Dopamine Receptor Antagonists in the Nucleus Accumbens Attenuate Analgesia Induced by Ventral Tegmental Area Substance P or Morphine and by Nucleus Accumbens Amphetamine

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ABSTRACT

In the present study, we examined the effects of dopamine (DA) receptor antagonists infused into the nucleus accumbens septi (NAS) on analgesia induced by intra-ventral tegmental area (VTA) infusions of the substance P (SP) analog, DiMe-C7 or morphine and intra-NAS infusions of amphetamine. Rats received intra-NAS infusions of either the mixed DA receptor antagonist flupenthixol (1.5 or 3.0 μg/0.5 μl/side; DiMe-C7 only), the DA D1/D5 receptor antagonist SCH 23390 (0.1 μg/0.5 μl/side; DiMe-C7 only) or the DA D2-type receptor antagonist raclopride (1.0, 3.0 or 5.0 μg/0.5 μl/side). Ten minutes later, rats received intra-VTA infusions of DiMe-C7 (3.0 μg/0.5 μl/side) or morphine (3.0 μg/0.5 μl/side) or intra-NAS infusions of amphetamine (2.5 μg/0.5 μl/side). Animals were then administered the formalin test for tonic pain. Intra-NAS raclopride prevented analgesia induced by intra-VTA DiMe-C7, intra-VTA morphine and intra-NAS amphetamine. Similarly, intra-NAS flupenthixol or SCH 23390 attenuated the analgesia induced by intra-VTA DiMe-C7. These findings suggest that tonic pain is inhibited, at least in part, by enhanced DA released from terminals of mesolimbic neurons. Furthermore, the evidence that SP and opioids in the VTA mediate stress-induced analgesia suggests that the pain-suppression system involving the activation of mesolimbic DA neurons is naturally triggered by exposure to stress, pain or both.

Midbrain ascending DA neurons, arising from the cell bodies of the VTA and projecting to various forebrain structures such as the prefrontal cortex, NAS and amygdala, play an important role in mediating the suppression of tonic or persistent pain. Infusions into the VTA of the tachykinin neuropeptide SP (Altier and Stewart, 1993, 1997) or morphine (Franklin, 1989; Manning et al., 1994) induce analgesia in the formalin test for tonic pain. Intra-VTA infusions of either an SP or an opioid receptor antagonist block the analgesia induced by exposure to stress in the formalin test (Altier and Stewart, 1996; N. Altier and J. Stewart, unpublished observations), suggesting that SP-DA and opioid-DA interactions are important in stress-induced analgesia. Amphetamine and morphine administered systemically induce analgesia in the formalin test, and these effects are attenuated by either pretreatment with DA receptor antagonists (Morgan and Franklin, 1991) or DA-depleting 6-OHDA lesions of the VTA (Morgan and Franklin, 1990).

There are several reasons to believe that the analgesia induced by SP, morphine and amphetamine in the formalin test is mediated, at least in part, by the release of DA from terminals of midbrain DA neurons projecting to the NAS (see Kalivas, 1985, for review), and intra-VTA infusions of SP enhance DA metabolism in the NAS (e.g., Cador et al., 1988), as do those of the metabolically stable SP analog DiMe-C7 (Elliott et al., 1986) and the highly selective SP (NK-1) receptor agonist GR-73632 (Elliott et al., 1991). Similarly, SP or DiMe-C7 administered systemically increase extracellular levels of DA in the NAS, as assessed by in vivo microdialysis (Boix et al., 1992a, 1992b). Other studies show that intra-VTA administration of SP, DiMe-C7 or GR-73632 stimulates locomotor activity (e.g., Eison et al., 1982; Elliott and Iversen, 1986; Elliott et al., 1991; Stinus et al., 1978) and that this response can be blocked by either intra-NAS infusions of the DA receptor antagonist haloperidol or 6-OHDA lesions of the NAS (Kelley et al., 1979).

There also is evidence that morphine and other μ-opioid receptor agonists act to increase DA release in the NAS. For example, DA metabolism in the NAS is increased after intra-

ABBREVIATIONS: VTA, ventral tegmental area; DA, dopamine; NAS, nucleus accumbens septi; SP, substance P; 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance.
VTA infusions of opioids (Kalivas et al., 1983; Kalivas and Richardson-Carlson, 1986; Latimer et al., 1987), as is extracellular DA in the NAS after systemic injections of morphine and other mu opioid receptor agonists (e.g., Di Chiara and Imperato, 1988; Spanagel et al., 1990). Intra-VTA infusions of mu opioid receptor agonists stimulate locomotor activity (Joyce et al., 1981; Kalivas et al., 1983; Latimer et al., 1987), and this effect is blocked by either intra-NAS DA receptor antagonists or lesions made to the NAS (Stinus et al., 1980; Kalivas et al., 1983). Studies have also shown that the rewarding effects of opioids are associated with the release of DA in the NAS from terminals of midbrain ascending neurons (e.g., Kiyatkin et al., 1993). Other studies show that systemic or intra-VTA administration of mu agonists elicit conditioned place preference, and that electrolytic or 6-OHDA lesions of the NAS block this effect (e.g., Phillips and LePiane, 1980; Kelsey et al., 1989; Bals-Kubik et al., 1993; Shippenberg et al., 1993).

Finally, amphetamine is known to elevate extracellular levels of DA in the NAS (e.g., Sharp et al., 1987; Di Chiara and Imperato, 1988) to enhance locomotor activity and to have rewarding properties. Amphetamine-induced hyperactivity and self-administration are greatly reduced by 6-OHDA lesions of the NAS (Kelly et al., 1975; Kelly and Iversen, 1976; Lyness et al., 1979) and by infusions of DA receptor antagonists into the NAS (Pijnenburg et al., 1975; van den Boss et al., 1988). It has also been shown that infusions of amphetamine into the NAS induce conditioned place preference and that electrolytic or 6-OHDA lesions of the NAS block this effect (e.g., Phillips and LePiane, 1980). Amphetamine-infused NAS neurons have been shown to project to the periaqueductal gray (Paxinos and Watson, 1986). The stereotaxic arms were angled laterally at 15 degrees from the perpendicular, and the skull was level between lambda and bregma (i.e., flat skull position). Five stainless-steel screws were secured to the skull, and the VTA cannulae were anchored to the skull with dental acrylic cement applied around them without blocking bregma from view. The incisor bar was then set 5.0 mm above the interaural line, and cannulae were angled at 10 degrees from the perpendicular. The SP analog DiMe-C7 (Sigma Chemical, St. Louis, MO) was dissolved in acid saline (pH 6.05) and infused bilaterally into the VTA at a dose of 3.0 pmol/side (Altier and Stewart, 1993). Other studies show that intra-VTA infusions of either the SP analog, DiMe-C7, or morphine and by infusions of amphetamine in the NAS.

**Materials and Methods**

**Subjects, housing and habituation.** Naive male Wistar rats weighing 350 to 375 g (Charles River Canada, St. Constant, Quebec, Canada) served as subjects. On arrival, they were housed individually in stainless-steel cages. They had continuous access to food and water and were maintained on a 12-hr light-dark cycle (lights on at 9:00 p.m. and off at 9:00 a.m.). All testing took place during the dark portion of the light-dark cycle. On each of the 4 days before testing, all animals were handled for at least 1 min. On each of the 2 days before formalin testing, all animals were handled and habituated to the various testing procedures to be used on the test day, except that neither intracranial nor formalin injections were administered.

**Apparatus.** The formalin test cubicles, 30 x 30 x 30 cm, were made of clear Plexiglas. A mirror was mounted beneath the floor of the cubic at a 45 degree angle to allow an unobstructed view of the rats’ paws.

**Surgery.** Seven to 10 days after arrival, rats were anesthetized with an injection of sodium pentobarbital (Somnotol, 65 mg/kg i.p.; MTC Pharmaceuticals, Cambridge, Ontario, Canada). When required, anesthesia was supplemented with methoxyflurane (Metofane; Pitman-Moore, Mississauga, Ontario, Canada). Animals receiving a DA receptor antagonist in the NAS followed by intra-VTA DiMe-C7 or morphine were implanted with four cannulae. First, 21-mm-long, 22-gauge guide cannulae (Plastics One, Roanoke, VA) were implanted bilaterally 1.0 mm above the VTA and aimed at the following coordinates: −5.7 mm posterior to bregma, +0.6 mm lateral from the midline and −7.4 mm ventral from the dura mater (Paxinos and Watson, 1986). The stereotaxic arms were angled laterally at 15 degrees from the perpendicular, and the skull was level between lambda and bregma (i.e., flat skull position). Five stainless-steel screws were secured to the skull, and the VTA cannulae were anchored to the skull with dental acrylic cement applied around them without blocking bregma from view. The incisor bar was then set 5.0 mm above the interaural line, and cannulae were angled at 10 degrees from the perpendicular. The 19-mm-long, 22-gauge guide cannulae were implanted bilaterally 1.0 mm above the NAS at the following coordinates: +3.0 mm anterior from bregma, +1.4 mm lateral from midline and −6.3 mm ventral from the skull surface (Pellegrino et al., 1967). Cannulae were anchored with dental acrylic cement. Animals receiving infusions into the NAS of raclopride followed by amphetamine were implanted with cannulae in the NAS using the same coordinates stated above. Before surgery, all animals received 0.6 mg/kg s.c. of atropine sulfate (Glaxo Laboratories, Montreal, Quebec, Canada). After surgery, 28-gauge stainless-steel ob- turator (Plastics One) were inserted into the guide cannulae. These extended 1.0 mm beyond the tip of the guide cannulae. On recovery under a heat lamp, rats were housed individually in standard plastic cages with wire tops and allowed a 7-day recovery period before habituation to testing procedures was begun.

**Formalin test.** Tonic pain was induced by a subcutaneous injec- tion of 0.05 ml of 2.5% formalin into the plantar surface of one hind paw. Pain responses were recorded once every 5 sec for 60 min using a time-sampling procedure. Thus, 12 observations were made per minute. The intensity of pain was rated according to four behavioral categories, using a scale of 0 to 3: a score of 0, if the rat walked or sat normally with weight placed equally on both hindpaws; 1, if the rat favored the injured paw (e.g., if it limped); 2, if the rat held the injured paw off the floor, with at most the nails touching the floor; and 3, if the rat chewed or licked the injured paw. The scores used for each time point were based on the mean for each animal of all the pain scores taken over a 3-min period. This weighted-scores method of rating formalin pain responses has been validated previously (Coderre et al., 1993). In all cases, the observer was blind as to which animals received a drug and which received the vehicle.

**Drugs.** The doses used to induce analgesia in these experiments were based on previous findings. The SP analog DiMe-C7 (Sigma Chemical, St. Louis, MO) was dissolved in acid saline (pH 6.05) and infused bilaterally into the VTA at a dose of 3.0 pmol/0.5 µl/side (Altier and Stewart, 1993, 1997). Stock volumes (5.0 µl) of this compound and its vehicle were aliquoted into polypropylene vials and frozen at −70°C. Solutions were thawed within 30 min of the infusions. Mor- phine sulfate (BDH, Quebec, Canada) was dissolved in saline and infused bilaterally into the VTA at a dose of 3.0 µg/0.5 µl/side (Manning et al., 1994). d-Amphetamine sulfate (SmithKline Beecham, Oakville, Ontario, Canada) was dissolved in saline and infused bilaterally into the NAS at a dose of 2.5 µg/0.5 µl/side (Altier and Stewart, 1993).

For dopamine receptor antagonists, the range of doses was chosen on the basis of previous studies with the effects of these drugs infus- ed into the NAS on other behaviors or was determined experimentally by testing one and then increasing or decreasing the dose depending on the effects seen. cis-Flupenthixol (Lundbeck, Copen-
hagen-Valby, Denmark) was dissolved in saline and infused bilaterally into the NAS at doses of 1.5 and 3.0 μg/0.5 μl/side. SCH 23390 (RBI, Natick, MA) was dissolved in saline and infused into the NAS at a dose of 0.1 μg/0.5 μl/side. The effects of intra-NAS pretreatment with higher doses of SCH 23390 on DiMe-C7-induced analgesia were not examined because of the motor impairment they induce (Nakajima and Wise, 1987). Raclopride (Astra Pharma, Mississauga, Ontario, Canada) was dissolved in saline and infused bilaterally into the NAS at doses of 1.0, 3.0 and 5.0 μg/0.5 μl/side.

Intracranial microinjections. After removal of the obturators, 28-gauge stainless-steel internal injector cannulae extending 1.0 mm beyond the tip of the guide cannulae were inserted and held in place in the guide cannulae by a brass screw cuff. The injector cannulae were connected via polyethylene tubing to 1-μl Hamilton syringes. Compounds were administered in unrestrained rats in a volume of 0.5 μl/side over 60 sec. The injectors remained in place for an additional 120 sec to allow diffusion of the solutions around the injection site. Obturators were immediately replaced after removal of the injectors. To prevent intracranial infections, all internal injector cannulae and obturators were wiped with 70% alcohol and dried immediately before being inserted into the guide cannulae.

Design and procedure. On the test day, rats received either bilateral intra-VTA infusions of DiMe-C7 (3.0 μg/0.5 μl/side) or morphine (3.0 μg/0.5 μl/side) or bilateral intra-NAS infusions of amphetamine (2.5 μg/0.5 μl/side). Ten minutes before these intracranial infusions, all rats were pretreated with bilateral intra-NAS infusions of either raclopride (1.0, 3.0 or 5.0 μg/0.5 μl/side), SCH 23390 (0.1 μg/0.5 μl/side; DiMe-C7 only), flupenthixol (1.5 or 3.0 or 5.0 μg/0.5 μl/side; DiMe-C7 only) or saline. Rats received a subcutaneous injection of 0.05 ml of 2.5% formalin into the plantar surface of one hindpaw immediately after the last intracranial infusion. Rats assigned to conditions flupenthixol-DiMe-C7 and saline-DiMe-C7 (fig. 1), SCH 23390-DiMe-C7 and saline-DiMe-C7 (fig. 2), raclopride-DiMe-C7 and saline-DiMe-C7 (fig. 3), raclopride-morphine and saline-morphine (fig. 4) and raclopride-amphetamine and saline-amphetamine (fig. 5) were tested in the formalin test using a counterbalanced within-subjects design. Thus, animals were tested twice in the formalin test at a 1-week interval. Either the right or left hindpaw was injected on successive tests. Rats assigned to all the remaining conditions were tested in the formalin test once, using a between-subjects design.

Histology. After completion of the experiments, rats were deeply anesthetized with chloral hydrate (1 ml i.p.) and perfused transcardially with the injectors in place with 0.9% saline followed by 10% formalin. Brains were then removed and stored in 10% formalin for at least 1 week. Histological verification of cannulae tip placements was subsequently determined on 30-μm thionine-stained coronal sections.

Statistical analyses. To verify whether DiMe-C7, morphine and amphetamine induced significant analgesia and whether pretreatment with the DA receptor antagonists had any effects on its own, separate two-way ANOVAs were conducted with treatment group (vehicle-drug vs. antagonist-vehicle vs. vehicle-vehicle) as the between-subjects variable and time (10 postinfusion time points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 min of testing, only the first 10 postinfusion time points were included in the analyses. All analyses were followed, if appropriate, by Tukey's post hoc test for overall differences between groups. Unless otherwise specified, two-way ANOVAs were conducted with pretreatment condition (antagonist-drug vs. vehicle-drug) and time (10 postinfusion time points) as within-subjects variables to examine the effects of pretreatment with the DA receptor antagonists on the analgesia induced by DiMe-C7, morphine and amphetamine. Tests for simple main effects were used, if appropriate, to analyze the differences at each time point between the two conditions. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

Effect of intra-NAS flupenthixol on DiMe-C7-induced analgesia. Figure 1 shows the effect of intra-NAS pretreatment with two doses (1.5 or 3.0 μg/0.5 μl/side) of the mixed DA D1/D5 and D2-type receptor antagonist flupenthixol on saline infused bilaterally into the NAS using a dose of either (A) 1.5 μg/0.5 μl/side or (B) 3.0 μg/0.5 μl/side. Significant differences between flupenthixol-DiMe-C7 and saline-DiMe-C7, *P < .05. Animals assigned to conditions flupenthixol-DiMe-C7 and saline-DiMe-C7 in A (n = 5) and B (n = 6) were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions flupenthixol (1.5 μg)-vehicle (n = 5), flupenthixol (3.0 μg)-vehicle (n = 5) and saline-vehicle (n = 7). Animals in the saline-vehicle condition were the same in A and B.

![Graph showing mean formalin pain scores](image-url)
Effect of intra-NAS SCH 23390 on DiMe-C7-induced analgesia. Figure 2 shows the effect of intra-NAS pretreatment with the DA D1/D5 receptor antagonist SCH 23390 (0.1 mg/0.5 μl/side) on the analgesia induced by intra-VTA DiMe-C7 (3.0 μg/0.5 μl/side). The ANOVA conducted for treatment group (saline-DiMe-C7 vs. SCH 23390-saline vs. saline-DiMe-C7) by time revealed a significant overall effect of treatment group, F(2,12) = 55.04, P < .0001. As shown by comparing groups saline-DiMe-C7 and saline-vehicle, intra-VTA infusions of DiMe-C7 induced significant analgesia for 30 min after the formalin injection (P < .05). The ANOVA conducted for pretreatment condition (SCH 23390-DiMe-C7 and saline-DiMe-C7) by time revealed a significant effect of pretreatment condition, F(1,5) = 10.85, P < .05, indicating that intra-NAS infusions of SCH 23390 significantly attenuated the analgesic effect of intra-VTA DiMe-C7.

Effect of intra-NAS raclopride on DiMe-C7-, morphine- and amphetamine-induced analgesia. Figure 3 shows the effect of intra-NAS pretreatment with escalating doses of raclopride on the analgesia induced by intra-VTA infusions of DiMe-C7 (3.0 μg/0.5 μl/side). The ANOVAs conducted for treatment group (saline-DiMe-C7 vs. SCH 23390-saline vs. saline-vehicle) by time revealed a significant overall effect of treatment group, F(2,12) = 55.04, P < .0001. As shown by comparing groups saline-DiMe-C7 and saline-vehicle, intra-VTA infusions of DiMe-C7 induced significant analgesia for ~30 min after the formalin injection (P < .05). The ANOVA conducted for pretreatment condition (SCH 23390-DiMe-C7 and saline-DiMe-C7) by time revealed a significant effect of pretreatment condition, F(1,5) = 10.85, P < .05, indicating that intra-NAS infusions of SCH 23390 significantly attenuated the analgesic effect of intra-VTA DiMe-C7.

At this dose, DiMe-C7-induced analgesia was blocked at all time points for 18 min after the formalin injection. As seen by comparing groups raclopride-vehicle (●) and saline-vehicle (○), raclopride pretreatment alone was without effect on pain scores at any of the doses tested. Because raclopride caused the most pronounced blockade of DiMe-C7-induced analgesia of the three antagonists tested, the effect of raclopride was examined further on the
Saline (50% of animals were assigned to conditions raclopride-saline (50%). Animals were tested in a counterbalanced within-subjects design. Separate groups assigned to the raclopride-morphine and saline-morphine conditions were at the same site. Significant differences between raclopride-amphetamine (2.5 μg/0.5 μl/side) and saline-infusions of morphine (3.0 μg/0.5 μl/side) were tested in a counterbalanced within-subjects design. Separate groups assigned to the raclopride-morphine and saline-morphine conditions were at the same site. Significant differences between raclopride-morphine and saline-morphine conditions: *P < .05; **P < .01; ***P < .001. Animals (n = 5) assigned to the raclopride-morphine and saline-morphine conditions were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions raclopride-saline (n = 8) and saline-saline (n = 9).

Fig. 4. Mean formalin pain scores (±S.E.M.) after bilateral intra-VTA infusions of morphine (3.0 μg/0.5 μl/side) or saline in animals pretreated with raclopride (5.0 μg/0.5 μl/side) or saline infused bilaterally into the NAS. Significant differences between raclopride-morphine and saline-morphine conditions: *P < .05; **P < .01; ***P < .001. Animals (n = 5) assigned to the raclopride-morphine and saline-morphine conditions were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions raclopride-saline (n = 8) and saline-saline (n = 9).

Fig. 5. Mean formalin pain scores (±S.E.M.) after bilateral infusions of amphetamine (2.5 μg/0.5 μl/side) or saline into the NAS in animals pretreated with raclopride (5.0 μg/0.5 μl/side) or saline infused bilaterally into the NAS. Significant differences between raclopride-amphetamine and saline-amphetamine conditions: *P < .05; **P < .001. Animals (n = 6) assigned to groups raclopride-amphetamine and saline-amphetamine were tested in a counterbalanced within-subjects design. Animals assigned to groups raclopride-saline (n = 5) and saline-saline (n = 6) were tested in a between-subjects design.

Analgesia induced by intra-VTA morphine and intra-NAS amphetamine. The effect of intra-NAS raclopride (5.0 μg/0.5 μl/side) pretreatment on the analgesia induced by intra-VTA morphine is shown in figure 4. The ANOVA conducted for treatment group (saline-morphine vs. saline-saline vs. raclopride-saline) by time revealed a significant group effect, F(2,22) = 46.38, P < .0001. As seen by comparing groups saline-morphine and saline-saline, intra-VTA infusions of morphine significantly reduced pain scores for ~30 min after the formalin injection (P < .05); this effect was completely blocked by raclopride pretreatment. The ANOVA conducted for pretreatment condition (raclopride-morphine vs. saline-morphine) by time revealed a significant effect of pretreatment condition, F(1,7) = 41.65, P < .0005.

Figure 5 shows the effect of pretreatment with raclopride (5.0 μg/0.5 μl/side) infused into the NAS on the analgesic effect induced by amphetamine (2.5 μg/0.5 μl/side) infused at the same site. The ANOVA yielded a significant overall effect of treatment group, F(2,14) = 29.15, P < .0001. As seen by comparing saline-amphetamine and saline-saline groups, amphetamine infused into the NAS significantly reduced formalin pain scores (P < .05). The ANOVA conducted for pretreatment condition (raclopride-amphetamine and saline-amphetamine) by time revealed a significant effect of pretreatment condition, F(1,5) = 35.56, P < .005, indicating that this amphetamine-induced analgesic effect was blocked by intra-NAS raclopride pretreatment.

Discussion

The present findings indicate that DA D2-type receptor antagonism in the NAS prevents the analgesia induced by SP, morphine and amphetamine in the formalin test for tonic pain. More specifically, it was found that pretreatment with the DA D2-selective receptor antagonist raclopride infused directly into the NAS blocks the analgesia induced by intra-VTA infusions of either the SP analog DiMe-C7 or morphine and of intra-NAS infusions of amphetamine. In the case of DiMe-C7, animals were pretreated with three different doses of raclopride, and a dose-dependent attenuation of DiMe-C7-induced analgesia was observed, with the highest dose (i.e., 5.0 μg/0.5 μl/side) resulting in a complete blockade of the analgesic effect. Likewise, this highest dose of raclopride caused a complete blockade of intra-VTA morphine- and intra-NAS amphetamine-induced analgesia. These findings are in accordance with those of previous studies implicating midbrain ascending DA neurons in the inhibition of tonic pain (Franklin, 1989; Morgan and Franklin, 1990; Manning et al., 1994; Anderson and Rompré, 1996) and, more specifically, implicating enhanced DA activity in the NAS in this effect (Clarke and Franklin, 1992; Altier and Stewart, 1993).

Previous studies have reported that selective DA D2-type agonists administered systemically induce analgesia in the formalin test (Morgan and Franklin, 1991) and the vocalization after discharge test (Carr, 1984), the latter of which induces pain associated with significant negative affect, as is the case with the formalin test. Selective DA D2-type receptor antagonists administered systemically attenuate the analgesia induced by systemic amphetamine, morphine and cocaine in the formalin test (Lin et al., 1989; Morgan and Franklin, 1991) and in the vocalization after discharge test (Paalzow and Paalzow, 1975; Carr, 1984). Our findings are consistent with those of these previous studies implicating DA D2-type receptors in the inhibition of tonic pain and indicate that the NAS is the neuroanatomical site where these DA receptor subtypes mediate this response. The present findings also extend those of previous studies by showing that DA D2-type receptors in the NAS mediate the analgesia induced by morphine and amphetamine when they are infused directly at sites within the mesolimbic system, namely, in the VTA and NAS, respectively. Finally, the re-
sults indicate that, as is the case for morphine and amphetamine, DA D2-type receptors in the NAS also mediate the analgesic effects induced by SP acting in the VTA.

It was also found that pretreatment with the selective DA D1/D5 receptor antagonist SCH 23390 infused into the NAS attenuates the analgesic effects induced by the SP analog DiMe-C7 infused into the VTA. Together with the findings on the effects of raclopride, this finding suggests that SP acting in the VTA inhibits tonic pain by causing the release of DA in the NAS from mesolimbic terminals, which, in turn, stimulates both DA D1/D5 and DA D2-type receptors at this site. The finding that DA D1/D5, in addition to DA D2-type, receptors in the NAS mediate the analgesic effects induced by SP acting in the VTA agrees with the results of other reports indicating that SCH 23390 administered systemically attenuates the analgesia induced by amphetamine, morphine and cocaine in the formalin test (Lin et al., 1989; Morgan and Franklin, 1991). It is likely that the NAS is the site where DA D1/D5 receptors also mediate the analgesia induced by morphine and amphetamine administered either systemically or into the VTA or NAS, respectively, given the similarities reported in the present study between the effects of DiMe-C7, morphine and amphetamine on tonic pain and on the involvement of DA D2-type receptors in the NAS in these effects. This remains, however, to be tested in the future.

Although the antagonism of either DA D1/D5 or D2-type receptors in the NAS attenuated the analgesia induced by intra-VTA DiMe-C7, the DA D2-type receptor antagonist raclopride appeared to be more effective than the DA D1/D5 receptor antagonist SCH 23390. These findings suggest that D2-type receptors may play a more important role than D1/D5 receptors in the NAS in the mediation of analgesia induced by the activation of DA neurons. It could be that the dose of SCH 23390 used was not high enough to induce a more potent attenuation of analgesia. However, higher doses were not used because we were concerned that they would induce sedation and motor impairment (Nakajima and Wise, 1987).

It was also found that intra-NAS infusions of the mixed DA receptor antagonist flupenthixol attenuates the analgesia induced by intra-VTA DiMe-C7. This finding is consistent with the results of previous studies showing that the mixed DA receptor antagonists flupenthixol and chlorpromazine administered systemically attenuate the analgesia induced by systemic amphetamine, morphine and cocaine in the formalin test (Lin et al., 1989; Morgan and Franklin, 1991). It is interesting to note, however, that the attenuation of DiMe-C7-induced analgesia by intra-NAS flupenthixol within the range of doses used was not as great as that seen after intra-NAS raclopride.

The present findings concerning the role of DA in the NAS on analgesia induced by intra-NAS amphetamine test are consistent with those of Clarke and Franklin (1992), who showed that bilateral 6-OHDA lesions of the NAS attenuated the analgesia induced by systemic amphetamine in the formalin test. In contrast to the present results, however, Clarke and Franklin found that 6-OHDA lesions in the NAS did not reduce the analgesic effect of systemically administered morphine in the formalin test, whereas in the present study, reduced DA neurotransmission in the NAS prevented the analgesia induced by intra-VTA morphine. There are two possible reasons for this difference. It may be that the intra-NAS infusions of the DA receptor antagonists used in the present experiments were more effective in reducing DA transmission than the 6-OHDA lesions used by Clarke and Franklin, which were incomplete and known to allow substantial recovery of DA function (see Castaneda et al., 1990). Differences in the routes of morphine administration might also account for the discrepancy. Indeed, it is possible that the manipulations carried out in the study of Clarke and Franklin aimed at decreasing DA transmission in the NAS did not block morphine analgesia because the opioid given systemically elicited analgesia by acting at several sites involved in modulating tonic pain. Morphine infused in the spinal cord (Malmberg and Yaksh, 1993) and at several supraspinal sites other than the VTA, such as the posterior hypothalamic area, periaqueductal gray and habenula, produces analgesia in the formalin test (Cohen and Melzack, 1985; Manning et al., 1994; Vaccarino and Chorney, 1994). In contrast, in the present study, morphine analgesia may have been prevented successfully by DA receptor antagonism in the NAS because the opioid was infused directly into the VTA, thereby restricting the effect of the opioid on tonic pain to midbrain ascending DA systems.

Finally, it was found that intra-NAS infusions of raclopride, SCH 23390 and flupenthixol had no effect when given alone on pain responses in the formalin test. Although these manipulations appeared to induce mild hyperalgesia (i.e., increased pain responsiveness), this effect was expected to be more pronounced given the evidence presented here that DA plays a role in modulating tonic pain. It is possible, however, that these manipulations were not effective at inducing the expected hyperalgesic effects because pain scores, recorded after an injection of 2.5% formalin, show a ceiling effect. In support of this idea, it has been reported in a previous study that pain scores recorded after the injection of 5.0% formalin do not differ from those recorded after the injection of 2.5% formalin (Coderre et al., 1993). These findings suggest that it might be worthwhile to reexamine the effects DA receptors antagonists in the NAS on tonic pain using a concentration of formalin lower than that used in the present study.

It was observed in the present study that intra-VTA DiMe-C7, intra-VTA morphine and intra-NAS amphetamine increased locomotor activity. The issue is often raised that analgesia induced by drugs that increase behavioral activation may be an artifact; that is, increased locomotor activity may compete and interfere with the animals' ability to display pain behavior, thereby accounting for the decreased pain scores. There are several lines of evidence to suggest, however, that locomotor activity and analgesia in the formalin test are dissociable. First, there is a discrepancy in the time course between the locomotor-stimulant and analgesic effects induced by SP, opioids and amphetamine. More specifically, the locomotor-activating effects of intra-VTA SP or DiMe-C7 (Kalivas, 1985), intra-VTA mu receptor agonists (e.g., Joyce et al., 1981; Kalivas and Richardson-Carlson, 1986) and intra-NAS amphetamine (e.g., van den Bosse et al., 1988) greatly outlast the analgesic effects induced by similar pharmacological manipulations. We compared the time course of the behavioral effects of intra-VTA DiMe-C7 in formalin-treated animals and found that the SP analog increased locomotor activity for at least 110 min, whereas analgesia lasted for ~30 min (Altier, 1993), indicating that animals can display pain behaviors despite high levels of...
locomotor activity. Other evidence that secondary motor effects do not interfere with an animal's ability to display pain behavior comes from studies showing that animals are either cateleptic (Matthies and Franklin, 1992) or hypactive due to DA receptor antagonist pretreatment (Morgan and Franklin, 1991) can display high pain scores in the formalin test. Based on all these findings, it is unlikely that the analgesic effects reported here are confounded by secondary motor effects.

In summary, the results of the present study indicate that blockade of DA D2-type receptors in the NAS by raclopride prevents the analgesic effects induced by either infusions into the VTA of the SP analog DiMe-C7 or morphine or infusions of amphetamine into the NAS. It was also found in tests with intra-VTA DiMe-C7 that intra-NAS infusions of either the mixed DA receptor antagonist flupenthixol or the DA D1/D5 receptor antagonist SCH 23390 attenuate analgesia, although less effectively. Together, these findings suggest that a mechanism underlying the inhibition of tonic pain is the stimulation of DA receptors in the NAS by DA released from terminals of mesolimbic neurons. The evidence that SP and opioid receptors in the VTA play a role in mediating stress-induced analgesia in the formalin test (Altier and Stewart, 1996; N. Altier and J. Stewart, unpublished observations) further suggests that the pain-suppression system involving the activation of mesolimbic DA neurons is naturally triggered by exposure to stress, pain or both through the release of SP and opioids in the VTA.

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