

Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats

(amphetamine/cocaine/ethanol/nicotine/opiates)

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ABSTRACT The effect of various drugs on the extracellular concentration of dopamine in two terminal dopaminergic areas, the nucleus accumbens septi (a limbic area) and the dorsal caudate nucleus (a subcortical motor area), was studied in freely moving rats by using brain dialysis. Drugs abused by humans (e.g., opiates, ethanol, nicotine, amphetamine, and cocaine) increased extracellular dopamine concentrations in both areas, but especially in the accumbens, and elicited hypermotility at low doses. On the other hand, drugs with aversive properties (e.g., agonists of κ opioid receptors, U-50,488, tifiuadom, and bremazocine) reduced dopamine release in the accumbens and in the caudate and elicited hypomotility. Haloperidol, a neuroleptic drug, increased extracellular dopamine concentrations, but this effect was not preferential for the accumbens and was associated with hypomotility and sedation. Drugs not abused by humans [e.g., imipramine (an antidepressant), atropine (an antimuscarinic drug), and diphenhydramine (an antihistamine)] failed to modify synaptic dopamine concentrations. These results provide biochemical evidence for the hypothesis that stimulation of dopamine transmission in the limbic system might be a fundamental property of drugs that are abused.

Since it was established that drugs abused by humans are rewarding (i.e., give an interoceptive pleasurable effect) for humans and for animals (1), a great deal of research has been devoted to clarifying the biological mechanism of drug abuse. Drugs that are abused are from diverse and apparently antithetic classes (central depressants, central stimulants, narcotic analgesic drugs, etc.), suggesting that they act through various primary mechanisms. This, however, does not exclude the possibility that these drugs might secondarily activate a final common pathway that mediates their rewarding properties.

Among central nervous system neurotransmitters and neuromodulators, dopamine is a candidate to transmit the rewarding properties of drugs of abuse (2, 3). According to this hypothesis, drugs of abuse would act by stimulating dopamine-mediated transmission along specific pathways. This hypothesis, however, has been challenged because (i) experimental studies utilizing lesions or pharmacological manipulations to explore the role of dopamine in drug reward have provided highly conflicting results (4-7), except for studies with amphetamine (8, 9); and (ii) direct *in vivo* evidence that drugs of abuse indeed stimulate dopamine transmission *in vivo* is lacking, again except for studies with amphetamine (10, 11), as a result of the difficulties inherent in the *in vivo* quantitation of dopaminergic transmission.

Brain dialysis has been developed for measuring extracellular synaptic dopamine concentrations, which can be used as an index of dopamine transmission in freely moving animals

(10-12). With this method, we have studied the effects of various drugs of abuse on the extracellular concentration of dopamine and its metabolites in two anatomically and functionally distinct subdivisions of the dopaminergic system: the nucleus accumbens (accumbens), the major terminal area of the mesolimbic dopaminergic system, and the dorsal caudate nucleus (caudate), a site of projection of the nigrostriatal dopaminergic system (13).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories) (180-200 g) were housed in groups of five rats per cage for at least 5 days before use. Food and water were freely available and animals were maintained under an artificial 12-hr light-dark cycle (light beginning at 0600). Experiments were started between 0800 and 1000.

Surgery. Dialysis tubes (Vitafiber; Amicon; 300- μ m o.d.) were implanted in rats under halothane anesthesia by inserting tubing transversally through the accumbens (coordinates A 9.6 and V 7.0 from temporal bone) (14) and inserting tubing through the dorsal caudate nucleus (coordinates A 7.4 and V 5.5 from temporal bone). The technique used to prepare and to implant the dialysis tube was essentially as described (11, 12). Fig. 1 shows a diagram of the dialysis tubes implanted in the accumbens and in the caudate.

Analytical Procedure. Twenty-minute samples of the dialysate, without any purification, were injected into a high-performance liquid chromatograph equipped with a reverse-phase octadecylsilica column (Supelcosil, Supelco, Bellefonte, PA) and an electrochemical detector (BAS, Lafayette, IN) to quantitate dopamine, dihydroxyphenylacetic acid, and homovanillic acid (11).

Twenty-four hours after the implantation of the dialysis tube, rats were transferred from their cages to the wire-mesh floor of a Perspex cylinder (40 cm in diameter \times 40 cm in height) and perfused through the implanted dialysis tubes with Ringer's solution at a constant rate of 2 μ l/min. After \approx 2 hr of perfusion, when the output of dopamine and of its metabolites became stable, saline or drugs were administered.

Drugs and Reagents. Amphetamine sulfate, cocaine hydrochloride, imipramine, and nicotine hydrogen tartrate (CIBA-Geigy); atropine sulfate and diphenhydramine (Parke, Davis); bremazocine and tifiuadom (Sandoz Pharmaceutical); fentanyl and haloperidol (Janssen Pharmaceutica, Beerse, Belgium); *dl*-methadone (Tosi Farmaceutici, Milan); morphine hydrochloride (S.A.L.A.R.S. Farmaceutici, Como, Italy); and U-50,488 (Upjohn) were dissolved in saline and administered. Ethanol was dissolved in saline [20% (vol/vol) solution] and given intraperitoneally. Methadone was administered intraperitoneally (0.5 ml/100 g). All other drugs were administered subcutaneously in the neck in a volume of 0.1 ml/100 g. For drugs supplied as salts, doses refer to the amount of the base administered. All reagents were analytical grade. Water was twice distilled and filtered

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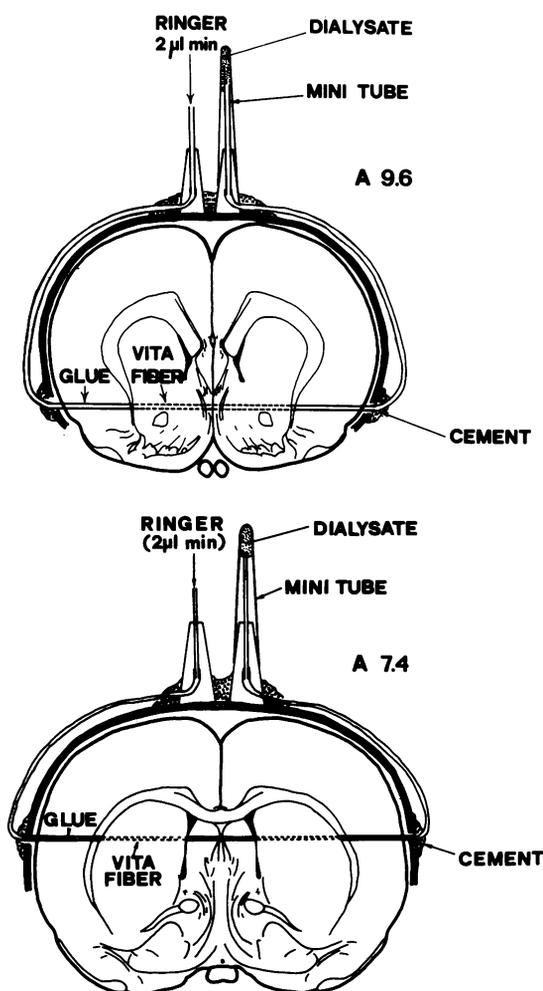


FIG. 1. Diagram of the dialysis tubes implanted at the level of the nucleus accumbens (A 9.6) and of the dorsal caudate nucleus (A 7.4) (14). The stippled portion of the dialysis tube corresponds to the part that is not covered by glue and where dialysis takes place.

through Millipore all-glass filter apparatus (filter type, GSTF; pore size, 0.22 μ m).

Statistics. A two-way analysis of variance for repeated measures and a Newman-Keuls multiple comparison test or a Tukey test were applied to evaluate the statistical significance of the results obtained.

RESULTS

Amphetamine and Cocaine. The effects of rewarding (5, 7, 9) doses of amphetamine [1 mg/kg (s.c.)] and cocaine [5.0 mg/kg (s.c.)] on the output of dopamine, dihydrophenylacetic acid, and homovanillic acid in the accumbens and in the caudate are shown in Figs. 2 and 3, respectively. Amphetamine and cocaine increased synaptic dopamine concentrations preferentially in the accumbens. Amphetamine stimulated dopamine release maximally by a factor of 10 in the accumbens and by a factor of ≈ 5.5 in the caudate ($P < 0.01$). Cocaine increased synaptic dopamine concentrations maximally by a factor of 3.5 in the accumbens and by a factor of 2.5 in the caudate ($P < 0.01$). At these doses amphetamine and cocaine induced behavioral stimulation characterized by hypermotility and rearing.

Opiates: μ Agonists Versus κ Agonists. Morphine at a dose of 1.0 mg/kg (s.c.), which is rewarding in rats (7, 15), increased synaptic dopamine concentrations preferentially in the accumbens and also increased dopamine concentrations when tested over a wide range of doses (Fig. 4A). The ability to increase

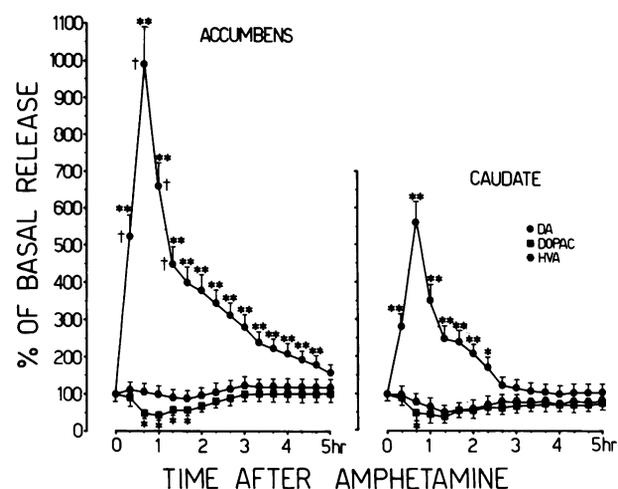


FIG. 2. Effect of amphetamine [1.0 mg/kg (s.c.)] on the output of dopamine (DA), of dihydroxyphenylacetic acid (DOPAC), and of homovanillic acid (HVA) from the accumbens and from the caudate. Basal release (20 min) from the accumbens was as follows: dopamine, 0.20 ± 0.02 pmol; dihydroxyphenylacetic acid, 22.5 ± 2.5 pmol; and homovanillic acid, 19.4 ± 2.3 pmol. Basal release (20 min) from the dorsal caudate was as follows: dopamine, 0.40 ± 0.04 pmol; dihydroxyphenylacetic acid, 35.3 ± 4.2 pmol; and homovanillic acid, 26.7 ± 3.3 pmol. Results are expressed as mean \pm SEM of values obtained from five rats. $P < 0.05$ (*) and $P < 0.001$ (**) are with respect to basal values.

extracellular dopamine preferentially in the accumbens appears to be a property of other opiates that preferentially stimulate μ opiate receptors, such as methadone (Fig. 4B) and fentanyl [0.05–0.1 mg/kg (s.c.)], data not shown]. A 24-hr pretreatment with 10 nmol (intracerebroventricularly) of the μ -receptor-specific antagonist β -funaltrexamine (16) or a low dose of naloxone [0.1 mg/kg (s.c.)] abolished the effect of morphine at 1.0 mg/kg in the accumbens (data not shown). κ receptor agonists, in contrast to μ receptor agonists, are aversive in animals (17, 18). U-50,488 (19), a selective agonist of κ opiate receptors, reduced extracellular dopamine concentrations in the accumbens and in the caudate by the same extent and elicited hypomotility (Fig. 5A). This property was common to other opiate agonists with preferential activity on κ receptors, such as bremazocine (20) (Fig. 5B) and tifluadom (21) (data not shown). The effect of κ agonists on dopamine output was not modified by a 24-hr pretreatment with β -funaltrexamine [10 nmol intracerebroventricularly] but was

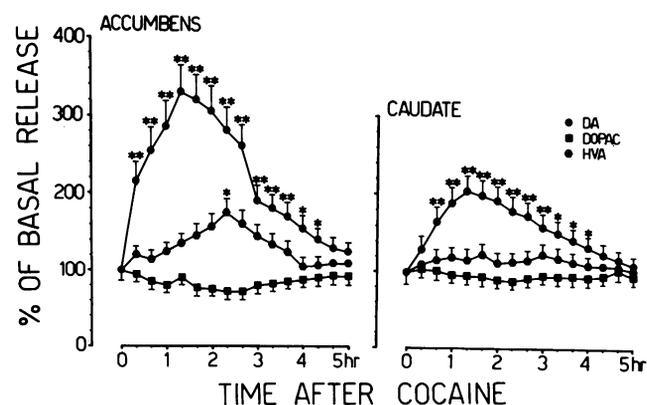


FIG. 3. Effect of cocaine [5 mg/kg (s.c.)] on the output of dopamine (DA), of dihydroxyphenylacetic acid (DOPAC), and of homovanillic acid (HVA) from the accumbens and from the caudate. Results are expressed as percent of basal output. (mean \pm SEM of values obtained from four rats). $P < 0.05$ (*) and $P < 0.001$ (**) are with respect to basal values.

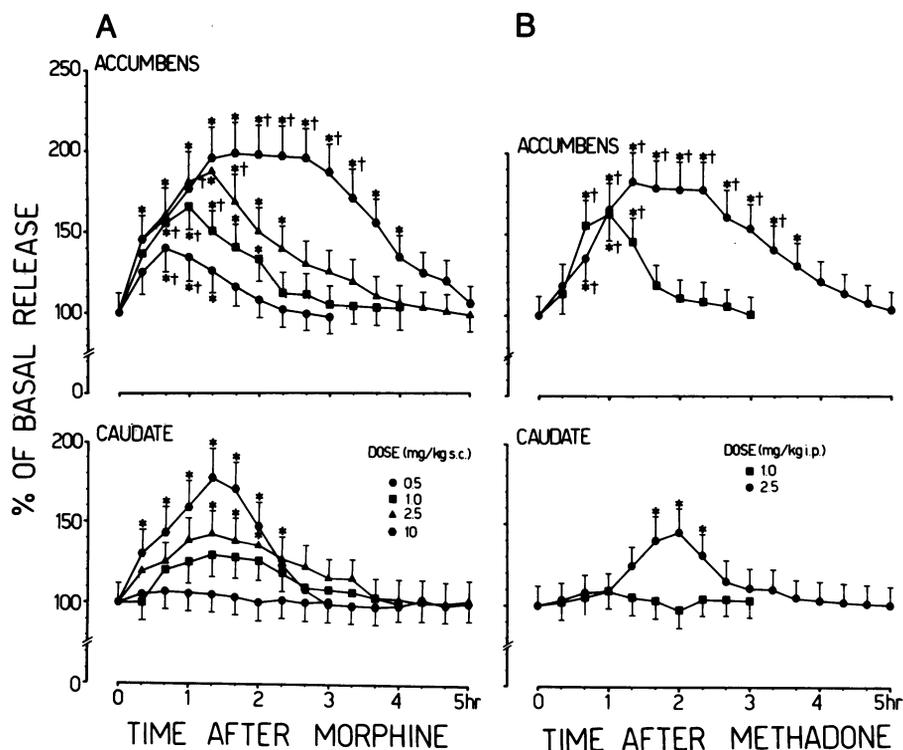


FIG. 4. Effect of morphine (A) and methadone (B) on dopamine output from the accumbens and from the caudate. Results are expressed as percent of basal output (mean \pm SEM of values obtained from at least four animals). $P < 0.05$ (*) is with respect to basal values; $P < 0.05$ (†) is with respect to the corresponding values of the caudate.

reduced by a rather large dose of naloxone [2.5 mg/kg (s.c.)] (data not shown).

Ethanol. As shown in Fig. 6, ethanol, which is rewarding in rats under selected experimental conditions (22, 23), increased synaptic dopamine concentrations preferentially in the accumbens over a wide range of doses [0.25–2.5 g/kg (i.p.)].

Nicotine. Nicotine, a rewarding drug (24, 25), at 0.6 mg/kg (s.c.) increased synaptic dopamine concentrations by $\approx 100\%$

in the accumbens and by 50% in the caudate and elicited behavioral stimulation characterized by marked rearing, locomotion, and grooming (Fig. 7). The nicotine-induced increase of extracellular dopamine was dose-related, being $\approx 100\%$ after the highest doses tested [0.8 mg/kg (s.c.)]. The effect of nicotine [0.6 mg/kg (s.c.)] on dopamine concentrations and behavior was not influenced by blockade of peripheral nicotine receptors with hexamethonium [1 mg/kg (s.c.), 40 min before nicotine], although it was significantly

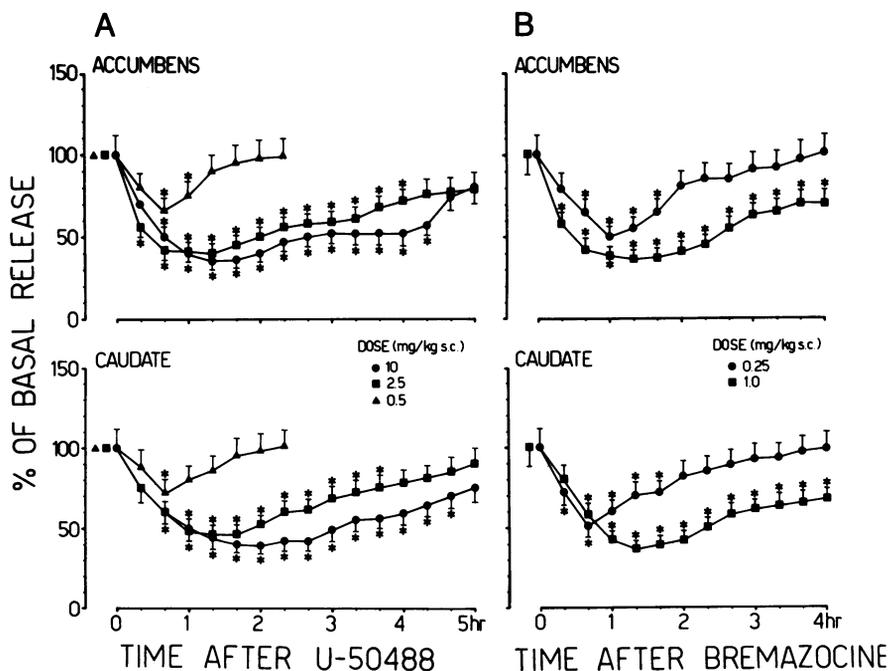


FIG. 5. Effect of two κ -opiate agonists [U-50,488 (A) and bremazocine (B)] on dopamine output from the accumbens and from the caudate. Results are expressed as percent of basal release (means \pm SEM of values obtained from at least four rats). $P < 0.05$ (*) is with respect to basal values.

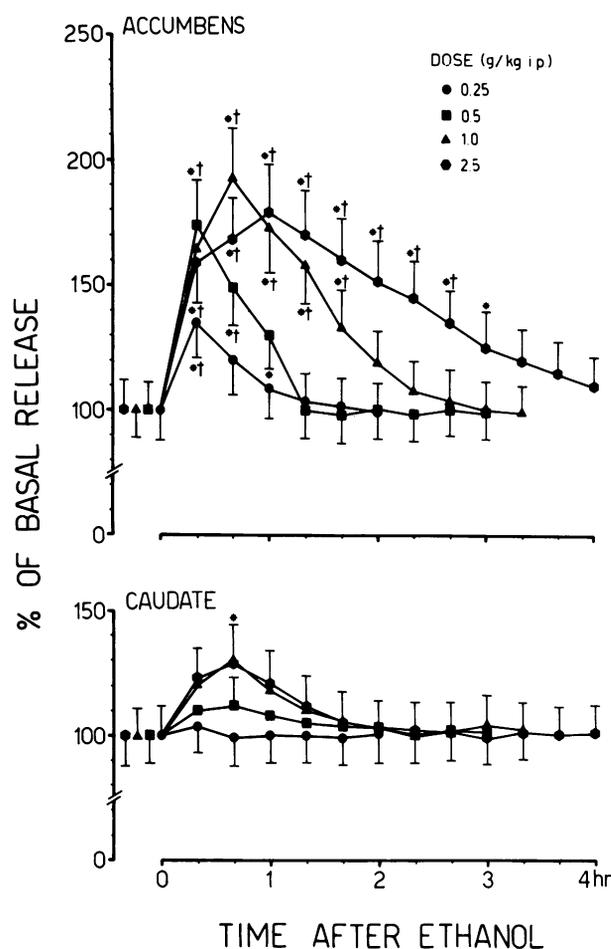


FIG. 6. Effect of ethanol on dopamine output from the accumbens and from the caudate. Results are percent of basal release (mean \pm SEM of values obtained from at least four rats). $P < 0.05$ (*) is with respect to basal values. $P < 0.05$ (†) is with respect to the corresponding values of the caudate.

reduced by an antagonist of central nervous system nicotinic receptors, such as mecamylamine [1 mg/kg (s.c.), 40 min before nicotine]. The following results for a 2-hr accumulation of dopamine output from the accumbens were obtained: for the saline control, 0.83 ± 0.10 pmol; for nicotine, 1.375 ± 0.16 pmol; for mecamylamine plus nicotine, 0.92 ± 0.12 pmol (not significant); and for hexamethonium plus nicotine,

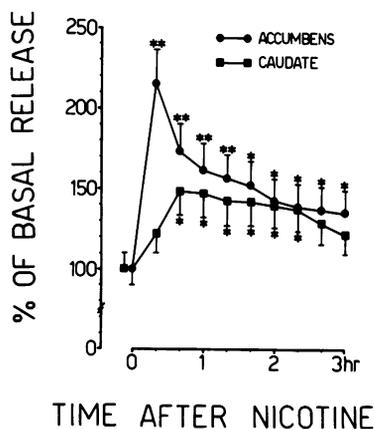


FIG. 7. Effect of nicotine [0.6 mg/kg (s.c.)] on the output of dopamine from the accumbens and from the caudate. Results are expressed as percent of basal values (means \pm SEM of values obtained from six animals). $P < 0.05$ (*) and $P < 0.001$ (**) are with respect to basal values.

1.429 ± 0.18 pmol (mean \pm SEM of the results obtained from five rats).

Nonabused Drugs. Neuroleptics are not rewarding *per se* and block the rewarding properties of typical reinforcers, such as amphetamine (7) and food (26). Haloperidol, a typical neuroleptic, increased extracellular dopamine concentrations in the caudate and in the accumbens by the same extent. For the first 3 hr after administration of haloperidol at 0.1 mg/kg (s.c.), dopamine output from the accumbens was $135 \pm 15\%$ (mean \pm SEM) of the basal level and from the caudate was $145 \pm 16\%$ of the basal level. For the same period after administration of haloperidol at 0.5 mg/kg (s.c.), dopamine output from the accumbens was $180 \pm 20\%$ of the basal level and from the caudate was $193 \pm 22\%$ of the basal level. Hypomotility and sedation were observed at all the doses tested (12). No changes in dopamine output from the accumbens and from the caudate were observed after other drugs not abused by humans (25)—e.g., imipramine (a tricyclic antidepressant) at 20 mg/kg, atropine (an antimuscarinic drug) at 50 mg/kg (s.c.), and diphenhydramine (an antihistamine) at 25 mg/kg (s.c.) (data not shown).

DISCUSSION

The present results show that drugs belonging to different pharmacological classes but sharing the characteristic of being rewarding in animals and humans share the properties of preferentially increasing synaptic dopamine concentrations in the mesolimbic dopaminergic system and of stimulating behavior.

The drugs showing these properties include central stimulants (e.g., amphetamine and cocaine), opiates (e.g., morphine, methadone, and fentanyl), central depressants (e.g., ethanol), and cholinergic agonists (e.g., nicotine). Whereas amphetamine and cocaine increase extracellular dopamine by displacing it from presynaptic sites and by blocking dopamine reuptake, respectively, opiates, ethanol, and nicotine increase extracellular dopamine by stimulating the firing of dopaminergic neurons (27–29).

At low doses of the various drugs of abuse there is a correlation between stimulation of behavior and increase of synaptic dopamine concentrations in the accumbens; however, this is not the case at higher doses of opiates and ethanol that elicit motor inhibition with rigidity (opiates) or sedation and hypnosis (ethanol) in spite of the fact that they further stimulate dopamine output in the accumbens. This apparent lack of correlation between the increase of synaptic dopamine and behavioral stimulation might result from the fact that opiates and ethanol act at independent sites located downstream from the dopaminergic system that interfere with the behavioral expression of dopaminergic stimulation.

The μ opioid receptors mediate narcotic-stimulated dopamine release, as indicated by their sensitivity to β -funaltrexamine. Nicotine stimulation of dopamine transmission is not due to a peripheral action since it was blocked by central but no peripheral nicotine antagonists.

Drugs without rewarding properties (e.g., neuroleptics, tricyclic antidepressants, antimuscarinic drugs, and antihistamines) or with aversive properties [e.g., κ opiate receptor agonists (17, 18)] are devoid of the properties of the abused drugs outlined above. Thus, neuroleptics increase synaptic concentrations of dopamine in the accumbens but not in a preferential manner compared to the dorsal caudate. Moreover, neuroleptics induce sedation and motor inhibition with catalepsy even at low doses as a result of blockade of post-synaptic dopamine receptors (12). Indeed, for neuroleptics, the increase in synaptic dopamine is a feedback response to a primary impairment of dopamine transmission induced by the blockade of dopamine receptors (12). The κ opiate receptor agonists on the other hand reduce synaptic

dopamine to a similar extent in the accumbens and in the caudate and induce sedation and hypomotility.

It appears, therefore, that, among drugs active in the central nervous system, the ability to act as a rewarding stimulus, to activate motor behavior, and to increase synaptic dopamine concentrations in the mesolimbic system are in some way linked to one another. It is unlikely that the rewarding properties of abused drugs are secondary to their ability to induce behavioral activation since drugs such as opiates, ethanol, and barbiturates retain their rewarding properties at doses eliciting a depression of motor behavior (2, 3); as shown by our results, at these doses the drugs are still able to increase synaptic dopamine concentrations. Therefore, we suggest that the rewarding and motor stimulating properties often coincide because they are both dependent, at least in part, on the activation of the mesolimbic dopaminergic system. Thus, activation of the mesolimbic dopaminergic system would result in or contribute to an interoceptive pleasurable effect (reward) as well as to a motor-activating effect; motor activation, however, does not seem to be essential for experiencing pleasure.

This hypothesis is supported by experimental evidence on the role of the mesolimbic dopamine system in the motor-stimulant properties as well as in the rewarding properties of amphetamine (2, 3, 30). Our results provide biochemical *in vivo* evidence for a role of dopamine in the rewarding properties not only of amphetamine but also of cocaine, opiates, ethanol, and nicotine.

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