesia; tolerance then developed so that analgesia was no longer significant after 2 weeks. Morphine withdrawal at this point induced a hyperalldynia that required approximately a week to dissipate. When morphine treatment was instead continued, no significant hyperalloydina was apparent, indicating that pain sensitivity had been rendered morphine-dependent. This finding of morphine-induced hyperalldynia extends evidence (see Introduction) of opioid-induced hyperalgesia and allodynia.

F 13640 prevented and reversed morphine-induced hyperalldynia. To the extent that 5-HT\textsubscript{1A} activation mimics the central effects of nociceptive stimulation (Colpaert et al., 2002; Buritova et al., 2003), these findings can be understood from a concept of nociceptive signal transduction. The concept (Colpaert, 1996) suggests that opioid pain and analgesic tolerance result from a single process of sensitization to nociceptive input and that this sensitization is counteracted by nociceptive stimulation. These F 13640 actions persisted upon discontinuation of treatment. This is consistent with the concept attributing these actions to a desensitization to nociceptive input that should outlast the (5-HT\textsubscript{1A}) receptor activation much in the same manner as opioid tolerance outlasts (\mu-opioid) receptor activation. In support of this, the intrinsic analgesic action of F 13640 persisted upon treatment discontinuation and at a time that plasma levels have become undetectable (Wu et al., 2003).

Fig. 3. Effects of F 13640 on morphine-induced conditioned place preference. A, acquisition: rats were implanted with a 2-week osmotic pump releasing saline (S) or 0.63 mg/day of F 13640 (F); using a three-compartment apparatus, i.p. injections of saline or 7.5 mg/kg morphine were associated with the placement of the rat in the saline and the morphine compartment, respectively. Data (mean ± 1 S.E.M.) are the time (in seconds) spent in the morphine-associated compartment during test sessions that took place before (white columns) or after conditioning had been administered (gray columns). After-conditioning (i.e., acquisition) tests were conducted upon the injection of either saline (s; in Ss and Fs groups) or that of 7.5 mg/kg morphine (m; in Sm and Fm groups; n in parentheses). B, maintenance: rats that had acquired CPP while being infused with saline-releasing pumps were reimplanted with 2-week pumps releasing either saline (S-S) or 0.63 mg/day F 13640 (S-F) and tested 14 days later (black columns) for CPP after an injection of either saline (s; in Ss-Ss and Fs-Fs groups) or 7.5 mg/kg morphine (m; in Ss-Sm and Fs-Fm groups). *, p < 0.05; **, p < 0.01; ***, p < 0.001 (after-conditioning test data are shown but not included in data analysis as they all pertain to animals having similarly developed CPP while saline-infused).
the very outset of opioid receptor activation and emerge even upon initial exposure (Colpaert, 1996). Thus, any opioid treatment, such as a single morphine injection, produces long-lasting pain (Colpaert, 1996; Bruins Slot and Colpaert, 1999a). In addition, any subsequent injection is expected to produce a temporary but appreciable relief from opioid pain, albeit only to aggravate this pain thereafter in a spiraling manner. Thus, to the extent that morphine’s positive-afffect action results from the opioid temporarily relieving opioid pain, the analgesic effect of F 13640 on opioid pain (Fig. 1) can be expected to counteract that action. Indeed, F 13640 counteracts pain-associated conditioned taste preference for an opioid in arthritic rats (Colpaert et al., 2002). Our data indicate that F13640 both prevents and reverses morphine-induced CPP, suggesting this CPP to be mediated by opioid pain. Consistent with this, neuronal systems that have traditionally been associated with pain processing also mediate bidirectional affective states (Hirakawa et al., 2000; Laviolette et al., 2004).

Opioid CPA/CPP is commonly considered to represent a core feature of opioid addiction (Koob and Le Moal, 1997). Indeed, it has been hypothesized (Colpaert, 1996; Bruins Slot and Colpaert, 2003) that opioid pain, and the memory retrieval thereof, may underlie addiction. The following supports a relationship between opioid pain and addiction. The affective quality of pain acts as a motivational drive (Scholz and Wolf, 2002); addiction is similarly characterized by affective qualities (Frenois et al., 2002) that drive opioid self-administration (Shippenberg, 1993). Conversely, in the presence of pain, opioid self-administration is driven by pain relief (Colpaert et al., 2001). That opioids induce the affective/motivational quality of pain complements evidence that opioids induce the sensory quality of pain and like its sensory proalgesic effects, aversive affective/motivational effects of morphine in rat persist hours after a single injection (Parker et al., 2002). The affective quality of severe chronic pain is prevalent and persistent in opioid addicts (Jamison et al., 2000; Rosenblum et al., 2003). Like opioid addiction (Koob and Le Moal, 1997), opioid pain develops in a progressive, insidious manner (Colpaert, 1996); much as the desire for opioids in addicts can eventually become “compulsive” (Berke and Hyman, 2000), evidence suggests that anatomically diffuse opioid pain in humans can become “excruciating” (Bruins Slot et al., 2002).

Memory plays an important role in both addiction (Berke and Hyman, 2000) and pain (Malcangio and Lessmann, 2003). Indeed, central sensitization in pain-processing somatosensory neurons and long-term potentiation share similar mechanisms of synaptic plasticity (Ji et al., 2003). In particular, as morphine produces first order analgesia and second order pain, it also produces two distinct memory states during which analgesia and pain, respectively, prevail (Bruins Slot and Colpaert, 1999a,c). The second order, long-lasting memory state of morphine sets the opportunity for the encoding of morphine-induced pain in that state; once this encoding is established, subsequent opioid administrations reinstate retrieval (e.g., of pain; Bruins Slot and Colpaert, 1999b,c) and render retrieval increasingly dependent on that state (Bruins Slot and Colpaert, 2003). This state-dependent retrieval may explain how subsequent opioid administrations reinstate pain in terms of both its sensory (Sokolowska et al., 2002), and, as here (Fig. 3B), affective/motivational qualities (i.e., in a CPP paradigm; Mueller et al., 2002).

Reinstatement also occurs when an opioid is administered to withdrawn subjects with a self-administration history, raising the question as to whether opioid pain co-mediates self-administration. Opioid pain occurs even upon single administration and can be conditioned to “morphine onset cues” to cause intra-administration associations (Sokolowska et al., 2002). Indeed, previous experience with heroin in withdrawal—at a time that opioid pain likely prevails—is necessary for subsequent heroin-seeking behavior to be enhanced when rats again experience withdrawal (Hutcheson et al., 2001). Although it is felt (Hutcheson et al., 2001) that intrinsic reward is required for self-administration at least at the time of its acquisition, evidence cited above indicates that even a first opioid injection causes lasting pain. In drug-naive volunteers, opioid self-administration occurs only to the extent that they receive painful stimulation, and the latter abolishes opioid-induced, positive-affect “elation” (Zaeny et al., 1996). Thus, as suggested above with opioid CPP, opioid self-administration may at least partly be driven by opioid onset cues that predict the relief, however short-lived, of opioid pain. Although not excluding that somatic withdrawal (Shippenberg, 1993) and intrinsic reward (Hutcheson et al., 2001) may exert motivational effects, we suggest that opioid addiction to some extent represents self-therapy of opioid-induced pain the affective quality of which persists because of it being encoded in a long-term memory state.

Both a theory and evidence (Colpaert, 1996; Colpaert et al., 2002) suggest that the neuroadaptive actions on pain processing that evolve upon μ-opioid or 5-HT1A receptor activation arise from the mechanisms of signal transduction in central pain-processing systems. Any input to such systems causes both a first order as well as a contraddirectional second order effect; with chronicity, the second order effect becomes amplified, masks the first order effect, and induces long-term hyperalgesia and apparent tolerance or hyperalgesia and inverse tolerance (with μ-opioid and 5-HT1A receptor activation, respectively).

With opioids, this neuroadaptive reversal of effect occurs also with large in vivo doses or in vitro concentrations and likely originates at a subcellular level; during continued opioid exposure, a (stimulatory-to-inhibitory) reversal of effect can be observed with the release of unbound Ca2+ in CHO-Ki cells transfected with G protein-coupled μ-opioid receptors (Bruins Slot et al., 2002). The molecular and cellular mechanisms of the sign reversal of opioid effects involve a shift from predominantly Gαi inhibitory to Gβγ stimulatory adenyl cyclase signaling following chronic morphine (Chakrabarti et al., 2005) and also operate (Varga et al., 2003) in the ventral tegmental area where opioid receptor activation reverses reward signaling by switching the conductance properties of a discrete population of GABA receptors from an inhibitory to an excitatory mode (Laviolette et al., 2004).

5-HT1A receptor-mediated cellular adaptations have been studied less extensively. However, autoreceptors in the anterior raphe nuclei and postsynaptic receptors in hypothalamic nuclei and the amygdala, but not hippocampal and cerebral cortex receptors, have been found to desensitize upon repeated or continuous activation (Hensler, 2003).

Nociceptive stimulation powerfully modulates the activity...
of supraspinal, mesolimbic and other neuroanatomical regions that are thought to govern reward (i.e., amygdala, ventral tegument/periaqueductal gray, and nucleus accumbens; Becerra et al., 2001). In the rostral ventromedial medulla, ON, OFF and neutral cells projecting to the spinal and trigeminal dorsal horn modulate pain processing; manipulations of these neurons produce bidirectional, pro- and hypoalgesic actions the sensory and affective qualities of which seem to be closely matched (Hirakawa et al., 2000). Such modulation can be reinstated by re-exposure to the environment to which pain-induced aversion had previously been conditioned (Lei et al., 2004). The amygdala is involved in the formation of affective memories (Richardson et al., 2004). Serotonin plays an important role in amygdala function; acting via 5-HT<sub>1A</sub> receptors, 5-HT depresses excitatory synaptic transmission and depolarization-evoked Ca<sup>2+</sup> influx in the basolateral amygdala (Cheng et al., 1998), and 5-HT<sub>1A</sub> activation in amygdala slice preparations inhibits the induction of long-term potentiation (Pollandt et al., 2003). Intramygdaloid 5-HT<sub>1A</sub> activation in vivo modulates affective memory processing (Liang, 1999), suggesting that amygdaloid 5-HT<sub>1A</sub> receptors may constitute one site of action of F 13640 on opioid affective conditioning. In conclusion, a 5-HT<sub>1A</sub> agonist prevents and reverses the sensory allodynia that develops in rats after infraorbital nerve injury; F 13640 also prevents and reverses opioid-induced hyperalldynia. In experiments examining opioid affective/motivational actions, F 13640 similarly prevents and reverses opioid CPP and CPA. Thus, 5-HT<sub>1A</sub> receptor activation reverses pathological pain that is induced by nerve injury or opioids. Future work will examine the extent to which opioid addiction represents self-therapy of opioid-induced pathological pain.

References


