Altered Nucleus Accumbens Circuitry Mediates Pain-Induced Antinociception in Morphine-Tolerant Rats

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We investigated the effect of chronic administration of morphine on noxious stimulus-induced antinociception (NSIA) produced by intraplantar capsaicin injection. In the untreated (naïve) rat, we previously found that NSIA depends on activation of dopamine, nicotinic acetylcholine, and μ- and δ-opioid receptors in nucleus accumbens. Rats chronically implanted with subcutaneous morphine pellets demonstrated tolerance to the antinociceptive effects of acute systemic morphine administration but did not show cross-tolerance to NSIA. Morphine pretreatment, however, significantly reduced NSIA dependence on intra-accumbens opioid receptors but not on dopamine or nicotinic acetylcholine receptors. As observed in naïve rats, intra-accumbens microinjection of either the dopamine receptor antagonist flupentixol or the nicotinic receptor antagonist mecamylamine blocked NSIA in rats tolerant to the antinociceptive effects of morphine, but, in contrast to naïve rats, intra-accumbens microinjection of either the μ-receptor antagonist Cys^2,Tyr^3,Orn^5,Pen^7 amide or the δ-receptor antagonist naltrindole failed to block NSIA. These findings suggest that although NSIA is dependent on nucleus accumbens opioid receptors in the naïve state, this dependence disappears in rats tolerant to the antinociceptive effects of morphine, which may account for the lack of NSIA cross-tolerance. In separate experiments, intra-accumbens extracellular dopamine levels were measured using microdialysis. Dopamine levels increased after either capsaicin or systemic morphine administration in naïve rats but only after capsaicin administration in morphine pretreated rats. Thus, intra-accumbens dopamine release paralleled antinociceptive responses in naïve and morphine pretreated rats.

Key words: nucleus accumbens; morphine; tolerance; antinociception; dopamine release; noxious stimulation; capsaicin; pain; analgesia; μ opioid receptors; δ opioid receptors; microdialysis; jaw-opening reflex

Evidence is accumulating that nucleus accumbens is an important neural substrate for opioid-mediated pain modulation. For example, we recently demonstrated that noxious stimuli can induce antinociception (NSIA) similar in magnitude to that induced by high-dose morphine and that this effect is blocked by intra-accumbens injection of either the nonselective opioid antagonist naloxone (Gear et al., 1999) or by a selective antagonist for either μ- or δ-opioid receptors (Schmidt et al., 2002). Direct microinjection of opioids into nucleus accumbens also induces antinociception (Dill and Costa, 1977; Yu and Han, 1990; Schmidt et al., 2002), and the antinociceptive effect of systemically administered morphine can be attenuated by intra-accumbens naloxone administration (Dill and Costa, 1977).

Non-opioid receptors in nucleus accumbens play a role in antinociception as well. For example, intra-accumbens injection of the nicotinic acetylcholine receptor antagonist mecamylamine

blocks NSIA and also inhibits the antinociceptive effect of systemically administered morphine (Schmidt et al., 2001). Dopaminergic mechanisms have also been implicated in nociceptive modulation (Altier and Stewart, 1999). Intra-accumbens microinjection of a dopamine antagonist blocks NSIA (Gear et al., 1999) as well as the antinociceptive effect of intraventral tegmental area morphine (Altier and Stewart, 1998).

In the current study we investigated the effect of chronic morphine administration on NSIA. Opioid tolerance is a well known phenomenon that results from chronic exposure to an opioid agonist such as morphine (Harrison et al., 1998; Borgland, 2001; Williams et al., 2001). Tolerance induced by exposure to an agonist can produce cross-tolerance to a different agonist that acts at the same receptors. Morphine and heroin, both of which act at μ-receptors, can produce cross-tolerance to each other, and effects mediated by endogenous opioids can also become cross-tolerant to exogenous opioids (Lewis et al., 1981; Christie et al., 1982; Girardot and Holloway, 1984). Because NSIA depends on intra-accumbens opioid receptors, we tested the hypothesis that rats tolerant to the antinociceptive effects of morphine would exhibit cross-tolerance to NSIA.

Other goals of this study were to evaluate the response of nucleus accumbens dopamine levels to noxious stimulation or to acute morphine administration in naïve rats and in rats tolerant to antinociceptive effects of morphine, and to determine whether tolerance alters the requirement for dopaminergic and nicotinic neurotransmission in NSIA.

MATERIALS AND METHODS

Animals. Experiments were performed on 280–380 gm male Sprague Dawley rats (Bantin and Kingman, Fremont, CA). These animals were housed in groups of two per cage under a 12 hr light/dark cycle (lights on at 7:00 A.M.) in the animal care facility of the University of California, San Francisco. Food and water were available *ad libitum*. Experimental protocols were approved by the University Committee on Animal Research.

Nociceptive assay. Changes in nociception were measured as attenuation (i.e., antinociception) or enhancement (i.e., hyperalgesia) of the trigeminal jaw-opening reflex (JOR) electromyographic (EMG) signal (Mason et al., 1985; Gear and Levine, 1995; Gear et al., 1999; Schmidt et al., 2001). This assay was chosen because it is segmentally remote from the site at which the noxious stimulus is applied in the hindpaw, thus allowing separation of heterosegmental effects from any intrasegmental effects that might influence assays such as the paw withdrawal reflex or the tail flick reflex. Although the rats were anesthetized in this study, we have shown that NSIA occurs in the awake animal (Gear et al., 1999). In groups of awake rats, capsaicin administered into the fore paw significantly increased hindpaw withdrawal thresholds, indicating antinociception, and this effect was blocked by intra-accumbens administration of either the dopamine receptor antagonist flupentixol or the opioid receptor antagonist naloxone.

Morphine tolerance protocol. Antinociceptive tolerance to morphine was induced by subcutaneous implantation of two morphine base pellets (75 mg; National Institute on Drug Abuse) (Gold et al., 1994). The antinociceptive action of two morphine pellets, as measured by tail flick latency, returns to baseline value by 12–36 hr (Yoburn et al., 1985; Gold et al., 1994). Implantation of pellets was performed under isoflurane anesthesia (Abbott Laboratories, Chicago, IL). Experiments were performed 72 hr after pellet implantation. Vehicle pellets (from the same source) were similarly implanted in one group.

Anesthesia. All experiments were performed in rats anesthetized with an intraperitoneal injection of urethane (0.9 gm/kg) and α-chloralose (45 mg/kg; both from Sigma-Aldrich, St. Louis, MO). This method provides a stable level of anesthesia (Buelke-Sam et al., 1978) and JOR EMG signal over the time period required to complete the experiments (Gear and Levine, 1995).

Electrode implantation. To evoke the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, South San Francisco, CA). A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

JOR electromyogram. At the beginning of each experiment, stimulation current was set at three times the threshold for eliciting a JOR. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive jaw-opening reflex EMG signals evoked by stimulating the tooth pulp with 0.2 msec square wave pulses at a frequency of 0.33 Hz. Baseline amplitude was defined as the average of the last three data points, recorded at 5 min intervals, before an experimental intervention. As is customary for JOR studies (Chiang et al., 1990, 1991; Banks et al., 1992; Gear and Levine, 1995; Ahn et al., 1998; Takeda et al., 1998; Zhang et al., 1998; Gear et al., 1999; Zhang et al., 1999; Belforte et al., 2001; Schmidt et al., 2001), data were normalized for differences in baseline by calculating the percentage change from baseline for each postintervention data point. These values were plotted in the figures and used in the statistical analyses.

Cannula placement. For nucleus accumbens injections, 23 gauge stainless steel guide cannulas were stereotactically positioned bilaterally and cemented with orthodontic resin (L. D. Caulk Co., Milford, DE) to allow injections via insertion of a 30 gauge stainless steel injection cannula, which extended 2 mm beyond the guide cannulas, connected to a 2 μl microsyringe (Hamilton, Reno, NV). Injection volumes were 0.5 μl in all experiments and were performed over a period of 120 sec; the injection cannula was left in place for an additional 30 sec. The stereotaxic coordinates for nucleus accumbens injections were (from bregma) 1.3 mm rostral, 7.2 mm ventral, and ±1.8 mm lateral. Injection sites were verified by histological examination (100 μm sections stained with cresyl
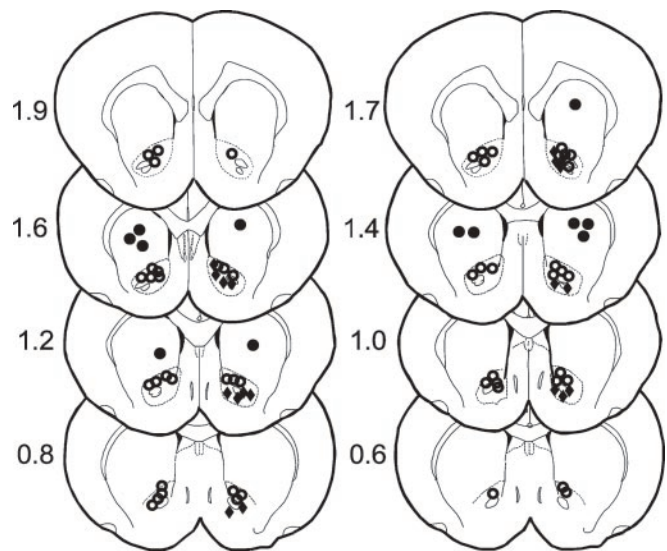

Figure 1. Location of injections. *Open circles* are considered to be within the target area of nucleus accumbens; note that they mostly fall within the area of the core. *Filled circles* designate offsite injections. *Filled diamonds* designate microdialysis probe location. Because some injections were mapped to identical locations, there are fewer symbols shown than the total number of injections performed.

violet acetate) and plotted on coronal sections adapted from the atlas of Paxinos and Watson (1986) (Fig. 1).

In vivo microdialysis. Seventy-two hours before each experiment, a 12 mm, 21 gauge guide cannula was stereotactically positioned and cemented with orthodontic resin (L. D. Caulk Co.) into the right nucleus accumbens: (from bregma) 1.3 mm rostral, 7.2 mm ventral, and 1.8 mm lateral. For this procedure rats were anesthetized with pentobarbital sodium, 50 mg/kg (Abbott Laboratories, North Chicago, 60064). On the day of the experiment, rats were anesthetized with the α-chloralose/urethane combination, and a CMA/11 microdialysis probe (CMA/Microdialysis AB, Stockholm, Sweden) was inserted through the guide cannula such that the 2 mm active membrane extended beyond the tip of the cannula. The microdialysis perfusate consisted of artificial CSF (148 mM NaCl, 1.2 mM CaCl₂, 2.7 mM KCl, 0.85 mM MgCl₂, pH 7.4). The flow rate was set at 2 μl/min with a CMA/102 microdialysis pump (CMA/Microdialysis AB). After a 2 hr equilibration period, six baseline fractions were collected at 10 min intervals. The mean basal dopamine levels for naïve and morphine pretreated rats were 0.400 and 0.454 ng/ml, respectively, which were not significantly different ($t(61) = -0.352$; $p = 0.726$). The experimental interventions were then performed, and dialysis samples were collected every 10 min and analyzed for dopamine using HPLC.

HPLC analysis. Dopamine was measured by HPLC using electrochemical detection. Dopamine was isolated by injecting dialysate samples with a CMA/200 microsampler (CMA/Microdialysis AB) through a 150×3 mm column (ESA, MD-150, Chelmsford, MA). Dopamine was quantified by an ESA Coulochem II detector and an analytical cell (ESA model 5011) with two electrodes in series: an oxidizing electrode (+220 mV) and a reducing electrode (−60 mV). The mobile phase consisted of 75 mM sodium phosphate, 1.7 mM 1-octanesulfonic acid, 100 μl/l triethylamine, 25 μM EDTA, and 10% acetonitrile; the pH was adjusted to 3.0 with phosphoric acid. The flow rate was pumped at a rate of 0.4 ml/min with a Shimadzu LC-10ADVP (Shimadzu Corporation, Kyoto, Japan).

Drugs and doses. Capsaicin was dissolved in Tween 80 (50%) and ethanol (50%) to an initial concentration of 50 μg/μl and diluted with 0.9% saline to a concentration of 5 μg/μl; subdermal capsaicin injection volume was 50 μl (250 μg) in all experiments. Cys²,Tyr³,Orn⁵,Pen⁷ amide (CTOP) 1 μg (Ableitner and Schulz, 1992; Devine et al., 1993; Badiani et al., 1995) was dissolved in PBS. Naltrindole 1 μg (Kelley et al., 1996), (R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH-23390) (Caine et al., 1995; Moses et al., 1995; Okamura et al., 1997) and nor-binaltorphimine dihydrochloride 1.8 μg (Bodnar et al., 1995; Kelley et al., 1996) were dissolved in distilled water.

All drugs and reagents were obtained from Sigma-Aldrich or from Sigma-RBI (Natick, MA).

Because it has been reported that *nor*-binaltorphimine may not be selective for κ-opioid receptors until several hours after administration (Horan et al., 1992; Spanagel et al., 1994; Wettstein and Grouhel, 1996), intranucleus accumbens cannulas were placed under pentobarbital anesthesia, and *nor*-binaltorphimine was administered 1 d before the experiment. On the day of the experiment, the rats were anesthetized with α-chloralose/urethane, and the usual experimental protocols were followed.

Data analysis. A two-way repeated measures ANOVA with one between-subjects factor (i.e., treatment) and one within-subjects factor (i.e., time) was used to determine whether there were significant ($p > 0.05$) differences in responses (expressed as percentage change from baseline) among the groups. For each ANOVA, the Mauchley criterion was used to determine whether the assumption of sphericity for the within-subjects effects was met; if the Mauchley criterion was not satisfied, Greenhouse–Geisser-adjusted p values are presented. If there was a significant between-subjects main effect of treatment group, *post hoc* contrasts, using the Tukey test, were performed to determine the basis of the significant difference.

RESULTS

Morphine tolerance

Although the protocol that we used to induce antinociceptive tolerance to morphine is well established, we compared the antinociceptive effect of morphine (10 mg/kg) in rats chronically exposed to morphine (see Materials and Methods) and previously untreated (i.e., "naïve") as well as sham treated (i.e., implantation of vehicle pellets) rats (Fig. 2) (all statistical results are shown in Table 1). The difference in antinociception between these groups was highly significant, confirming that pretreatment with morphine pellets, but not with vehicle pellets, induces tolerance to the antinociceptive effects of high-dose morphine.

NSIA in morphine-tolerant rats

We also compared the antinociceptive effect of subdermally administered capsaicin (250 μg) into the plantar surface of a hindpaw in morphine-tolerant rats and naïve rats. The antinociceptive effect of this treatment was not significantly different in these two groups, indicating that chronic morphine treatment does not produce cross-tolerance to NSIA (Fig. 3).

Involvement of nucleus accumbens opioid receptors

We previously observed in naïve rats that NSIA is mediated in nucleus accumbens by both μ- and δ- but not κ-opioid receptors (Schmidt et al., 2002). To determine whether this is the case in rats tolerant to the antinociceptive effects of morphine, we administered either CTOP or naltrindole, selective antagonists for μ- and δ-opioid receptors, respectively, to nucleus accumbens 10 min before the administration of intraplantar capsaicin. The long-lasting selective κ-receptor antagonist *nor*-binaltorphimine was administered the day before the experiment to avoid the nonselective action that is reported to occur after acute administration (see Materials and Methods). The antinociceptive effect of capsaicin after these antagonists was not significantly different from its effect when administered alone (Fig. 4). Thus, although NSIA is unchanged in rats tolerant to the antinociceptive effects of morphine, this form of antinociception does not depend on nucleus accumbens opioid receptors as is the case in morphine naïve rats. Neither CTOP nor naltrindole administered alone into nucleus accumbens (i.e., without subsequent capsaicin administration) affected the JOR (data not shown).
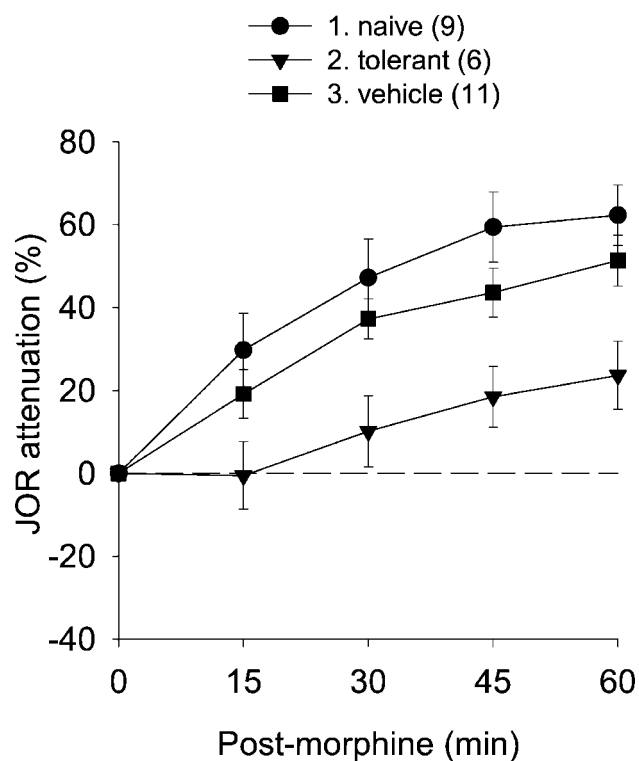

Figure 2. The antinociceptive effect of acute subcutaneous morphine administration in naïve rats and in rats pretreated with either morphine or vehicle pellets. The ability of pretreatment protocol using morphine pellets to induce tolerance is indicated by the virtually complete disappearance of antinociception after acute morphine administration. In this and subsequent figures, antinociception is plotted as percentage attenuation from baseline of the JOR EMG amplitude on the *y*-axis (i.e., greater antinociception is represented as higher positive numbers). Baseline JOR recordings were obtained before interventions. Time 0 on the *x*-axis represents the time at which the last (or only) treatment was given for each group. Data are plotted as mean ± SEM. The number of rats in each group is shown in *parentheses*. *Group numbers*, preceding *group names*, refer to the Tukey *post hoc* analyses in Table 1.

Involvement of nucleus accumbens dopamine and nicotinic cholinergic receptors

Lack of participation by nucleus accumbens opioid receptors in NSIA in rats tolerant to the antinociceptive effects of morphine could indicate either that nucleus accumbens itself no longer plays a role in this phenomenon or that intra-accumbens circuits are reorganized to eliminate dependence on opioid receptors. To distinguish between these possibilities, we tested whether NSIA in morphine pretreated rats is dependent on either dopamine or acetylcholine nicotinic receptors in nucleus accumbens as was shown previously to be the case in naïve rats (Gear et al., 1999; Schmidt et al., 2001). Intra-accumbens administration, but not offsite administration, of the selective D_1-receptor antagonist SCH-23390 10 min before intraplantar capsaicin blocked NSIA; intra-accumbens administration of the nonselective dopamine antagonist flupentixol, which has been tested previously in naïve rats, also blocked NSIA (Fig. 5a). Intra-accumbens administration, but not offsite administration, of the acetylcholine nicotinic receptor antagonist mecamylamine 10 min before intraplantar capsaicin also blocked NSIA (Fig. 5b), as was observed previously in naïve rats (Schmidt et al., 2001). These results indicate that although opioid receptors are no longer involved, nucleus accumbens itself is still an important neural substrate for NSIA. We also

Table 1. Statistical summary

	ANOVAs				Tukey *post hoc*	
	Effects	DF	*F*	*p*	Groups	*p*
Figure 2	Tx	1,23	6.442	0.006	1 versus 2	0.004
	Time	3,69	34.764	<0.001	1 versus 3	0.384
	Time × tx	6,69	0.444	0.778	2 versus 3	0.049
Figure 3	Tx	1,20	0.740	0.400		
	Time	3,60	0.944	0.393	n/a*	
	Time × tx	3,60	0.143	0.854		
Figure 4	Tx	3,32	1.139	0.348		
	Time	3,96	6.040	0.004	n/a*	
	Time × tx	9,96	1.413	0.224		
Figure 5*a*					1 versus 2	0.016
					1 versus 3	0.006
	Tx	3,24	6.614	0.002	1 versus 4	0.967
	Time	3,72	0.063	0.951	2 versus 3	0.833
	Time × tx	9,72	1.160	0.941	2 versus 4	0.133
					3 versus 4	0.045
Figure 5*b*	Tx	2,20	7.626	0.003	1 versus 2	0.003
	Time	3,60	9.781	<0.001	1 versus 3	0.642
	Time × tx	6,60	1.084	0.379	2 versus 3	0.021
Figure 5*c*	Tx	2,19	31.321	<0.001	1 versus 2	<0.001
	Time	3,57	0.229	0.732	1 versus 3	<0.001
	Time × tx	6,57	0.571	0.638	2 versus 3	0.959
Figure 6 (DA)	Tx	1,12	0.486	0.499		
	Time	5,60	3.379	0.040	n/a*	
	Time × tx	5,60	3.133	0.050		
Figure 6 (JOR)	Tx	1,7	0.018	0.896		
	Time	5,35	1.528	0.249	n/a*	
	Time × tx	5,35	0.095	0.192		
Figure 7 (DA)	Tx	1,9	16.512	0.003		
	Time	5,45	2.195	0.161	n/a*	
	Time × tx	5,45	2.344	0.147		
Figure 7 (JOR)	Tx	1,9	42.717	<0.001		
	Time	5,45	0.900	0.431	n/a*	
	Time × tx	5,45	0.402	0.693		

The discussion and conclusions of this study are based primarily on the main effect of treatment (Tx) and the Tukey *post hoc* analyses shown in the extreme right column; however, the main effect of time (Time) and the time × treatment interaction (Time × tx) are shown for completeness. The identity of the groups in the *post hoc* column is indicated by the numbers that are given in each of the respective figures. In some experiments, the JOR was measured in parallel with the collection of microdialysis fractions. Figure 6 (DA) and Figure 7 (DA) show the statistical analyses of dopamine release induced by capsaicin or morphine, respectively (shown in Figs. 6 and 7). Figure 6 (JOR) and Figure 7 (JOR) show the statistical analyses for the JOR measurements taken in parallel with the microdialysis samples; some microdialysis experiments were done without simultaneous JOR measurements. See Figures 2 and 3 for the effect of morphine or capsaicin, respectively, on the JOR in morphine naïve and tolerant rats.

Post hoc analyses were not done because there were only two groups or because there was no significant main effect of treatment (i.e., Fig. 4).

confirmed the ability of SCH-23390 to block NSIA in naïve rats (Fig. 5*c*).

Noxious stimulation and nucleus accumbens dopamine levels

Because NSIA is dependent on intra-accumbens dopamine receptors in both morphine naïve and morphine pretreated rats, we performed microdialysis experiments to measure the effect of capsaicin administration (250 μg) on nucleus accumbens dopamine release. To correlate the effect of capsaicin on nucleus accumbens dopamine levels with its effect on nociceptive responses, the JOR was measured simultaneously in some experiments. Intra-accumbens dopamine levels increased after capsaicin injection in both groups (Fig. 6). Although there appeared to be a spike of dopamine in the naïve group at the 20 min time point (that likely accounts for the significant time × treatment interaction) (Table 1), the overall effect of capsaicin on dopamine

was not significantly different, however, between the two groups. Similarly, the antinociceptive effect of capsaicin in these two groups was not significantly different (Table 1), confirming the finding shown in Figure 3. Taken together, these findings support the suggestion that NSIA induces dopamine release in nucleus accumbens and that this release correlates closely with antinociception.

Systemic morphine and nucleus accumbens dopamine levels

The effect of subcutaneous injection of morphine (10 mg/kg) on nucleus accumbens dopamine release in rats tolerant to the antinociceptive effects of morphine and naïve rats was assessed in experiments parallel to those above with capsaicin. Morphine induced antinociception as well as increased intra-accumbens dopamine levels in naïve rats but did not induce either effect in rats tolerant to the antinociceptive effects of morphine (Fig. 7,
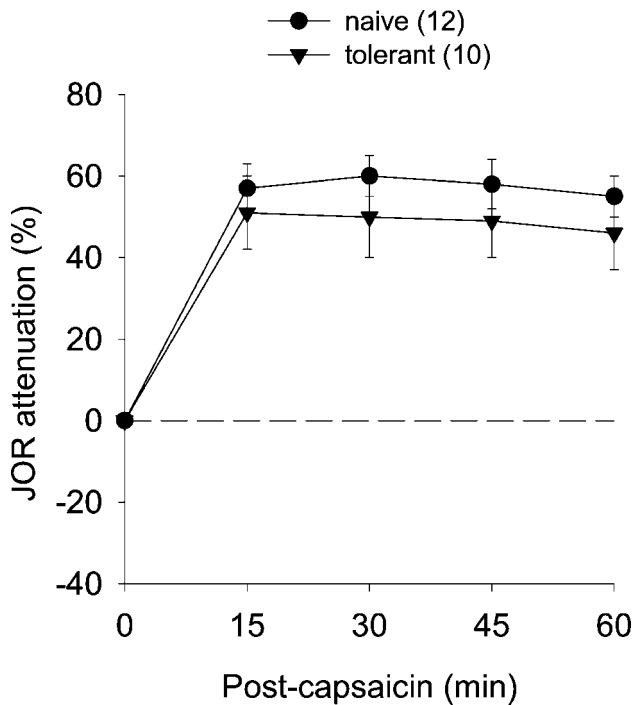
Figure 3. The antinociceptive effect of intraplantar capsaicin administration in morphine-tolerant and naïve rats. Absence of cross-tolerance is indicated by the ability of capsaicin to induce a similar degree of antinociception in naïve and morphine-tolerant rats.

Table 1), thus further supporting the suggestion that dopamine release in nucleus accumbens correlates with the antinociceptive effect.

DISCUSSION

In the naïve rat, intra-accumbens administration of either a selective μ- or δ-opioid receptor antagonist blocks NSIA, but antinociception induced by direct intra-accumbens administration of opioid agonists requires both μ- and δ-agonists in combination (Schmidt et al., 2002). These results strongly support the dependence of NSIA on nucleus accumbens opioid receptors in the naïve rat and also suggest that to induce antinociception, μ- and δ-opioid receptors must act cooperatively as suggested by other studies (Porreca et al., 1987; Heyman et al., 1989; Negri et al., 1995; Loh et al., 1998; Matthes et al., 1998). Despite opioid receptor dependence of NSIA in the naïve state, NSIA is undiminished in rats that are tolerant to the antinociceptive effects of morphine, implying lack of cross-tolerance. Thus, unlike the naïve rat, NSIA was not blocked in morphine-tolerant rats by intra-accumbens administration of either μ- or δ-opioid receptor antagonists.

Such a switch from dependence on nucleus accumbens μ- and δ-opioid receptors to independence of these receptors could result either from a change in intra-accumbens NSIA circuitry or from extra-accumbens circuit adaptations that bypass nucleus accumbens altogether. To determine whether nucleus accumbens mediates NSIA in rats tolerant to the antinociceptive effects of morphine, we microinjected antagonists for either nicotinic cholinergic receptors or dopamine receptors, both of which are known to mediate NSIA (Gear et al., 1999; Schmidt et al., 2001). NSIA was blocked by the nicotinic receptor antagonist mecamylamine as well as by the dopamine receptor antagonist flupentixol. In addition, because flupentixol may act at non-
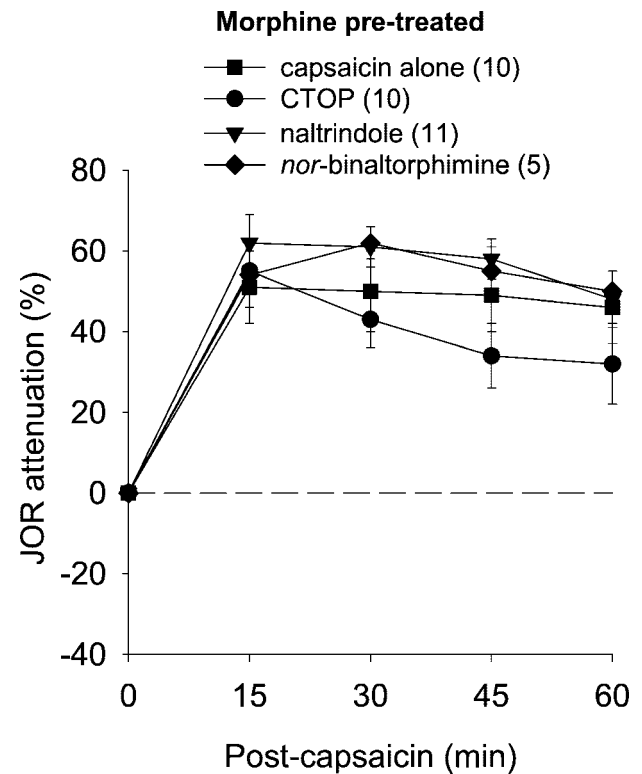

Figure 4. Effect of selective opioid receptor antagonists administered into nucleus accumbens to block the NSIA in morphine pretreated rats. None of these antagonists significantly reduced capsaicin-induced antinociception, indicating lack of participation of opioid receptors in nucleus accumbens in NSIA during morphine tolerance.

dopamine receptors, we administered the D_1-receptor selective antagonist SCH-23390, which also blocked NSIA in both naïve and tolerant rats. These results indicate that nucleus accumbens still mediates NSIA in rats chronically exposed to morphine and, of note, bear a striking parallel to our earlier study in which we found that nicotinic receptors no longer mediate NSIA in rats chronically exposed to nicotine (Schmidt et al., 2001). The mechanism(s) by which NSIA is able to switch from opioid receptor dependence to independence in rats chronically exposed to morphine on the one hand, or from nicotinic receptor dependence to independence in rats chronically exposed to nicotine on the other hand, remains to be determined.

Nucleus accumbens dopamine mechanisms appear to be an important underlying feature of NSIA because intra-accumbens administration of a dopamine antagonist blocks NSIA in naïve rats (Gear et al., 1999) as well as in rats chronically exposed to either nicotine (Schmidt et al., 2001) or morphine (this study). We therefore measured intra-accumbens dopamine release in response to administration of either morphine or capsaicin in rats chronically exposed to morphine and in naïve rats. We found that dopamine release qualitatively paralleled the induction of antinociception in these groups. That is, capsaicin induced antinociception as well as dopamine release in rats chronically exposed to morphine as well as in naïve rats, whereas acute morphine (10 mg/kg) administration induced dopamine release and antinociception in naïve rats but not in rats chronically exposed to morphine.

Our finding that acutely administered morphine induced dopamine release in nucleus accumbens in the naïve rat is in
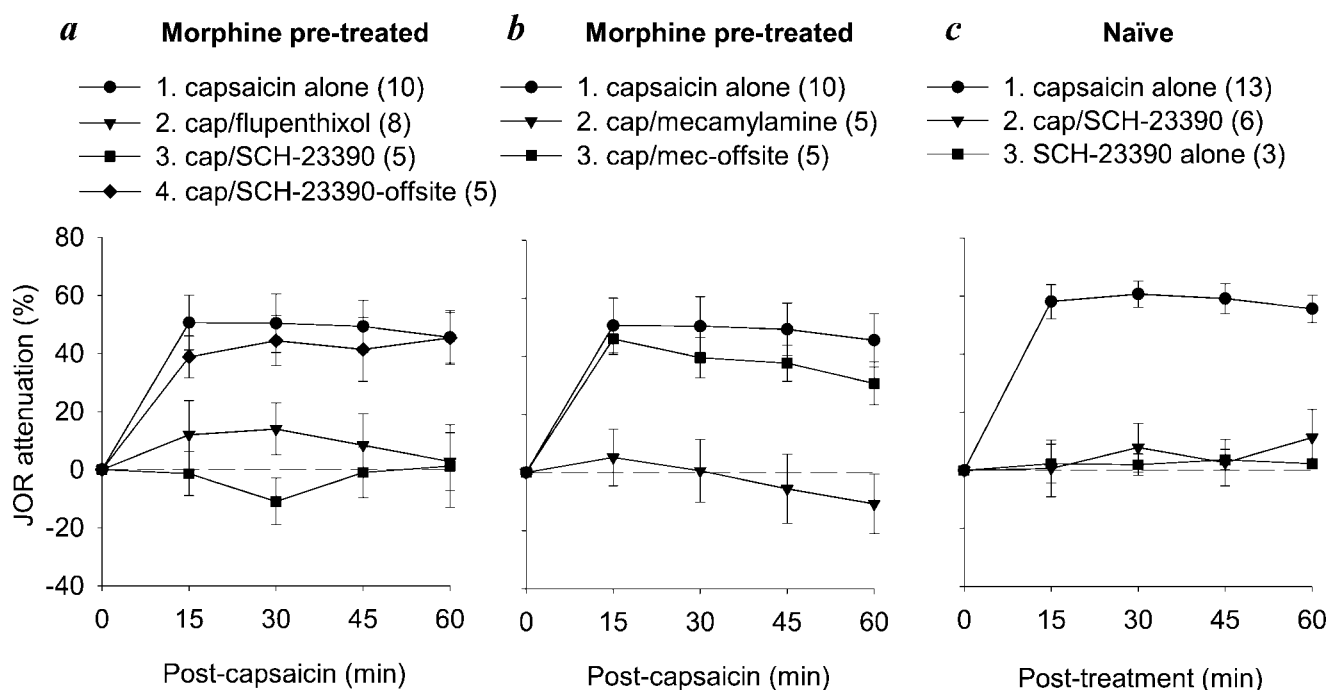
Figure 5. Effect of intra-accumbens administration of non-opioid receptor antagonists on NSIA in morphine pre-treated and naïve rats. *a*, NSIA was blocked by either flupentixol (nonselective dopamine receptor antagonist) or SCH-23390 (selective D_1-receptor antagonist) in morphine pretreated rats. *b*, NSIA was blocked by intra-accumbens administration, but not by offsite administration, of the nicotinic receptor antagonist mecamylamine in morphine pretreated rats. *c*, NSIA was blocked by SCH-23390 in naïve rats. SCH-23390 had no effect when administered alone. *cap*, Capsaicin. *Group numbers*, preceding *group names*, refer to the Tukey *post hoc* analyses in Table 1.
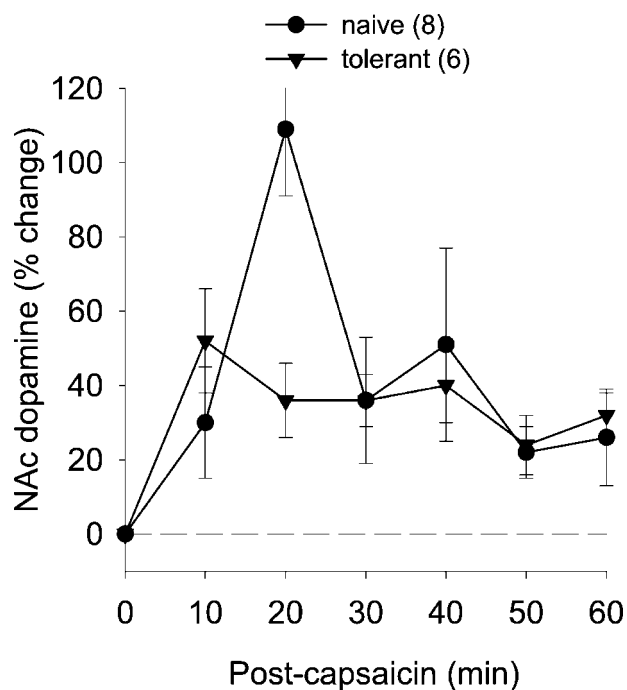

Figure 6. Effect of noxious stimulation on nucleus accumbens dopamine levels in naïve and morphine-tolerant rats. Although there was a spike in dopamine release in naïve rats at the 20 min time point, the overall effect of capsaicin on dopamine release was not significantly different.

Figure 7. Effect of morphine administration on nucleus accumbens dopamine levels in naïve or morphine-tolerant rats. Morphine induced an increase in dopamine in naïve rats, but neither effect was observed in tolerant rats.

agreement with other studies (Pothos et al., 1991; Borg and Taylor, 1997; Maisonneuve et al., 2001), but the observation that chronic morphine abrogated the ability of acute morphine to induce release of dopamine into nucleus accumbens may be
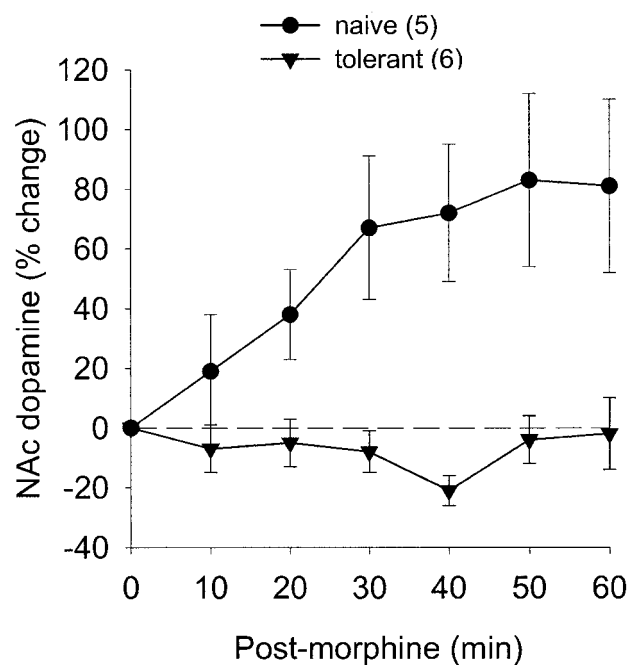
specific to the morphine pellet implantation protocol used in the present study; it was shown that acute morphine injection after seven daily injections of morphine (20 mg/kg) does not abolish morphine-induced dopamine release in nucleus accumbens

(Pothos et al., 1991). It has also been shown that naloxone-precipitated withdrawal in rats chronically implanted with pellets results in a significant decrease in dopamine levels, an effect that requires a high dose of morphine (i.e., 100 mg/kg, an order of magnitude higher than that given in the present study) to overcome (Rossetti et al., 1992).

A number of studies have shown that opioids and dopamine interact in nucleus accumbens in complex ways (for review, see Stinus et al., 1992). Dopamine receptor agonists injected into nucleus accumbens induce behavioral activation as do opioid receptor agonists (for review, see Kalivas et al., 1993), although these appear to be independent actions. However, neurotoxic depletion of dopamine terminals in nucleus accumbens has been shown in a number of studies to enhance opioid-induced motor activity (Kalivas and Bronson, 1985; Stinus et al., 1985; Churchill and Kalivas, 1992). Whether similar interactions occur between nucleus accumbens opioids and dopamine circuits in NSIA remains to be evaluated.

In summary, we demonstrate that although chronic morphine treatment results in tolerance to morphine antinociception, pain-induced antinociception is unchanged. The reliance on nucleus accumbens opioid circuitry is modified, whereas the dependence on nucleus accumbens nicotinic and dopamine receptors remains. The correlation between the antinociception and nucleus accumbens dopamine release in either morphine naïve or morphine-tolerant rats points to dopamine as a key neurotransmitter for production of antinociception. These findings suggest that a supraspinal, dopamine-mediated pain modulation system exists that might be effective in the management of intractable pain in patients tolerant to opioid analgesics.

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