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Effects of dextromethorphan on dopamine release in the nucleus accumbens: Interactions with morphine

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Abstract

Dextromethorphan has been reported to decrease the self-administration of several drugs of abuse, including morphine, methamphetamine, cocaine, and nicotine. Most drugs of abuse increase extracellular levels of dopamine (DA) in the shell of the nucleus accumbens. The effects of dextromethorphan on DA release in the nucleus accumbens of naïve rats and of rats treated acutely and chronically with morphine were studied using in vivo microdialysis. DA dialysate levels were evaluated by high-performance liquid chromatography with electrochemical detection. Acute morphine (5 mg/kg ip) treatment increased the levels of DA in the nucleus accumbens to approximately 175% of basal levels. Chronic morphine (20 mg/kg ip daily for 5 days) increased DA release in the nucleus accumbens to 250% of basal levels. Acute treatment with dextromethorphan (20 or 30 mg/kg sc) alone did not alter nucleus accumbens DA levels. Pretreatment with dextromethorphan (20 mg/kg sc, 20 min prior) potentiated the effects of acute morphine, while attenuating the effects of chronic morphine on nucleus accumbens DA levels. These results with dextromethorphan suggest that the mechanism mediating the effects of dextromethorphan on drug self-administration involves modulation of the dopaminergic mesolimbic pathway.

Keywords: α3β4 nicotinic receptor; Dextromethorphan; Dopamine; Microdialysis; Morphine; Nucleus accumbens; Sensitization

1. Introduction

Dextromethorphan, the active ingredient in most over-the-counter cough medicines, has been shown to have a variety of effects related to its ability to interact with multiple receptor types. Dextromethorphan has little or no opioid activity, but binds to sigma receptors (Klein and Musacchio, 1989), and acts as a noncompetitive antagonist at the α3β4 nicotinic receptor (Hernandez et al., 2000) and at the *N*-methyl-D-aspartate (NMDA) receptor (Murray and Leid, 1984; Ebert et al., 1998). Dextromethorphan has been reported to decrease the self-administration of many drugs of abuse, including cocaine (Pulvirenti et al., 1997), morphine, methamphetamine, and nicotine (Jun and Schindler, 2000; Glick et al., 2001).

The mechanisms of action of dextromethorphan have not yet been elucidated, in part due to its metabolism to dextrorphan. This problem is further obscured by the fact that route of administration plays a large role in metabolism of dextromethorphan to dextrorphan (Wu et al., 1995). Following intraperitoneal administration of dextromethorphan, approximately three times as much dextrorphan is formed compared to subcutaneous administration (Wu et al., 1995); and peak brain concentrations of dextrorphan are fivefold higher after intraperitoneal administration of dextromethorphan compared to subcutaneous administration. Dextromethorphan and dextrorphan have different affinities for various receptor sites. For example, dextrorphan is approximately 10 times more potent than dextromethorphan at blocking the NMDA receptor (Franklin and Murray, 1992) and dextrorphan is approximately three times less potent than dextromethorphan at blocking α3β4 nicotinic receptors (Hernandez et al., 2000). The ability of dextromethorphan to decrease self-administration of methamphetamine and cocaine has been attributed to antagonism of the NMDA receptor (Pulvirenti et al., 1997; Jun and Schindler, 2000). However, our laboratory (Glick et al., 2001) found that subcutaneous administration of dextromethorphan and dextrorphan were equipotent in decreasing self-administration of morphine, methamphetamine, and

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nicotine, indicating involvement of a non-NMDA receptor mechanism.

The mesolimbic pathway, often thought to be critically involved in reward, connects the ventral tegmental area (VTA; A10 cell group) with the nucleus accumbens, as well as the olfactory tubercle, amygdala, and prefrontal cortex (Wise and Bozarth, 1987; Xi and Stein, 1999). Most drugs of abuse increase extracellular levels of dopamine (DA) in the nucleus accumbens (Pontieri et al., 1995). However, the possible involvement of the dopaminergic mesolimbic pathway in mediating the decrease in self-administration of drugs of abuse observed with dextromethorphan has not yet been assessed. While it has been shown that destruction of DA terminals in the nucleus accumbens with 6-hydroxydopamine lesions do not affect opioid self-administration (Pettit et al., 1984), opioid self-administration does produce elevations in extracellular DA levels in the nucleus accumbens (Wise et al., 1995). Therefore, the present study investigated the effect of dextromethorphan (administered subcutaneously) on DA release in the nucleus accumbens using in vivo microdialysis in freely moving rats. Additionally, the effects of subcutaneous dextromethorphan on DA release in the nucleus accumbens in response to a single morphine injection and to repeated and intermittent administrations of morphine were also assessed.

2. Materials and methods

2.1. Animals and surgery

Subjects were naïve female Sprague–Dawley rats (Taconic, Germantown, NY, USA), weighing approximately 240–260 g. All animals were housed individually in polyurethane cages in a colony room controlled for temperature and humidity, and maintained on a normal light/dark cycle (lights on/off at 07:00/19:00). Food and water were available ad libitum. Under pentobarbital anesthesia (50 mg/kg ip), rats were implanted stereotaxically with guide cannulae (CMA Microdialysis: 8309010; North Chelmsford, MA, USA) either unilaterally or bilaterally at an angle of 14° from vertical, with tips aimed medially over the nucleus accumbens. The coordinates were chosen such that, when inserted, the tips of the dialysis probes were located in the medial portion of the shell of the accumbens (AP +1.6 mm, ML ±3.1 mm from bregma; DV −8.8 mm from surface of skull). The cannulae were fixed firmly to the skull using four anchor screws and cranioplastic cement. Animals were monitored for proper recovery but otherwise left undisturbed for a minimum of 4 days after surgery. The experimental protocol was approved by Albany Medical College Institutional Review Committee for the Use of Animal Subjects and was in compliance with *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, 1996).

2.2. Drugs

Morphine sulfate (Mallinckrodt, St. Louis, MO) and dextromethorphan hydrobromide (Sigma/RBI, St. Louis, MO) were dissolved in physiological saline. The doses used, expressed as the salt, were: morphine, 5 or 20 mg/kg ip, and dextromethorphan, 20 or 30 mg/kg sc.

2.3. In vivo microdialysis procedure

The afternoon before the microdialysis session, rats were briefly anesthetized with methohexital (45 mg/kg ip) and a dialysis probe (with 2 mm of active membrane) was inserted through the guide cannula. For the acute studies, probes were implanted unilaterally, and in the repeated morphine study a probe was implanted unilaterally on the day before the first microdialysis experiment and contralaterally for the second microdialysis experiment. Artificial CSF (146 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, and 1.0 mM $MgCl_2$) was delivered by a Harvard syringe pump at a flow rate of 1 μl/min.

Collection of perfusates began approximately 16 h after probes were inserted. Collection tubes were placed at the end of the outlet dialysis tubing and taped to the swivel tether such that samples could be taken without disturbing the animal. Twenty-minute fractions were collected in vials containing 2 μl of 1.1 M perchloric acid solution (containing 5 mg/l EDTA and 5 mg/l sodium metabisulfite). Up to seven baseline samples were collected prior to drug injections, and samples were collected for 3 h following the drug injection(s). Upon completion of a microdialysis experiment, rats were euthanized and histological analysis of each brain was performed to verify the location of the probes. Only dialysates of animals whose probe tracks were within the shell of the nucleus accumbens were analyzed.

2.4. Acute effects of dextromethorphan

Rats were injected with dextromethorphan (20 or 30 mg/kg sc) following the collection of baseline samples for 2 h. The drug effects were then monitored for 3 h.

2.5. Effects of dextromethorphan in rats treated acutely with morphine

Rats were injected with saline (subcutaneous) or dextromethorphan (20 mg/kg sc) immediately following the collection of the last baseline sample. Twenty minutes later, an injection of morphine (5 mg/kg ip) was administered and the effects of the drug combinations were monitored for 3 h.

2.6. Effects of dextromethorphan in rats treated repeatedly with morphine

During the repeated morphine treatment studies, each rat was included in two microdialysis sessions 1 week apart.

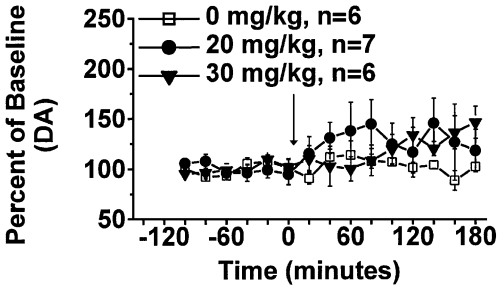

Fig. 1. Extracellular levels (mean ± S.E.M.) of DA in the nucleus accumbens, expressed as percentage of baseline, prior to and following dextromethorphan (0, 20, and 30 mg/kg sc) administered at time 0 (arrow). There were no significant effects of either dose of dextromethorphan (ANOVA, $P>.05$).

This provided a within-subject design and allowed each rat to be used as its own control in statistical analyses. In the first session, rats were injected with saline (subcutaneous) followed 20 min later by morphine (20 mg/kg ip) and samples were collected for 3 h. Following the first microdialysis session, rats were returned to their home cages. On each of the next four treatment days, the same rats were transported to the microdialysis room in their home cages and injected with 20 mg/kg ip morphine (procedure similar to that of Szumlinski et al., 2000). The rats remained in the microdialysis room in their home cages, but near the microdialysis chambers for 3 h after the morphine injection, and were then returned in their home cages to the colony room. This repeated morphine-treatment procedure was conducted to allow for the formation of morphine-context associations that promote the expression of DA sensitization on test day (Stewart, 1992). In preliminary studies, we have determined that repeated saline administration does not alter the effects of an acute morphine injection, and hence a repeated saline group was not included in the present study.

Following 48 h of withdrawal, the rats were set up for the second microdialysis session. The same microdialysis procedures were followed for the second microdialysis session; however, the probe was inserted into the opposite guide cannula as that for the first session (counterbalancing sides across pretreatment groups). Rats received the same drug injection schedule as used in the first microdialysis session except the first injection was dextromethorphan (20 mg/kg sc) in half the group and saline (subcutaneous) in the other half of the group.

2.7. Catecholamine assay

Perfusate samples were analyzed for DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), by high-performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system consisted of an ESA 540 autosampler, an ESA 580 solvent delivery system, an ESA small bore column (MD-150/RP-C18; diameter = 3.0 μm), and an ESA Coulochem II electrochemical detector with a working electrode set at a potential of 250 mV with respect to a reference electrode. The mobile phase was purchased from ESA (MD-TM). It consisted of 0.075 μM sodium dihydrogenphosphate, monohydrate, 0.0017 μM 1-octanesulfonic acid, 25 μM EDTA in 10% HPLC grade acetonitrile, and adjusted to pH = 3 with phosphoric acid. The mobile phase was used at room temperature and pumped at a flow rate of 0.053 ml/min. Chromatograms were integrated using Hewlett-Packard CHEMSTATION software.

2.8. Statistical analysis

Baseline data (expressed as pmol/10 μl) were examined by ANOVA with repeated measures for differences in each group. All data were expressed as percentage of baseline values and repeated measures ANOVAs were performed to evaluate differences in treatments. Newman–Keuls post hoc tests were performed to identify at which time points the effects were different. All data analyses were performed using version 6 of STATISTICA (StatSoft). $P<.05$ was considered significant.

3. Results

3.1. Acute effects of dextromethorphan

Baseline values for DA, DOPAC, and HVA did not significantly differ among the three groups of animals [saline (DA, 0.014 ± 0.003 pmol/10 μl; DOPAC, 6.99 ± 2.0 pmol/10 μl; HVA, 2.71 ± 0.43 pmol/10 μl); 20 mg/kg dextromethorphan (DA, 0.009 ± 0.002 pmol/10 μl; DOPAC, 10.80 ± 2.22 pmol/10 μl; HVA, 4.05 ± 1.14 pmol/10 μl); 30 mg/kg dextromethorphan (DA, 0.019 ± 0.005 pmol/10 μl; DOPAC,

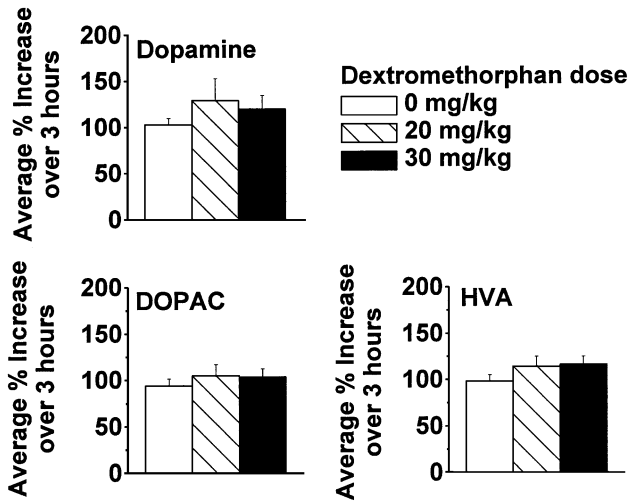

Fig. 2. Average (± S.E.M.) of the DA (top left panel), DOPAC (bottom left panel), and HVA (bottom right panel) increases over 3 h induced by dextromethorphan (0, 20, and 30 mg/kg sc) in the nucleus accumbens, expressed as percentage of baseline. There were no significant effects of either dose of dextromethorphan (ANOVAs, $P>.05$).

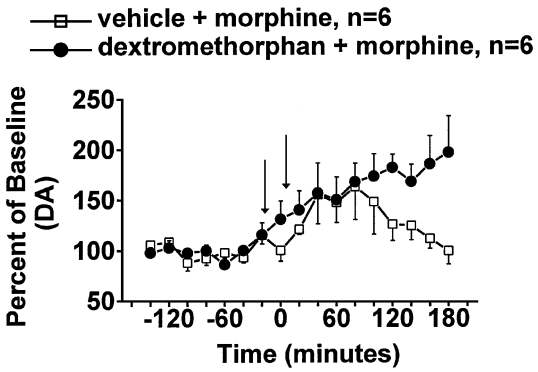

Fig. 3. Extracellular levels (mean ± S.E.M.) of DA in the nucleus accumbens, expressed as percentage of baseline, prior to and following dextromethorphan (0 or 20 mg/kg sc) pretreatment before an acute dose of morphine (5 mg/kg ip): vehicle or dextromethorphan administered at time −20 (first arrow), 5-mg/kg morphine administered at time 0 (second arrow). Dextromethorphan significantly enhanced the effects of morphine on DA (ANOVA, $P < .05$).

7.72 ± 1.21 pmol/10 μl; HVA, 2.79 ± 0.42 pmol/10 μl)]. As shown in Figs. 1 and 2, two-way ANOVA with repeated measures revealed no significant effects due to treatment (0, 20, or 30 mg/kg dextromethorphan) on extracellular levels of DA, DOPAC, or HVA in the nucleus accumbens.

3.2. Effects of dextromethorphan in rats treated acutely with morphine

Baseline values for DA, DOPAC, and HVA did not significantly differ between the two groups of animals [saline (DA, 0.011 ± 0.002 pmol/10 μl; DOPAC, 6.20 ± 1.25 pmol/10 μl; HVA, 1.99 ± 0.30 pmol/10 μl); 20 mg/kg dextromethorphan (DA, 0.011 ± 0.0007 pmol/10 μl; DOPAC, 9.11 ± 1.55 pmol/10 μl; HVA, 3.13 ± 0.47 pmol/10 μl]. As shown in Figs. 3 and 4, one-way ANOVA with repeated measures revealed that acute treatment with morphine (5 mg/kg ip) increased extracellular levels of DA [$F(16,80) = 2.36$, $P < .007$], DOPAC [$F(16,80) = 4.67$, $P < .000001$], and HVA [$F(16,80) = 11.32$, $P < .000001$] in the nucleus accumbens. Two-way ANOVA with repeated measures revealed that pretreatment with 20 mg/kg dextromethorphan enhanced the effects of morphine on extracellular levels of DA [$F(16,144) = 1.85$, $P < .03$], DOPAC [$F(16,144) = 1.71$, $P < .05$], and HVA [$F(16,144) = 1.72$, $P < .05$] in the nucleus accumbens.

3.3. Effects of dextromethorphan in rats treated repeatedly with morphine

Baseline values for DA, DOPAC, and HVA did not significantly differ between the two groups of animals on Day 1 [saline (DA, 0.020 ± 0.0039 pmol/10 μl; DOPAC, 13.60 ± 2.47 pmol/10 μl; HVA, 4.39 ± 0.79 pmol/10 μl); 20 mg/kg dextromethorphan (DA, 0.024 ± 0.013 pmol/10 μl; DOPAC, 12.91 ± 1.49 pmol/10 μl; HVA, 3.90 ± 0.57 pmol/10 μl)]. Two-way ANOVA with repeated measures revealed no significant differences between the two groups on Day 1 so the data were combined for Figs. 5 and 6. However, one-way ANOVA with repeated measures revealed that acute treatment with morphine (20 mg/kg) significantly increased extracellular levels of DA [$F(16,160) = 2.10$, $P < .01$], DOPAC [$F(16,160) = 6.24$, $P < .00001$], and HVA [$F(16,160) = 11.85$, $P < .00001$] in the nucleus accumbens as compared to baseline values (Figs. 5 and 6).

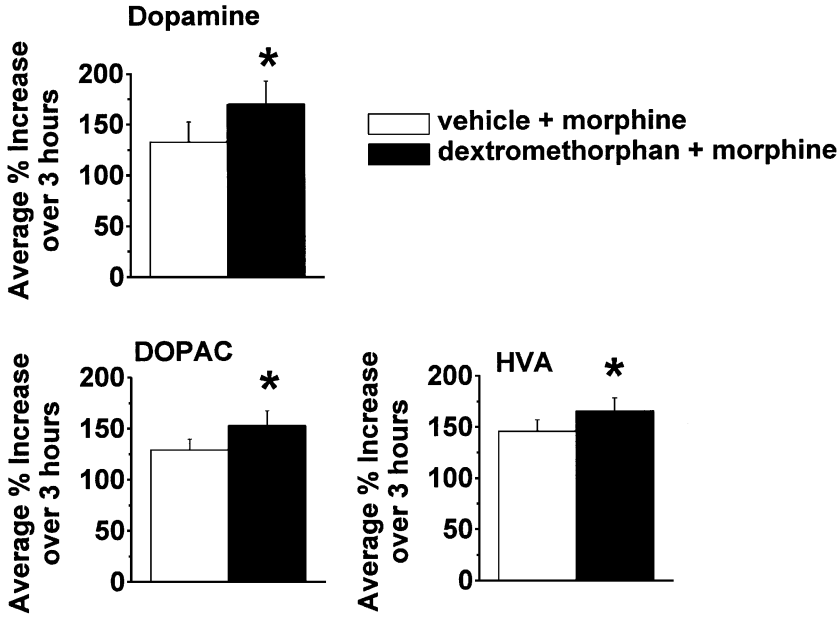

Fig. 4. Average (± S.E.M.) of the DA (top left panel), DOPAC (bottom left panel), and HVA (bottom right panel) increases over 3 h induced by dextromethorphan (0 or 20 mg/kg sc) pretreatment before an acute dose of morphine (5 mg/kg ip) in the nucleus accumbens, expressed as percentage of baseline. Dextromethorphan significantly enhanced the effects of morphine on DA, DOPAC, and HVA (ANOVAs, * $P < .05$).

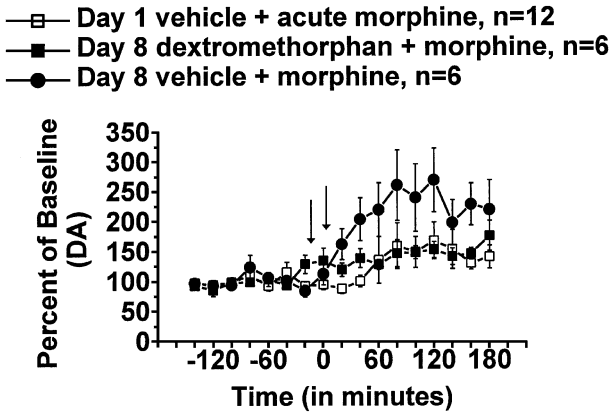

Fig. 5. Extracellular levels (mean ± S.E.M.) of DA in the nucleus accumbens following acute treatment with morphine (20 mg/kg ip) (Day 1 vehicle + acute morphine) vs. pretreatment with 0 (Day 8, vehicle + repeated morphine) or 20 mg/kg sc dextromethorphan (Day 8, dextromethorphan + morphine) in rats treated repeatedly with morphine (five daily injections of 20 mg/kg ip): vehicle or dextromethorphan administered at time −20 (first arrow), morphine (20 mg/kg) administered at time 0 (second arrow). In rats treated repeatedly with morphine, dextromethorphan blocked the expression of DA sensitization (ANOVA, $P < .005$).

Baseline values for DA, DOPAC, and HVA did not significantly differ between the two groups of animals on Day 8 [saline (DA, 0.013 ± 0.0018 pmol/10 μl; DOPAC, 15.39 ± 3.15 pmol/10 μl; HVA, 5.66 ± 1.12 pmol/10 μl); 20 mg/kg dextromethorphan (DA, 0.0095 ± 0.0018 pmol/10 μl; DOPAC, 13.01 ± 2.05 pmol/10 μl; HVA, 4.32 ± 0.64 pmol/10 μl)] (Figs. 5 and 6). Comparisons of baseline values of days 1 and 8 revealed no significant differences in basal levels of DA, DOPAC, or HVA for either treatment group (i.e., also no difference between left and right sides of the brain). Since the same animals were used following repeated treatment regimens, the acute data from Day 1 microdialysis were compared to the data from Day 8 microdialysis. Two-way ANOVA with repeated measures revealed that sensitization to morphine occurred in rats treated repeatedly with 20 mg/kg morphine [DA, $F(16,80) = 2.587$, $P < .003$]; however, similar trends with DOPAC and HVA were not significant (Fig. 6). Treatment with 20 mg/kg dextromethorphan blocked the expression of morphine-induced DA sensitization [$F(16,160) = 2.33$, $P < .005$; Treatment × Time Time interaction].

4. Discussion

The mesolimbic pathway is often thought of as the "reward pathway" and connects the VTA (A10 cell group) with the nucleus accumbens, as well as the olfactory tubercle, amygdala, and prefrontal cortex (Wise and Bozarth, 1987; Xi and Stein, 1999). Most drugs of abuse have been shown to increase extracellular levels of DA in the nucleus accumbens (Pontieri et al., 1995). Previous results in this laboratory (Glick et al., 2001) and others (Jun and Schindler, 2000; Pulvirenti et al., 1997) have shown that dextromethorphan decreases the self-administration of several drugs of abuse (e.g., morphine, methamphetamine, cocaine, and nicotine) but has no effect on responding for nondrug reinforcers (water and food). However, the involvement of the dopaminergic mesolimbic pathway in mediating the decrease in self-administration of drugs of abuse observed with dextromethorphan has not been shown.

In agreement with previous studies (DiChiara and Imperato, 1988; Pothos et al., 1991; Rada et al., 1991; Maison-

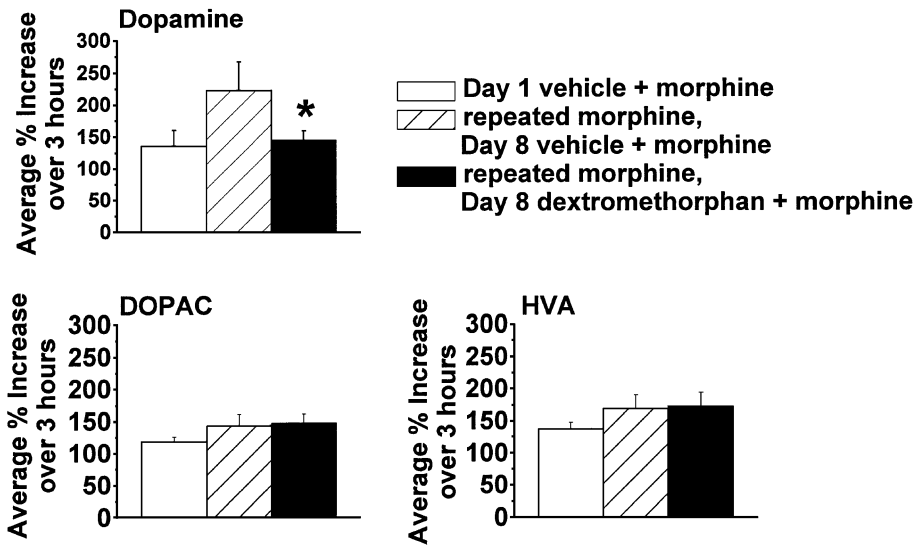

Fig. 6. Average (± S.E.M.) of the DA (top left panel), DOPAC (bottom left panel), and HVA (bottom right panel) increases over 3 h induced by acute treatment with morphine (20 mg/kg ip) (Day 1 vehicle + acute morphine) vs. pretreatment with 0 (Day 8, vehicle + repeated morphine) or 20 mg/kg sc dextromethorphan (Day 8 dextromethorphan + morphine) in rats treated repeatedly with morphine (five daily injections of 20 mg/kg ip), expressed as percentage of baseline. In rats treated repeatedly with morphine, dextromethorphan blocked the expression of DA sensitization (ANOVAs, * $P < .005$).

neuve et al., 1991; Pontieri et al., 1995; Borg and Taylor, 1997; Giorgi et al., 1997; Willins and Meltzer, 1998; Barrot et al., 1999; Di Giannuario et al., 1999; Szumlinski et al., 2000; Maisonneuve et al., 2001), acute administration of a low dose (5 mg/kg) of morphine increased levels of DA in the nucleus accumbens. This laboratory has previously shown that acute morphine administration produces a non-monotonic dose–response relationship with regard to extracellular levels of DA in the nucleus accumbens; the peak effect of morphine occurs at 20 mg/kg (Maisonneuve et al., 2001). The doses used in the present study were based on the previously determined dose–response relationship (Maisonneuve et al., 2001). Thus, a 5-mg/kg dose of morphine was used in the acute study so that increases, as well as decreases, in its efficacy could be measured. As done previously (Szumlinski et al., 2000), a dose of 20 mg/kg was used in the repeated morphine study so that evidence of sensitization could be clearly attributed to an increase in efficacy.

Dextromethorphan had little to no effect on extracellular levels of DA, DOPAC, and HVA in the nucleus accumbens of naïve rats. However, pretreatment with dextromethorphan potentiated the increase in extracellular DA and its metabolites induced by acute morphine administration. In contrast, the sensitizing effect of repeated administration of morphine on DA levels was totally blocked by pretreatment with dextromethorphan. This is in agreement with other studies showing that treatments which decrease opioid self-administration in rats usually attenuate opioid-induced increases in nucleus accumbens DA levels (e.g., Buckett, 1981; Gerasimov et al., 1999). Thus, the mechanism mediating the effects of dextromethorphan on the self-administration of morphine, and possibly other abused drugs as well, appears to involve modulation of the dopaminergic mesolimbic pathway. Additionally, since dextromethorphan was administered via the subcutaneous route, the effects of dextromethorphan reported here can be attributed to dextromethorphan and not to its metabolite dextrorphan.

Morphine produces its effects by binding with opioid receptors. Localization studies have found opioid receptors on GABA-ergic neurons located in the VTA. When stimulated these GABA-ergic interneurons inhibit the firing of dopaminergic cells in the VTA that project to the nucleus accumbens (Xi and Stein, 1999). Administration of opioids, such as morphine, inhibit these VTA GABA interneurons, which results in disinhibition of the DA cells producing an increase in DA release in the nucleus accumbens via increases in DA synthesis and dopaminergic cell firing rate (Xi and Stein, 1999; Devine et al., 1993; Kelley et al., 1980). The ability of dextromethorphan to attenuate this response in rats sensitized to morphine implies modulation of this pathway. While dextromethorphan has little or no opioid activity, but binds with sigma receptors (Klein and Musacchio, 1989), and acts as a noncompetitive antagonist at the $\alpha3\beta4$ nicotinic receptor (Hernandez et al., 2000) and at the NMDA receptor (Murray and Leid, 1984; Ebert et al., 1998), no direct linkage to the mesolimbic pathway has been shown. Thus, modulation of the dopaminergic mesolimbic pathway via other mechanisms is probably involved.

The involvement of other nondopaminergic mechanisms in mediating or modulating reward has been postulated. Jun and Schindler (2000) and Pulvirenti et al. (1997) have suggested that the decreases in drug self-administration observed in rats treated with dextromethorphan can be attributed to antagonism of the NMDA receptor. Dextrorphan is 10 times more potent than dextromethorphan at NMDA receptors, so the equal potencies of both compounds to alter self-administration argue against NMDA receptor antagonism as the mediator of this phenomenon. However, antagonism of the $\alpha3\beta4$ nicotinic receptor by dextromethorphan has also been suggested as a likely mechanism (Glick et al., 2002). In support of this latter idea, dextromethorphan and its metabolite dextrorphan were both more potent in decreasing the self-administration of nicotine than in decreasing morphine or methamphetamine self-administration (Glick et al., 2001); moreover, the relative potencies of dextromethorphan and dextrorphan in these studies were more consistent with their affinities at the $\alpha3\beta4$ receptor than at the NMDA receptor. Furthermore, several other NMDA antagonists (e.g., Marcus et al., 2001) have been reported to increase extracellular levels of DA in the nucleus accumbens shell and the lack of such an effect of dextromethorphan in the present study would also argue against glutamate antagonism as dextromethorphan's major mode of action. Support for a role of nicotinic mechanisms in modulating the effects of abused drugs has also been provided by studies with mecamylamine, a nonspecific nicotinic antagonist. In addition to reducing nicotine self-administration in rats (e.g., Corrigall and Coen, 1989; Watkins et al., 1999) and attenuating nicotine-induced "drug liking" in humans (Rose et al., 1995), mecamylamine has been shown to decrease the self-administration of cocaine (Levin et al., 2000) and ethanol (Ericson et al., 1998; Nadal et al., 1998) in rats, and to decrease cocaine craving in humans (Reid et al., 1995). Recently, Papke et al. (2001) reported that mecamylamine has preferential affinity for $\alpha3\beta4$ receptors vs. other nicotinic subtypes (e.g., $\alpha4\beta2$).

Although present in low density in the VTA, $\alpha3\beta4$ receptors are heavily localized to the medial habenula and interpeduncular nucleus (Klink et al., 2001; Quick et al., 1999). The interpeduncular nucleus receives its primary input from the medial habenula, thus, forming the habenulo-interpeduncular pathway (Sutherland, 1982). Nishikawa et al. (1986) demonstrated functional interactions between the habenulo-interpeduncular and mesolimbic pathways. It has been shown that the medial habenula receives input from the nucleus accumbens and has efferents to the VTA (Sutherland, 1982). The interpeduncular nucleus sends efferents to the raphe nucleus in the brainstem and the medial dorsal thalamic nucleus, both of which connect to the VTA (Sutherland, 1982).

The data presented here do not provide direct evidence of which mechanism of dextromethorphan is involved in the modulation of nucleus accumbens DA output in response to acute or chronic morphine. However, the present results with dextromethorphan are very similar to previous results obtained with 18-methoxycoronaridine (Szumlinski et al., 2000), suggesting a common mechanism mediates the interactions of these agents with morphine. Additional support for involvement of a nicotinic pathway in the modulation of reward has come from this laboratory (Glick et al., 2002): low-dose combinations of dextromethorphan with mecamylamine or 18-methoxycoronaridine, or of mecamylamine with 18-methoxycoronaridine, reduced morphine and methamphetamine self-administration at treatment doses that were ineffective individually. The only known common action of dextromethorphan, mecamylamine, and 18-methoxycoronaridine is antagonism at $\alpha 3\beta 4$ nicotinic receptors. The role of $\alpha 3\beta 4$ nicotinic receptors as mediators or modulators of drug reward, therefore, requires further investigation.

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