Endogenous Opioids in Dopaminergic Cell Body Regions Modulate Amphetamine-Induced Increases in Extracellular Dopamine Levels in the Terminal Regions

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ABSTRACT

Opioid antagonists attenuate behavioral effects of amphetamine and amphetamine-induced increases in extracellular dopamine levels in nucleus accumbens and striatum of rats but do not alter those effects of cocaine. This study was performed to determine 1) if the effect of opioid antagonists on the dopamine response to amphetamine is mediated in either the terminal or cell body region of the nigrostriatal and mesolimbic pathways, and 2) if the enkephalinase inhibitor thiorphan, which slows degradation of endogenous opioid peptides, increases the dopamine response to amphetamine but not to cocaine. Microdialysis probes were placed either into a dopaminergic terminal region or into both a terminal and cell body region of rats. Naloxone methiodide (1.0 μM), a lipophobic opioid antagonist, was administered into either the terminal or cell body region by reverse dialysis, whereas extracellular dopamine was collected in the terminal region. Increases in extracellular dopamine in nucleus accumbens and striatum caused by amphetamine (0.1–6.4 mg/kg, s.c.) were reduced significantly (28–39%) by naloxone methiodide administered into either substantia nigra or ventral tegmentum but not into terminal regions. Thiorphan (10 μM) administered into substantia nigra increased significantly the dopamine response to amphetamine in the ipsilateral striatum by as much as 42% but did not affect the dopamine response to cocaine (3.0–56 mg/kg, i.p.). These results suggest that amphetamine promotes release of endogenous opioids, which, through actions in the ventral tegmentum and substantia nigra, contribute to amphetamine-induced increases in extracellular dopamine in the nucleus accumbens and striatum.

There is considerable evidence that endogenous opioids modulate dopamine systems in the brain. Neurons containing opioids and opioids containing dopamine coexist in the ventral tegmental area and in the substantia nigra (Moore and Bloom, 1978; Johnson et al., 1980), and there are opioid receptors on dopamine neurons (Pollard et al., 1977; Llorens-Cortes et al., 1979). A high percentage of neurons in the nucleus accumbens that express precursors of opioid peptides coexpress mRNAs of dopamine receptors (Akil et al., 1998). Agonists selective for either the μ- or δ-opioid receptor increase extracellular concentrations of dopamine in the nucleus accumbens and striatum when administered systemically or directly into either the ventral tegmentum or substantia nigra (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Devine et al., 1993). This opioid-induced release of dopamine appears to be a secondary consequence of the drugs inhibiting GABAergic interneurons and thereby disinhibiting dopaminergic neurons (Johnson and North, 1992; Koob, 1992). Opioid antagonists, such as naloxone or naltrexone, block all of these effects of the opioid drugs (Di Chiara and Imperato, 1988; Johnson and North, 1992; Devine et al., 1993; Yoshida et al., 1999). Endogenous opioids not only modulate central dopamine systems, they also appear to modulate the effects of some drugs that act via those systems, based upon the results of studies in which opioid antagonists were tested. Naloxone and naltrexone reduce many behavioral effects of amphetamine, a nonopioid psychomotor stimulant drug, in rodents and nonhuman primates. For example, opioid antagonists attenuate amphetamine-induced increases in locomotor activity (Holtzman, 1974; Andrews and Holtzman, 1987; Winslow and Miczek, 1988) and in responding maintained by intraoral self-stimulation (Holtzman, 1976) or by aversive stimuli (Holtzman, 1974) and block amphetamine-induced place-preference conditioning (Trujillo et al., 1991).

Opioid antagonists probably block or attenuate effects of amphetamine on behavior by attenuating the increases in

ABBREVIATIONS: CSF, cerebral spinal fluid; GABA, γ-aminobutyric acid; HPLC, high performance liquid chromatography; ANOVA, analysis of variance.
extracellular dopamine that are believed to mediate those effects. Naloxone administered systemically attenuates the increase in extracellular dopamine induced by systemically administered amphetamine in the striatum and nucleus accumbens of rats (Hooks et al., 1992; Schad et al., 1995). Naltrindole, an antagonist selective for δ-opioid receptors, also attenuates amphetamine-induced stimulation of locomotor activity in rats and reduces amphetamine-induced increases in extracellular levels of dopamine in the striatum (Jones and Holtzman, 1992; Schad et al., 1996).

The current study was performed to characterize further the role of endogenous opioids in the dopamine response to amphetamine. One objective was to determine whether opioid antagonists attenuate this response to amphetamine through an action in either the terminal or cell body region of the nigrostriatal and mesolimbic dopamine pathways. Amphetamine was administered subcutaneously to conscious rats while extracellular dopamine in the nucleus accumbens or striatum was collected by microdialysis. Before and during the injections of amphetamine, the opioid antagonist naloxone methiodide was administered continuously by reverse dialysis into either the terminal regions or, in different rats, into the ventral tegmentum or substantia nigra. Naloxone methiodide is a quaternary derivative of naloxone that often is used in brain mapping studies of opioid effects because it is less likely than naloxone to diffuse from the site of administration (Schroeder et al., 1991; Maldonado et al., 1992). Consistent with low lipophilicity, naloxone methiodide attenuates amphetamine-induced stimulation of locomotor activity when it is administered centrally but not when it is administered systemically (Jones and Holtzman, 1992).

If drugs that block the actions of endogenous opioids reduce the dopamine response to amphetamine, drugs that enhance the actions of endogenous opioids may be expected to have the opposite effect. Enkephalins, which constitute one of the major families of opioid peptides, are inactivated rapidly by membrane-bound peptidases. A second objective of this study was to determine whether amphetamine-induced increases in extracellular levels of dopamine are enhanced by thiorphan, an inhibitor of neutral endopeptidase (EC 3.4.24.11, enkephalinase) (Malfroy et al., 1978; Roques et al., 1980). Results of the experiments with naloxone methiodide pointed to the cell body regions as sites of action of endogenous opioids. Therefore, we measured the concentration of extracellular dopamine in the striatum while thiorphan was administered by reverse dialysis into the ipsilateral substantia nigra and amphetamine was injected subcutaneously.

In contrast to how it interacts with amphetamine, naloxone does not reduce the increases in locomotor activity and extracellular dopamine in the striatum of rats by cocaine (Jones et al., 1993; Schad et al., 1995). This suggests that endogenous opioids do not contribute to these effects of cocaine, and thiorphan would not be expected to enhance the dopamine response to that drug. Thus, a third objective of the study was to test this prediction. Thiorphan was administered into the substantia nigra and extracellular dopamine levels in the ipsilateral striatum were measured before and during administration of cocaine intraperitoneally.

Amphetamine and cocaine were administered in cumulative doses so that dose-response curves could be determined in each animal within a single microdialysis session (Schad et al., 1995; Schad et al., 1996).

Materials and Methods

Subjects. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used. Between experiments they were group housed in a temperature-controlled room and maintained on a 12-h light/dark cycle. Food and water were always available in the home cage. The rats weighed 370 to 420 g at the time of surgery. Protocols of all experiments were approved by the Institutional Animal Care and Use Committee of Emory University and were in keeping with the 1996 Guide for the Care and Use of Laboratory Animals (National Academy of Sciences).

Surgeries. Rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital (Nembutal; Abbott Laboratories, Pomezia, Italy) and placed in a stereotaxic frame. For rats in experiments in which terminal regions were perfused with naloxone methiodide, a single 21-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was inserted to meet the dorsal surface of either the striatum or nucleus accumbens. For rats in other experiments, two guide cannulas were inserted to meet the dorsal surface of either the striatum and ipsilateral substantia nigra or the nucleus accumbens and ipsilateral ventral tegmental area. With the incisor bar set at +5 mm, the stereotaxic coordinates were: striatum, AP +2.5 and ML ±2.7 from bregma and −2.7 from dura; substantia nigra, AP −3.8 and ML ±2.3 from bregma and −8.3 from dura; nucleus accumbens, AP +3.4 and ML ±1.5 from bregma and −6.0 from dura; ventral tegmentum, AP −4.0 and ML ±0.7 from bregma and −8.5 from dura (Pellegrino et al., 1979). The guide cannulas were secured with skull screws and cranialplastic cement (Plastics One). Intramuscular penicillin (60,000 units) was administered immediately after surgery. Probes were implanted 3 to 5 days after surgery.

Apparatus. Rats were put into a Plexiglas cage (40 × 25× 25 cm) for microdialysis experiments. The cages were placed inside of a ventilated and sound-attenuating chamber to minimize external disturbances and were illuminated by a small (10-cm) fluorescent bulb on the ceiling.

Microdialysis probes consisted of two lengths of fused silica tubing (40 μm i.d.; 100 μm o.d.; Polymicro Technologies, Phoenix, AZ) inserted into a piece of cellulose dialysis fiber (220 μm o.d.; 6000 M cutoff; Spectrum Medical Industries, Houston, TX). The ends of the dialysis fiber were sealed with polyimide sealing resin (Alltech Associates, Deerfield, IL). The distance between the silica inlet and outlet lines was 2.0 mm for the nucleus accumbens, 4.0 mm for the striatum, and 1.0 mm for both the substantia nigra and ventral tegmentum. Approximately 30 min before probe implantation, the inlet line of the probe was connected to a 500-μl Hamilton syringe containing artificial CSF composed of 149 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl2, 1.2 mM CaCl2, 0.25 mM ascorbic acid, and 5.4 mM d-glucose and was adjusted to a pH of 7.2 to 7.4 with 0.5 M sodium hydroxide. All chemicals were obtained from Fisher Scientific (Atlanta, GA) except l-ascorbic acid, which was obtained from Sigma (St. Louis, MO).

Dialysate samples were analyzed by injecting 9.0 μl of perfusate onto a small-bore high performance liquid chromatography (HPLC) system with a loop size of 0.5 μl. The HPLC system consisted of a 0.5-mm-i.d. × 10-cm column (5-μm C-18 stationary phase). Electrochemical detection of dopamine was accomplished using a PerkinElmer Life Sciences (Boston, MA) amperometric detector using a working electrode (model MF-1000; Bioanalytical Systems Inc., West Lafayette, IN) with an applied potential of +700 mV versus a Ag/AgCl reference electrode (model RE1; Bioanalytical Systems Inc.). Dopamine standard solutions were used to generate calibration curves.

Experimental Procedure. The evening before the experiment, animals were transferred to the experimental cages and allowed to habituate for 2 to 3 h. The rats were then weighed, and the dialysis
probes were inserted. To facilitate the implantation of dual microdialysis probes into animals with two guide cannulas, these rats were lightly anesthetized with 0.1 to 0.15 cc of sodium pentobarbital, i.p. Rats were awake and walking 5 to 15 min after the pentobarbital injection. The initial perfusion flow rate of artificial CSF was 0.1 μl/min to allow dopamine concentrations to equilibrate after the disturbance of implanting the probe. Approximately 9 h after probe implantation, the artificial CSF flow rate was gradually increased to 0.6 μl/min, and the collection of baseline samples began.

The collection of baseline samples was followed by the addition of 1.0 μM naloxone methiodide to the perfusate of the probe in either the cell body region or the terminal region of some animals or by the addition of 10 μM thiorphan to the perfusate of the substantia nigra probe. In experiments with naloxone methiodide, cumulative doses of d-amphetamine (0.0, 0.1, 0.4, 1.6, and 6.4 mg/kg, s.c.) were administered, beginning 30 min later. In experiments with thiorphan, cumulative doses of either d-amphetamine or cocaine (0, 3, 10, 30, and 56 mg/kg, i.p.) were administered, beginning 1 h after thiorphan was added to the perfusate. Dialysate samples were collected from the striatum or nucleus accumbens every 15 min from 1 h before the start of drug delivery by reverse dialysis until 45 min after the administration of the final dose of amphetamine or cocaine. This resulted in the collection of four samples before the start of reverse dialysis, four (naloxone methiodide) or six (thiorphan) more samples before the first dose of amphetamine or cocaine, and 10 samples in the presence of amphetamine or cocaine.

**Histology.** After the experiments, animals were anesthetized with 400 mg/kg chloral hydrate and perfused transcardially with 0.9% saline followed by 10% formalin. The brain was removed and stored in 10% formalin. Probe placement was verified by examining 40-μm coronal sections that were stained with thionine. Any animal found to have a probe placement lying outside the desired brain region(s) was discarded from data analysis. Striatum and nucleus accumbens probe placements were satisfactory in all animals. However, six rats were discarded from the experiments with naloxone methiodide for having unsatisfactory substantia nigra or ventral tegmentum probe placements and three were discarded from the experiments with thiorphan for having unsatisfactory substantia nigra probe placements.

**Drugs.** Naloxone methiodide (RBI, Natick, MA), and thiorphan (Sigma) were dissolved in artificial cerebrospinal fluid to make a 1.0 and 10 μM concentration, respectively. d-Amphetamine sulfate and cocaine hydrochloride (Sigma) were dissolved in 0.9% saline and injected volume of 1.0 ml/kg body weight either s.c. (amphetamine) or i.p. (cocaine). Doses of each drug are expressed as the free base.

**Data Analysis.** Dialysate dopamine concentrations (expressed as nanomolar dopamine) were subjected to a two-factor (perfusion and time) analysis of variance (ANOVA), with repeated measures on time. Separate ANOVAs were performed on the period before the start of cocaine or amphetamine administration (baseline) and on the period during and after the administration of those drugs. Post hoc tests were performed, where appropriate, using Tukey’s and Fisher’s LSD tests. The α level was set at 0.05.

**Results**

**Naloxone Methiodide in Terminal Regions**

**Striatum.** Baseline dopamine levels were not affected by the administration of naloxone methiodide into the striatum. Analysis of variance indicated no significant difference between the preamphetamine (−60 to 45 min) dialysate dopamine concentrations of rats perfused with only CSF and those perfused with naloxone methiodide \[F(1, 8) = 1.21, p = 0.3033\].

The administration of cumulative doses of amphetamine (0.1–6.4 mg/kg) caused a dose-dependent increase in the extracellular levels of dopamine in the striatum of both CSF- and naloxone methiodide-perfused animals. In both groups, the highest level of dopamine in the dialysate was reached 30 min after the administration of 6.4 mg/kg amphetamine: 448 ± 61 nM (mean ± S.E.M.) and 469 ± 52 nM for CSF and naloxone methiodide perfusions, respectively (Fig. 1). There was no significant difference between the dialysate dopamine concentrations of the two groups over the period (60–195 min) of amphetamine administration [\(F(1, 8) = 0.041, p = 0.844\)].

**Nucleus Accumbens.** Similar to what was observed in the striatum, adding naloxone methiodide to the perfusate did not affect baseline (−60 to 45 min) levels of extracellular dopamine in the nucleus accumbens \[F(1, 10) = 1.71, p = 0.220\].

The administration of cumulative doses of amphetamine (0.1–6.4 mg/kg) caused a dose-dependent increase in the extracellular levels of dopamine in the nucleus accumbens of both CSF- and naloxone methiodide-perfused animals. In this region, too, the highest level of dopamine in the nucleus accumbens was reached 30 min after the administration of 6.4 mg/kg amphetamine: 134 ± 11 and 156 ± 28 nM for CSF and naloxone methiodide perfusions, respectively (Fig. 2). There was no significant difference between the dialysate dopamine concentrations of the two groups over the period (60–195 min) of amphetamine administration \[F(1, 10) = 0.292, p = 0.601\].

**Naloxone Methiodide in Cell Body Regions**

A control experiment was conducted to ensure that extracellular dopamine levels in the terminal regions were not affected by the addition of a second probe in the corresponding ipsilateral cell body regions. The amphetamine-induced increase in extracellular dopamine was measured in the striatum of a group of animals with a single probe in the stria-
tum (n = 3) and in a second group of animals with a probe in both the striatum and substantia nigra (n = 4). All animals were perfused with artificial CSF. There was no difference between the dialysate dopamine concentrations in the striatum of animals with single versus dual microdialysis probes [F(1, 5) = 0.558, p = 0.489] (data not shown). This outcome is consistent with the finding that a second microdialysis probe located in the ventral tegmentum had no effect on dialysate dopamine concentrations in the ipsilateral nucleus accumbens (Parsons and Justice, 1993).

**Substantia Nigra/Striatum.** Baseline dopamine levels in the striatum were not affected by the administration of naloxone methiodide into the substantia nigra. There was no significant difference between the preamphetamine (60 to 45 min) dialysate dopamine concentrations of rats perfused with only CSF and those perfused with naloxone methiodide [F(1, 11) = 0.425, p = 0.840].

The administration of cumulative doses of amphetamine (0.1–6.4 mg/kg) caused a dose-dependent increase in the extracellular levels of dopamine in the striatum of both groups of rats. The curve for the group perfused with drug-free CSF (Fig. 3) was similar to the curves obtained in animals with a single probe (Fig. 1). The peak of extracellular dopamine levels was 465 ± 66 nM at 30 min after the administration of 6.4 mg/kg amphetamine. In contrast, the effect of amphetamine was blunted in rats perfused with naloxone methiodide (Fig. 3); peak extracellular levels of dopamine reached only 335 ± 20 nM, 39% lower, in rats perfused with naloxone methiodide. ANOVA confirmed that there was a significant main effect of perfusion [F(1, 12) = 15.8, p = 0.002] and of time [F(9,108) = 8.48, p = 0.002] and a significant perfusion × time interaction [F(9,108) = 140, p < 0.001].

**Ventral Tegmentum/Nucleus Accumbens.** ANOVA indicated that there was no significant difference between the baseline (60 to 45 min) levels of extracellular dopamine of the two groups [F(1, 12) = 3.85, p = 0.073]. Furthermore, within each group of animals, there was no difference between the first four and second four time points, indicating that baseline dopamine levels in the nucleus accumbens were not affected by the administration of naloxone methiodide.

As had also occurred when the substantia nigra was perfused with naloxone methiodide, perfusion of the ventral tegmentum with naloxone methiodide attenuated the effect of amphetamine (0.1–6.4 mg/kg; Fig. 4). Peak extracellular levels of dopamine in the nucleus accumbens averaged 120 ± 12 nM in rats perfused with CSF but only 73 ± 6 nM, 39% lower, in rats perfused with naloxone methiodide. ANOVA confirmed that there was a significant main effect of perfusion [F(1, 12) = 15.8, p = 0.002] and of time [F(9,108) = 8.48, p = 0.002] and a significant perfusion × time interaction [F(9,108) = 140, p < 0.001].
Baseline dopamine levels in the striatum were unaffected by the administration of thiorphan into the substantia nigra. ANOVA indicated no significant difference between the pre-drug (−60 to 75 min) dialysate dopamine concentrations of rats in which drug-free CSF was perfused and those in which thiorphan was perfused, either in the experiment with amphetamine [F(1, 10) = 0.731, p = 0.412] or in the one with cocaine [F(1, 11) = 0.421, p = 0.530].

Amphetamine (0.1–6.4 mg/kg) dose dependently increased extracellular levels in the striatum of both groups, although this effect was smaller than that obtained in the other experiments and the peak increase occurred 15 min after 6.4 mg/kg instead of 30 min after (Fig. 5). Thiorphan administered into the substantia nigra enhanced amphetamine-induced increases in extracellular levels of dopamine in the striatum. The maximum extracellular concentration of dopamine averaged 230 ± 27 nM in rats in which only CSF was perfused compared with 340 ± 33 nM, a 42% increase, in rats in which thiorphan was perfused (Fig. 5). ANOVA revealed a significant difference between the dopamine concentrations in the dialysate of the two groups over the period (90–225 min) during which amphetamine was administered [F(1,10) = 4.99, p = 0.049], a significant main effect of time [F(9,90) = 76.7, p < 0.001], and a perfusion × time interaction [F(9,90) = 4.12, p < 0.001].

Cocaine administered in cumulative doses (3.0–56 mg/kg) dose dependently increased extracellular levels of dopamine in the striatum of all rats (Fig. 6) but was less efficacious than was amphetamine. At the time of the peak effect, 15 min after 56 mg/kg cocaine, extracellular dopamine levels averaged 49 ± 5 nM in rats perfused with only CSF. Thiorphan did not alter the effects of cocaine (Fig. 6). The maximum extracellular levels of dopamine averaged 45 ± 9 nM in the striatum of rats perfused with thiorphan and the curves for the two groups from 90 to 225 min did not differ from each other [F(1, 11) = 0.198, p = 0.665].

**Discussion**

The results of the experiments with naloxone methiodide indicate that opioid antagonists attenuate amphetamine-induced increases in extracellular dopamine in the terminal regions of the nigrostriatal and mesolimbic pathways of rats through actions at the cell body regions of those pathways. Naloxone methiodide administered by reverse dialysis into the striatum or nucleus accumbens did not affect the increases in extracellular dopamine levels elicited in those regions by systemically administered amphetamine. In contrast, the same concentration of naloxone methiodide administered into the substantia nigra or ventral tegmentum significantly reduced amphetamine-induced increases in extracellular dopamine in the corresponding ipsilateral terminal region.

The regional specificity of the effects of naloxone methiodide has several implications. First, it rules out the possibility that effects of the drug were a consequence of diffusion throughout the brain or into the systemic circulation, even though naloxone was administered continuously for several hours. Second, it makes it unlikely that opioid antagonists, whether administered systemically or centrally, attenuate neurochemical and behavioral effects of amphetamine by a direct action at the dopamine transporter to inhibit neuronal uptake of amphetamine and/or release of dopamine. Third, it suggests that although δ- and (especially) μ-opioid receptors are present on dopaminergic nerve terminals (Mansour et al., 1995; Olive et al., 1997; Svingos et al., 1999), they do not have a major role in the attenuation of effects of amphetamine by opioid antagonists.

The results of a study with receptor-selective ligands suggest that different types of opioid receptors in each of the two dopamine pathways mediate the attenuation of effects of amphetamine by opioid antagonists. Naltrindole, a δ-opioid receptor antagonist, attenuated amphetamine-induced increases in extracellular dopamine in the striatum but not in
the nucleus accumbens, whereas β-funaltrexamine, a μ-opioid receptor antagonist, had the converse effect (Schad et al., 1996). Therefore, it appears that μ-opioid receptors have the principal role in opioid-induced modulation of effects of amphetamine in the mesolimbic pathway and δ-opioid receptors have the principal role in the nigrostriatal pathway. The results of the experiments with naloexone methiodide are consistent with this interpretation. The affinity of naloexone methiodide for μ-opioid receptors is three to four times higher than it is for δ-opioid receptors (Deviche, 1997). Naloxone methiodide had a greater effect on amphetamine-induced increases in extracellular dopamine when it was administered into the ventral tegmentum than it did when it was administered into the substantia nigra: a 39% decrease in the peak effect versus a 28% decrease. Validation of this conclusion awaits further experiments with receptor-selective opioid antagonists.

Evidence from studies with opioid antagonists suggests that endogenous opioids contribute to effects of amphetamine on behavior and brain dopamine systems but not to those effects of cocaine (Jones et al., 1993; Schad et al., 1995). The results of the experiment with thiorphan support and extend the findings of those studies. The substantia nigra contains a relatively high level of neutral endopeptidase (Roques et al., 1993). Perfusing this region with thiorphan increased the peak effect of amphetamine on extracellular levels of dopamine in the ipsilateral striatum by 42% but did not affect the increases in extracellular dopamine levels produced by cocaine. By inhibiting their enzymatic degradation, thiorphan increases extracellular levels and prolongs activity of enkephalins in brain regions where those opioid peptides are released (Roques et al., 1980, 1993). Which endogenous opioids are released after amphetamine administration and the source of their release remain to be determined. That neither thiorphan nor naloexone methiodide changed baseline levels of extracellular dopamine in the striatum indicates that opioids in the substantia nigra do not tonically modulate dopamine release in this pathway.

The amphetamine-induced increases in extracellular dopamine levels in striatum were smaller in the control group for the experiments with thiorphan than they were in the control groups for the experiments with naloexone methiodide. These differences likely are due to the fact that the separate sets of experiments were performed months apart, with different batches of animals and probes, and with different HPLC conditions. Quantitative differences in drug effects on extracellular levels of neurotransmitters in microdialysis studies are not uncommon under these circumstances, and data often are transformed to percent of baseline to compensate for such variation (Westerink, 1995). In fact, baseline levels of extracellular dopamine in striatum were significantly lower in the experiments with thiorphan than they were in those with naloexone methiodide (9.8 ± 0.6 versus 13.1 ± 1.0 nM; t(44) = 2.91, p = 0.006). The control group perfused with drug-free artificial CSF was tested concurrently with the group perfused with thiorphan, providing an appropriate contemporaneous comparator. That thiorphan did not enhance cocaine-induced increases in extracellular dopamine levels serves as a positive control for the effect thiorphan had on the increases in extracellular dopamine levels induced by amphetamine.

It is unclear why endogenous opioids in the nucleus accumbens and substantia nigra contribute to the increases in extracellular levels of dopamine in the terminal areas produced by amphetamine but not those produced by cocaine. One possibility is the difference in the mechanism by which each drug raises synaptic dopamine levels. Cocaine blocks the reuptake of neurally released dopamine into presynaptic terminals whereas amphetamine causes the release of dopamine from presynaptic terminals (Heikkila et al., 1975; Arnold et al., 1977), and increases dopamine levels considerably more than cocaine does (e.g., Figs. 5 and 6). Moreover, in contrast to the effects of cocaine, which are eliminated when neurons are rendered inactive by tetrodotoxin, the effects of amphetamine persist in quiescent neurons (Westerink et al., 1987, Nomikos et al., 1990). In addition, amphetamine causes large increases in extracellular levels of norepinephrine (Kuczenski and Segal, 1989). Therefore, amphetamine may promote the release of endogenous opioids, too, either by a direct action (that cocaine lacks) on opioid-containing neurons or as an indirect consequence of the release of a high concentration of another neurotransmitter.

Dopaminergic neurons in the mesolimbic and nigrostriatal pathways are under the inhibitory influence of GABAergic neurons (Roth and Elsworth, 1995). μ- and δ-opioid agonists appear to increase extracellular concentrations of dopamine in the terminal regions of those pathways by inhibiting GABAergic neurons, thereby disinhibiting dopaminergic neurons. μ-opioid agonists hyperpolarize nondopaminergic neurons in the substantia nigra and ventral tegmentum while leaving the membrane potential of dopaminergic neurons unchanged (Lacey et al., 1989; Johnson and North, 1992). Morphine administered via a microdialysis probe directly into the ventral tegmental area increases extracellular concentrations of dopamine and decreases those of GABA, effects that are blocked by systemically administered naloxone (Kliteneick et al., 1992). The results of the current study are the most direct evidence to date that extracellular levels of dopamine can be modulated by endogenous opioids as well as by exogenous ones, and that amphetamine does, indeed, cause release of endogenous opioids. These results together with those of studies cited above suggest that increases in extracellular dopamine produced by amphetamine in the nucleus accumbens and striatum are not due solely to the action of amphetamine on the dopamine transporter. Rather, this drug effect reflects the sum of two different events: the direct action of amphetamine on dopamine transporters on dopaminergic nerve terminals and the action of endogenous opioids released by amphetamine on GABAergic neurons in the cell body regions.

References


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