Progressive Enhancement of Delayed Hyperalgesia Induced by Repeated Heroin Administration: A Sensitization Process

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It is difficult to conceive that tolerance and sensitization processes, two apparently opposite phenomena, can concomitantly modify one given biological process, i.e., the processing of pain. We have shown recently that opiates produce not only analgesia but also long-lasting hyperalgesia in rats. This suggests that tolerance to the analgesic effect of an opiate, especially heroin, could be in part the result of an actual sensitization of pronociceptive systems. Here, we show that both magnitude and duration of heroin-induced delayed hyperalgesia increase with intermittent heroin administrations, leading to an apparent decrease in the analgesic effectiveness of a given heroin dose. Our observation that a small dose of heroin which is ineffective for triggering a delayed hyperalgesia in non-heroin-treated rats induced an enhancement in pain sensitivity for several days after a series of heroin administrations is in agreement with the

sensitization hypothesis. The effectiveness of the opioid receptor antagonist naloxone to precipitate hyperalgesia in rats that had recovered their pre-drug nociceptive value after single or repeated heroin administrations indicates that heroin-deprived rats were in a new biological state associated with a high level balance between opioid-dependent analgesic systems and pronociceptive systems. Because the NMDA receptor antagonist dizocilpine maleate (MK-801) prevented both heroin-induced long-lasting enhancement in pain sensitivity and naloxone-precipitated hyperalgesia, these findings further suggest that tolerance, sensitization, and one withdrawal symptom, hyperalgesia, are issued from a neuroadaptive process in which NMDA systems play a critical role.

Key words: heroin; delayed hyperalgesia; pain sensitization; NMDA receptors; opiate tolerance; rats

From a pharmacological viewpoint, sensitization refers to the “increased response to a drug that follows its repeated intermittent administration” (Post, 1980; Robinson and Becker, 1986; Antelman et al., 1987). This phenomenon has also been described as “reverse tolerance,” because tolerance is usually defined as a decreased response to a drug with repeated administrations (Jaffe, 1990). Tolerance and sensitization processes are two effective strategies developed by living organisms for maintaining adaptive responses to repeated changes in the internal or external environment. As stated by Stewart and Badiani regarding tolerance (1993), it is misleading to say that sensitization develops with respect to a drug. Sensitization develops to one particular effect of a drug and not to another. Although tolerance and sensitization are considered as two opposite processes, they might be concomitantly observed with some various effects of opiates: tolerance develops to the analgesic and sedative effects, while at the same time sensitization develops toward activating effects such as locomotor hyperactivity (Jaffe, 1990; Stewart and Badiani, 1993).

Although it is difficult to conceive that one drug may induce two opposite effects on the same biological process, a growing body of evidence suggests that opiates produce not only analgesia

but also hyperalgesia in animals (Yaksh and Harty, 1988; Laulin et al., 1998, 1999; Célèrier et al., 2000) and humans (Ali, 1986; Arner et al., 1988; De Conno et al., 1991; Devulder, 1997). It is now believed that pain modulation results from a balance between the activity of antinociceptive and pronociceptive systems (Rothman, 1992; Fields and Basbaum, 1994; McNally, 1999). We proposed that opiates concomitantly activate antinociceptive systems and a NMDA-dependent pronociceptive system, leading to long-lasting hyperalgesia (Larcher et al., 1998; Laulin et al., 1998; Célèrier et al., 1999, 2000). We also observed in rats that once-daily heroin administrations induce a persistent increase of basal pain sensitivity that progressively masks a sustained heroin analgesic action, the sum of both effects giving the impression of less analgesia, i.e., apparent tolerance (Laulin et al., 1999). Interestingly, the noncompetitive NMDA receptor antagonist MK-801, which prevents the gradual lowering of the nociceptive threshold, also prevents the apparent decrease in the effectiveness of heroin (Mao et al., 1994; Laulin et al., 1998). This suggests that repeated opiate administrations could induce an actual sensitization of pronociceptive systems, leading to a more sustained decrease of nociceptive threshold, which if not taken into account gives the impression of less analgesia.

The present experiments were designed to determine whether heroin induces an actual sensitization to nociceptive inputs (Robinson and Becker, 1986; Antelman et al., 1987). We first studied duration and magnitude of both analgesia and hyperalgesia induced by intermittent or once-daily administrations of the same dose of heroin. Second, we tested the ability of a small heroin dose to induce delayed hyperalgesia and the ability of naloxone to precipitate hyperalgesia in rats treated previously with higher

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heroin doses. Third, the consequences of NMDA receptor blockade on these phenomena were also examined.

MATERIALS AND METHODS

Animals. Experiments were performed on adult male Sprague Dawley rats (IFFA-CREDO, L'Arbresle, France), weighing 350–400 gm, housed in groups of five per cage under a 12 hr light/dark cycle (lights on at 8:00 A.M.) in a constant room temperature of $22 \pm 2^\circ\text{C}$. The animals had *ad libitum* access to food and water. Pharmacological tests and care of the animals were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Drugs. Diacetyl morphine hydrochloride (heroin) was purchased from Francopia (Gentilly, France), naloxone hydrochloride was from Sigma (Saint-Quentin Fallavier, France), and MK-801 (dizocilpine maleate) was from Research Biochemicals International (Natick, MA). All drugs were dissolved in normal saline (0.9%) and administered subcutaneously (100 μl /100 gm body weight). Control animals received equivalent saline injections.

Nociceptive tests. Nociceptive thresholds were evaluated by using a modification of the Randall-Selitto method (Kayser et al., 1990), the paw-pressure vocalization test, in which a constantly increasing pressure is applied to the hindpaw until the rat squeaks. The Basile analgesimeter (Apelex, Massy, France; tip diameter of the stylus: 1 mm) was used. A 600 gm cutoff value was set to prevent tissue damage.

General procedure. After arrival in the laboratory, animals were allowed 5 d to become accustomed to the colony room. To avoid stress from the experimental conditions, which might affect measurement of the nociceptive threshold by producing stress-induced analgesia (Amir and Amit, 1978), the experiments were performed by the same experimenter in quiet conditions in a test room close to the colony room. For 2 weeks before the start of the experiments, the animals were weighed daily, handled gently for 5 min, and placed in the test room for 2 hr to become accustomed to the nociceptive apparatus. To ensure basal value stability, a nociceptive threshold measurement was also performed on the day preceding the scheduled experimental day. All experiments began at 11:00 A.M. and were performed on groups of five to nine animals during the light part of the cycle.

Experimental protocols. Because we observed previously that the early analgesic effect of opiates was followed by a delayed hyperalgesic effect for several days (Laulin et al., 1998; Célèrier et al., 2000), we examined both the short-term (2–4 hr) and long-term (1–8 d) changes in nociceptive threshold induced by repeated heroin administrations. Two kinds of heroin administration protocols (intermittent or once daily) were used in this study. In the first protocol (protocol 1), five heroin administrations were performed according to an intermittent procedure: each heroin administration (1.25 mg/kg) was performed only when the delayed hyperalgesia induced by the preceding heroin administration had ended. According to this protocol, nociceptive threshold was measured every 30 min after each heroin injection until the end of the pharmacological effect on the heroin injection day. The nociceptive threshold was also measured once daily until recovery of the pre-drug value. In the second protocol (protocol 2), 12 once-daily administrations of heroin (0.3 or 2.5 mg/kg) were performed. According to this protocol, nociceptive threshold was measured every 30 min after the 1st and 12th heroin injection until the end of the pharmacological effect on the heroin injection day. The nociceptive threshold was also measured once daily between the 1st and 12th heroin injections and after the cessation of heroin injection until recovery of the pre-drug value. In the two protocols, basal nociceptive threshold measurement was performed just before every heroin administration.

At the end of these two protocols, when all animals had recovered their pretreatment nociceptive threshold value, two types of tests (separate groups) were performed: (1) a heroin test that consisted of comparing the effectiveness of a low heroin dose (0.2 or 0.3 mg/kg) for inducing early analgesia and delayed hyperalgesia in saline- and heroin-treated rats and (2) a naloxone test to assess the magnitude of naloxone-precipitated hyperalgesia in saline- and heroin-treated rats by evaluating the decrease of the nociceptive threshold 5 min after administration (1 mg/kg, s.c.).

Statistical analysis. One-way and two-way ANOVAs were used for assessing time effects of drugs and individual group comparisons. When significant effect was observed, *post hoc* analyses were performed using Dunnett's test. Student's *t* tests were used for assessing paired comparison between basal nociceptive threshold values and area under the curves (AUCs), or for evaluating changes in nociceptive threshold in-

duced by naloxone. AUCs for analgesic effects were calculated using the trapezoidal method as performed previously (Célèrier et al., 2000). Statistical significance criterion was $p < 0.05$.

RESULTS

Changes in pain sensitivity associated with intermittent heroin administrations

As shown in Figure 1A, no change in the analgesic effect of 1.25 mg/kg heroin was observed when using the intermittent heroin injection according to protocol 1 (AUC comparison; $F_{(4,56)} = 0.80$, NS). Further statistical analysis of the time course (0–4 hr) of the changes in nociceptive threshold showed that the analgesic effect of the fifth heroin administration on day 25 is similar to the analgesic effect of the first heroin administration on day 1 ($F_{(9,252)} = 1.13$, NS). Moreover, no difference in the analgesic effect of 0.30 mg/kg heroin was observed in saline- and heroin-treated rats when the opiate was injected at the end of protocol 1 on day 37 (Fig. 1A) (AUC comparison; Student's *t* test, NS).

Conversely, the intermittent heroin administrations induced a progressive enhancement in both amplitude and duration of the heroin-induced delayed hyperalgesia (Fig. 1B). Twenty-four hours after the first 1.25 mg/kg heroin administration, a marked decrease in the nociceptive threshold was observed for 2 d in the heroin group as compared with the saline group threshold ($F_{(1,23)} = 8.60$; $p < 0.01$; Dunnett's test, $p < 0.05$). The second, third, fourth, and fifth heroin administrations produced more and more hyperalgesia because significant decreases in nociceptive threshold were observed for 3, 6, 5, and 6 d, respectively ($F_{(1,23)} = 5.77$, $p < 0.05$; $F_{(1,23)} = 13.53$, $p < 0.01$; $F_{(1,23)} = 6.34$, $p < 0.05$; $F_{(1,23)} = 9.29$, $p < 0.01$, respectively; for the first, second, third, fourth, and fifth administration, Dunnett's test, $p < 0.05$). In contrast, the five intermittent saline administrations produced no change in the basal nociceptive threshold ($F_{(3,27)} = 0.95$, $F_{(5,45)} = 0.47$, $F_{(8,72)} = 0.39$, $F_{(8,72)} = 0.56$, and $F_{(8,72)} = 0.79$, respectively, for the first, second, third, fourth, and fifth administration; NS). To further evaluate sensitization of heroin-induced delayed hyperalgesia, we injected a low heroin dose (0.30 mg/kg) in both saline- and heroin-treated rats on day 37 (when heroin-treated rats had recovered their pre-drug nociceptive threshold value). In saline-treated rats the administration of the low heroin dose was ineffective for inducing a delayed hyperalgesia (Fig. 1B) ($F_{(5,45)} = 0.24$, NS). In contrast, 0.3 mg/kg heroin provoked a large decrease of the nociceptive threshold 24 hr after the opiate injection in heroin-treated rats (Fig. 1B) ($F_{(4,56)} = 10.25$, $p < 0.0001$; Dunnett's test as compared with saline group, $p < 0.05$).

Naloxone-precipitated hyperalgesia was also studied in both saline- and heroin-treated rats. When animals had recovered their pre-drug nociceptive threshold after the first or fifth 1.25 mg/kg heroin administrations (days 4 and 33, respectively), injection of naloxone produced an immediate lowering of the nociceptive threshold below the basal value (–32 and –46%, respectively) (Fig. 1C) (Student's *t* test, $p < 0.05$). In contrast, no significant effect of naloxone was observed when it was administered in saline-treated groups (Student's *t* test, NS).

Changes in pain sensitivity associated with once-daily administration of heroin

Although no significant change in the basal nociceptive threshold was observed 24 hr after a first exposure to 0.3 mg/kg heroin (Dunnett's test, NS), the 12 repeated once-daily administrations (protocol 2) induced a gradual lowering of the basal nociceptive threshold (hyperalgesia), which peaked at –30% after the 12th heroin injection (Fig. 2A) ($F_{(22,396)} = 5.74$ as compared with

Figure 1. Effects of five intermittent heroin (1.25 mg/kg, s.c.) or saline administrations (black arrows) on nociceptive threshold in rats ($n = 10$ – 15 rats per group). **A**, Histograms represent the analgesic index (AUC) of each heroin administration evaluated on days 1, 4, 9, 17, and 25 and the analgesic effect of a low heroin dose (0.3 mg/kg, s.c.; white arrow) on day 37 in both the saline- and heroin-treated groups. **B**, Changes in basal nociceptive threshold determined by once-daily measurement of the nociceptive threshold. Mean pressure values for triggering vocalization ($\pm$ SEM) were expressed in grams. $*p < 0.05$ and $**p < 0.01$ with Dunnett's test as compared with saline group. **C**, Effects of naloxone (1 mg/kg, s.c.; white arrow) on basal nociceptive threshold in saline- or heroin-treated rats. Naloxone was injected when animals had recovered their initial nociceptive threshold value after the first or fifth heroin administrations (days 4 and 33, respectively). The nociceptive threshold was measured 5 min after naloxone injection. Separate experiments were conducted for each naloxone test. Mean pressure values ($\pm$ SEM) were expressed as a percentage of basal value. $**p < 0.01$ with Student's t test as compared with pre-naloxone basal nociceptive value on day 4 or day 33.

saline group, $p < 0.0001$; Dunnett's test, $p < 0.01$). As shown in Figure 2A, the basal nociceptive threshold value returned to the saline group value on day 20, i.e., 8 d after cessation of heroin treatment (Dunnett's test, NS). On the contrary, the basal nociceptive threshold was not altered by 12 repeated once-daily saline administrations (Fig. 2A) ($F_{(22,198)} = 0.30$, NS).

Figure 2. **A**, Delayed effects of 12 once-daily heroin (0.3 mg/kg, s.c.) or saline administrations on basal nociceptive threshold in rats ($n = 10$ rats per group). Basal nociceptive threshold was determined daily before each heroin or saline administration and after the heroin treatment was stopped. Mean pressure values for triggering vocalization ($\pm$ SEM) were expressed in grams. $**p < 0.01$ with Dunnett's test as compared with saline group. **B**, Effects of naloxone (1 mg/kg, s.c.; white arrow) on basal nociceptive threshold in saline- or heroin-treated rats. Naloxone was injected on day 25 when animals had recovered their pre-drug nociceptive threshold after the heroin treatment. The nociceptive threshold was measured 5 min after naloxone injection. Mean pressure values for triggering vocalization ($\pm$ SEM) were expressed as a percentage of basal value. $**p < 0.01$ with Student's t test as compared with pre-naloxone basal nociceptive value.

Twelve repeated once-daily administrations of a higher dose of heroin (2.5 mg/kg) induced a more marked decrease in the nociceptive threshold below the saline group value (Fig. 3A) ($F_{(26,442)} = 8.37$, $p < 0.0001$; Dunnett's test, $p < 0.01$). Such a decrease is already observed 24 hr after the first 2.5 mg/kg heroin injection, and it peaked at -54% after the 12th heroin injection (Dunnett's test, $p < 0.01$). As observed previously, the basal nociceptive threshold is not altered by 12 repeated once-daily saline administrations (Fig. 3A) ($F_{(27,243)} = 0.54$, NS). In a separate experiment, we also compared the analgesic effect of 0.2 mg/kg heroin before and after 12 once-daily administrations of 2.5 mg/kg heroin. As shown in Figure 4 and as described previously (Laulin et al., 1999), we observed no change in both time course and AUC relating to the analgesic effect of heroin despite the large shift in the basal nociceptive threshold (group effect: $F_{(1,16)} = 40.96$, $p < 0.0001$; time effect: $F_{(4,64)} = 45.21$, $p < 0.0001$; no interaction: $F_{(4,64)} = 0.68$, NS; Dunnett's test, $p < 0.01$). After the cessation of 12 once-daily 2.5 mg/kg heroin administrations, the basal nociceptive threshold value returned to the saline group value on day 21 (Fig. 3A) (Dunnett's test, NS).

When injected in animals that had recovered their pre-drug nociceptive threshold after 12 once-daily 2.5 mg/kg heroin administrations (day 33), a small heroin dose (0.2 mg/kg) produced

Figure 3. *A*, Delayed effects of 12 once-daily heroin (2.5 mg/kg, s.c.) or saline administrations on basal nociceptive threshold in rats (black arrows; $n = 9$ –10 rats per group). The basal nociceptive threshold was determined daily before each heroin or saline administration and after the heroin treatment was stopped. Delayed effects of a low heroin dose (0.2 mg/kg, s.c.; white arrow) on days 33 when animals had recovered their pre-drug nociceptive threshold after the heroin treatment. Mean pressure values for triggering vocalization ($\pm$ SEM) were expressed in grams. $**p < 0.01$ with Dunnett's test as compared with basal nociceptive value on day 1. *B*, Effects of naloxone (1 mg/kg, s.c.; white arrow) on basal nociceptive threshold in saline- or heroin-treated rats. Naloxone was injected on day 29 when animals had recovered their pre-drug nociceptive threshold value after the heroin treatment. The nociceptive threshold was measured 5 min after naloxone injection. Mean pressure values for triggering vocalization ($\pm$ SEM) were expressed as a percentage of basal value. $**p < 0.01$ with Student's t test as compared with pre-naloxone basal nociceptive value. *C*, *D*, Results obtained in experiment similar to *A* and *B*, respectively, in rats receiving 12 coadministrations of MK-801 (0.15 mg/kg, s.c.) and heroin (or saline). MK-801 was administered 30 min before each 2.5 mg/kg heroin administration.

Figure 4. Changes of the nociceptive threshold induced by 0.2 mg/kg heroin on days 1 and 13 before and after 12 once-daily 2.5 mg/kg heroin administrations ($n = 9$ –15 rats per group). Mean pressure values ($\pm$ SEM) were expressed in grams. *Inset* indicates comparison of areas under the curve. $**p < 0.01$ with Dunnett's test as compared with saline group.

a marked decrease of the nociceptive threshold 24 hr after the opiate injection (Fig. 3*A*). Such a decrease lasted 3 d ($F_{(11,84)} = 19.22$, $p < 0.0001$; Dunnett's test as compared with saline group value, $p < 0.01$). This low heroin dose was ineffective in producing a delayed hyperalgesia in saline-treated rats ($F_{(7,63)} = 0.65$, NS).

When once-daily heroin-treated animals had recovered their

pre-drug nociceptive thresholds (days 25 and 29 for 0.3 and 2.5 mg/kg heroin injections, respectively), naloxone induced a significant decrease of 26 and 41% in the nociceptive threshold below the basal value (Figs. 2*B*, 3*B*) (Student's t test, $p < 0.01$). On the contrary, no significant effect of naloxone was observed in once-daily saline-treated rats (Student's t test, NS). Strikingly, naloxone still precipitated hyperalgesia in 2.5 mg/kg heroin-treated rats on day 70, i.e., 2 months after cessation of the heroin treatment (Table 1) (Student's t test, $p < 0.05$).

Preventive effect of MK-801 on both heroin-induced delayed hyperalgesia and naloxone-precipitated hyperalgesia

When injected 30 min before each 12 once-daily administrations of 2.5 mg/kg heroin, the noncompetitive NMDA receptor antagonist MK-801 (0.15 mg/kg) totally prevented the progressive decrease in the nociceptive threshold (Fig. 3*C*) ($F_{(27,216)} = 1.05$, NS). In a control experiment, MK-801 treatment induced no change in the basal nociceptive threshold value when it was performed on the saline-treated group (Fig. 3*C*) ($F_{(27,243)} = 0.48$, NS).

In the MK-801–heroin coadministration schedule, a low heroin dose (0.2 mg/kg) was ineffective in inducing delayed hyperalgesia when injected on day 33, i.e., 21 d after the heroin treatment had ceased (Fig. 3*C*) ($F_{(7,56)} = 0.56$ and $F_{(7,63)} = 0.73$ in MK-801–heroin- and MK-801–saline-treated groups, respectively; NS).

Moreover, naloxone was also unable to precipitate hyperalgesia when it was injected on day 29 in rats that have received 12 once-daily MK801–heroin coadministrations (Fig. 3*D*) (Student's

Table 1. Changes in basal nociceptive threshold induced by naloxone on day 70, i.e., 58 d after cessation of the 12 once-daily heroin or saline administrations ($n = 8$ –9 rats per group)

Treatment (for 12 d)	Day 70	
	Basal value	After naloxone
Saline group	323 ± 16	298 ± 15 (–7%)
Heroin group	323 ± 22	210 ± 20 ^{a,b} (–35%)

^a $p < 0.05$ with Student's t test as compared with pnaloxone basal nociceptive threshold value on day 70.

^b $p < 0.05$ with Student's t test as compared with saline group on day 70.

t test, NS). No naloxone effect was observed in the MK-801–saline-treated group (Fig. 3D) (Student's t test, NS).

DISCUSSION

The first result of this study is that intermittent administrations of the same dose of heroin induced not only an analgesic effect but also a long-lasting enhancement in pain sensitivity (hyperalgesia), as observed with the progressive emergence of a delayed decrease of the nociceptive threshold for several days. This hyperalgesia cannot be explained by an excess of nociceptive inputs induced by behavioral testing associated with heroin administration because we reported previously that long-lasting hyperalgesia is also observed in opiate-treated rats unexposed to repeated nociceptive stimuli on the day of opiate administration (Laulin et al., 1998; Célérier et al., 2000). This phenomenon appears an actual sensitization of pronociceptive systems because both magnitude and duration of hyperalgesia increased as a function of heroin administrations. Indeed, we observed that the first 1.25 mg/kg heroin administration induced moderate hyperalgesia for 2 d, whereas the fifth injection of the same heroin dose was followed by a larger hyperalgesia for 6 d. Our study also shows that repeated 12 once-daily subcutaneous heroin injections induced a gradual lowering of the nociceptive threshold that progressively disappeared after the cessation of the heroin treatment. Thermal hyperalgesia has also been reported 48 hr after cessation of a series of 8 once-daily intrathecal injections of morphine (Mao et al., 1994). Our observation that the administration of a small heroin dose (0.2 or 0.3 mg/kg), which was ineffective in inducing a delayed hyperalgesia after the first exposure in rats, triggered substantial delayed hyperalgesia after a series of intermittent or once-daily heroin administrations is in agreement with the sensitization hypothesis. Considered as a whole, these results clearly indicate that a repeated heroin administration schedule induced a sensitization to heroin-induced delayed hyperalgesia.

The second result of this study is that pain hypersensitivity progressively disappeared in heroin-treated rats after the cessation of heroin administrations, as demonstrated by the slow return of the nociceptive threshold to the pre-drug value. Interestingly, the larger the decrease of the nociceptive threshold, the larger was the delay to return to basal pain sensitivity. Two types of processes might account for this phenomenon. The first one would be a progressive deactivation of pronociceptive systems according to a mere homeostatic process. The second one would be a sustained and prolonged activity of the pronociceptive systems progressively opposed by an active and opposite counteradaptation that is isodirectional to the first effect of the opiate (Poulos and Cappell, 1991; Ramsay and Woods, 1997), i.e., pain inhibition by endogenous analgesic systems (Fig. 5). Although a progressive deactivation of pronociceptive systems after the ces-

Figure 5. Simplified schematic model illustrating the putative neuroadaptive continuum model linking sensitization, apparent tolerance, and hyperalgesic withdrawal symptom. Before the first exposure to heroin, an initial equilibrium (*homeostasis*) is associated with a low level balance between opioid-dependent analgesic systems (*a*) and NMDA-dependent pronociceptive systems (*b*) as indicated by the naloxone ineffectiveness in precipitating hyperalgesia. Dotted line represents the sum of the systems activity of *a* and *b*. Functioning levels of the latter are represented by the column height. Repeated heroin administrations induces a gradual decrease in the nociceptive threshold (*Pronociceptive systems sensitization*) leading to hyperalgesic state. This progressively shifts the unchanged analgesic response, giving the impression of less analgesia (apparent tolerance). After heroin treatment is stopped (*Withdrawal*), the return to pre-drug nociceptive threshold is not underlain by a deactivation of pronociceptive systems but is supported by an endogenous opioid system counteradaptation. The new equilibrium (*allostasis*) is associated with a high-level balance between opioid-dependent analgesic systems and NMDA-dependent pronociceptive systems leading to a long-term pain vulnerability.

sation of heroin administrations could not be excluded totally, our results strongly suggest a critical role for the second process. This is supported by the effectiveness of naloxone in precipitating hyperalgesia in rats that had recovered their pre-drug nociceptive threshold value after stopping heroin administration. Although the effectiveness of naloxone in precipitating hyperalgesia was only slightly increased between the first and fifth heroin injections (32 and 48% decrease of the nociceptive threshold, respectively) in the intermittent heroin injection schedule, our observation that the naloxone-precipitated hyperalgesia was maintained for 2 months after a series of 12 daily heroin administrations had ended (35% decrease of the nociceptive threshold) provides evidence that compensatory mechanisms permitting maintenance of the pre-drug nociceptive threshold value were sustained for a long time. Because an opioid receptor antagonist, which was ineffective in control heroin naive rats, induced a pharmacological effect such as hyperalgesia, this means either that opioid receptors were stimulated by a compensatory increase of endogenous opioid ligands or that signaling activity of opioid receptors is enhanced. Indeed, it has been reported that opioid agonist stimulation results in a gradual conversion of the μ opioid receptor into a sensitized or constitutively active state (Wang et al., 1994; Bilsky et al., 1996) and upregulation of the cAMP pathway (Nestler, 1992). Although these three mechanisms may account for the naloxone-precipitated hyperalgesia, the unmodified analgesic effects of heroin in this model of discontinuous administration favors the hypothesis of an increase of endogenous opioid ligands. Studies are in progress in our laboratory to identify the nature of endogenous opioids that could be involved in this adaptive process, permitting a return to basal nociceptive threshold. Taken

together, these studies suggest that heroin-deprived animals were, for a very long-time, in a new biological state associated with a high-level balance between opioid-dependent analgesic systems and pronociceptive systems that mask one another (Fig. 5). This is in agreement with the compensatory response hypothesis (Wise, 1988; Schulteis and Koob, 1996; Robinson and Berridge, 2000), especially the opponent process theory (Solomon, 1980).

The third result of our study is that, unlike sensitization of heroin-induced hyperalgesia observed after repeated administration, a change in heroin-induced analgesia was never observed. As reported earlier (Laulin et al., 1999), we observed that both time course and AUC related to the analgesic effect of heroin were unchanged when the opiate was injected during the hyperalgesic phase induced by heroin. During the hyperalgesic period, the shift of the nociceptive threshold is actually what produced the impression of less analgesia, i.e., apparent tolerance. Moreover, this study showed that the analgesic effect of heroin was also unchanged when heroin is administered in heroin-treated animals that recovered their pre-drug nociceptive threshold value after cessation of heroin administrations, and it is in agreement with some studies showing that intermittent exposure may lead to sensitization of a drug effect, whereas continuous exposure to a drug may lead to tolerance of the same drug effect (Post, 1980). This could explain why controlled clinical studies report that the dose of opiates that is required in chronic pain patients to alleviate pain (intermittent exposure) may remain constant for years on end (Twycross and McQuay, 1989; Foley, 1991; Portenoy, 1996). Taken together, these data indicate that the increases of the opiate doses that are sometimes required to alleviate pain in suffering patients are caused either by disease progression leading to aggravation of pain, as suggested previously (Collin et al., 1993; Colpaert, 1996), or by an excessive enhancement of pain sensitivity induced by repeated opiate administration, as observed in this study. In this respect, apparent tolerance to the opiate analgesic effect observed in intermittent heroin-treated rats appears as a by-product resulting from a pain sensitization process.

The relationships between apparent tolerance, pain sensitization, and naloxone-precipitated hyperalgesia observed in intermittent heroin-treated rats lead to the assumption that if the sensitization process was prevented before the drug effect was initiated, apparent tolerance, pain sensitization, and naloxone-precipitated hyperalgesia could not be expressed. Numerous studies demonstrated that the NMDA receptor antagonist may prevent the expression of sensitization processes (Stewart and Badiani, 1993), especially pain sensitization leading to hyperalgesia, allodynia, and spontaneous pain (Haley and Wilcox, 1992; Mao et al., 1995; Coderre and Katz, 1997). Of note is the observation that μ -opioid receptor stimulation triggers the activation of NMDA receptors by reducing Mg^{2+} blocking via intracellular protein kinase C (PKC) activation (Chen and Huang, 1991, 1992). It has been suggested that the subsequent increase of intracellular Ca^{2+} concentration further stimulates PKC activity leading to a lasting enhancement of glutamate synaptic efficiency in a positive feedback (Mao et al., 1995; Coderre and Katz, 1997). The present study shows that the NMDA receptor antagonist MK-801, when administered just before heroin, not only precluded sustained heroin-induced delayed hyperalgesia as described previously (Laulin et al., 1998, 1999) but also prevented the effectiveness of a small heroin dose to induce a sustained hyperalgesia in heroin-deprived rats, a critical criterion for sensitization. No apparent tolerance was observed. Moreover, MK-801 also prevented

naloxone effectiveness in precipitating hyperalgesia in heroin-deprived rats that had recovered their pre-drug nociceptive threshold value. This indicates that naloxone-precipitated hyperalgesia is the result of the sharp breakdown of an equilibrium between opioid-dependent analgesic systems and NMDA-dependent pronociceptive systems. Because NMDA receptor antagonists can prevent the development of sensitization and long-term potentiation (Kullmann and Siegelbaum, 1995; Hudspeth, 1997), our results lead to the hypothesis that pain sensitization and some signs of withdrawal such as hyperalgesia are issued from a neuroadaptive continuum triggered by opioid receptor stimulation in which NMDA-dependent pronociceptive systems play a critical role.

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