4-Ethoxyamphetamine: Effects on Intracranial Self-Stimulation and *in vitro* Uptake and Release of ^{3}H-Dopamine and ^{3}H-Serotonin in the Brains of Rats

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Experiments were conducted to compare the effects of 4-ethoxyamphetamine, a novel "designer" amphetamine, with (+)-amphetamine and an earlier "designer" amphetamine, 4-methoxyamphetamine, on rats. (+)-Amphetamine significantly decreased frequency threshold measures in an intracranial self-stimulation (ICSS) procedure using medial forebrain bundle electrodes, while 4-methoxyamphetamine and 4-ethoxyamphetamine increased these ICSS frequency thresholds. 4-Methoxyamphetamine and 4-ethoxyamphetamine had more potent effects on inhibition of uptake and stimulation of spontaneous release of 5-hydroxytryptamine (serotonin) than of dopamine. It is concluded that the neuropsychopharmacological profile of 4-ethoxyamphetamine is unlike that of (+)-amphetamine, but similar to that of 4-methoxyamphetamine, a potent hallucinogen in humans.

Key Words: amphetamine, 4-ethoxyamphetamine, 4-methoxyamphetamine, intracranial self-stimulation, neurotransmitter amines, uptake and release

INTRODUCTION

Designer drugs are substances with molecular structures designed to circumvent existing drug regulations (Lodge 1991). In 1987, Canadian police seized from an illicit laboratory a quantity of a chemical that was later identified as 4-ethoxyamphetamine by the Drug Identification Division of Health and Welfare Canada (Lodge 1991). 4-Ethoxyamphetamine was subsequently synthesized in quantity and characterized (By et al 1991). Concerns about its possible abuse and toxic effects led to a series of experiments to determine the characteristics of this designer amphetamine. It seemed likely that 4-ethoxyamphetamine shares properties with either (+)-amphetamine, a psychomotor stimulant, or with 4-methoxyamphetamine, another abused designer amphetamine characterized by potent hallucinogenic effects but without psychomotor stimulation (Tseng et al 1976). After a series of experiments in which these amphetamines were administered to rats with or without previous treatment with reserpine, Martin-Iverson et al (1991) concluded that 4-ethoxyamphetamine is not a psychomotor stimulant, but that many of its effects are similar to those of 4-methoxyamphetamine. The effects of these three drugs on intracranial self-stimulation (ICSS) (Greenshaw and Wishart 1987) and on the uptake and release of dopamine and 5-hydroxytryptamine (5-HT, serotonin) in striatal prisms (Martin et al 1978; Baker et al 1980) were investigated in the present study. A number of drugs of abuse, such as (+)-amphetamine, can alter the reinforcing properties of

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ICSS, and this property may predict the abuse potential of both stimulant and opioid compounds (Wise and Rompre 1989). It has been established that the behavioral effects of (+)-amphetamine are dependent primarily on its capacity to increase the release of a newly synthesized pool of dopamine from neuronal terminals through what appears to be a carrier-mediated exchange diffusion process (Arnold et al 1977; Fischer and Cho 1979; Kuczenski et al 1990; Miller and Shore 1982; Raiteri et al 1979; Scheel-Kruger 1971; Westerink et al 1989). However, (+)-amphetamine also blocks uptake of catecholamines by the pre-synaptic terminal and, at higher doses, inhibits monoamine oxidase, a primary catabolic enzyme for monoamines. Although much is still unknown about the specific mechanisms of action of hallucinogens, evidence suggests that these drugs produce their effects through actions on 5-HT-containing neurons or on cells post-synaptic to 5-HT-releasing terminals (Jacobs and Trulson 1981). Because 4-methoxyamphetamine is structurally so closely related to 4-ethoxyamphetamine and is an hallucinogen, it was of interest to compare the effects of 4-ethoxyamphetamine and 4-methoxyamphetamine on the uptake and release of both dopamine and 5-HT.

METHODS

Animals and drugs

Male Sprague-Dawley rats were housed under conditions of controlled temperature and humidity and a 12 hour light/12 hour dark cycle. The protocols for all experiments were approved by the Health Sciences Animal Welfare Committee of the University of Alberta. (+)-Amphetamine, (±)-4-methoxyamphetamine and (±)-4-ethoxyamphetamine were obtained from the Bureau of Drug Research, Health and Welfare Canada.

Radiochemicals

^{3}H-Dopamine HCl (sp. act. 47.0 Ci/mmol) and ^{3}H-5-HT binoxalate (38.1 Ci/mmol) were used.

Surgery and ICSS testing

Animals were anesthetized with pentobarbital (40 mg/kg to 50 mg/kg) and, using a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA), each rat was implanted unilaterally with a monopolar stainless steel electrode (200 μm) directed to the medial forebrain bundle at the level of the lateral hypothalamus (König and Klippel 1963) and a silver reference electrode in the frontal bone of the skull. The coordinates for stimulating electrode placement (mm from bregma) were AP + 1.5, lateral + 1.5, and ventral –8.5 relative to skull surface. The electrodes were secured to the skull with stainless steel screws and dental acrylic.

After a seven-day recovery period, the animals allocated to the self-stimulation condition were trained to press a response lever on a continuous reinforcement schedule of electrical hypothalamic stimulation in standard test chambers (Coulborn Instruments, Lehigh Valley, PA), each equipped with house light and a light above the response lever. Hypothalamic stimulation (train duration = 1 s, cathodal pulse width = 0.2 ms) was provided from a constant current source (Acadia Instruments, Saskatoon, SK), which was connected to the rats through gold track slip-rings (Stoelting Inc., Chicago, IL). Throughout the test sessions, the current intensity for each subject was monitored on an oscilloscope as the voltage drop on a 10 kΩ precision resistor connected in series with the rat. Microcomputers (CBM, Palo Alto, CA) served to control stimulation parameters and response-contingent delivery of stimulation and to record the behavioral responses on line.

The animals were trained on a schedule of varying stimulation frequency, based on the procedure developed by Gallistel and Karras (1984). The training procedure was described in detail by O'Regan et al (1987). In frequency-response tests, the effects of stimulation frequency on the subject's response rate were assessed in each test session. In these tests, the stimulation was initially set at 160 Hz and lowered in 0.1 log units until the response was extinguished. It was then increased in 0.1 log units up to 160 Hz. The animals had access for 60 s to each frequency step. The rates achieved with the ascending and descending frequencies were averaged for each step, and half-maximal rates were calculated with linear regression (rate on log frequency) as described above for the current-response assessment. Threshold measures were the frequency that maintained half-maximal rates (M_{50}) and the minimum frequency at which a response occurred (M_0).

For drug testing, the animals were injected with a range of doses of each drug in random order. Each drug test day was preceded by at least three control days. On the drug test days, a drug dose was administered i.p. 5 minutes prior to testing in a volume of 1 mL/kg. An equivalent volume of the 0.9% NaCl vehicle was administered on each control day. Each animal's performance on each drug per day was expressed as a percentage of its performance on the previous control day. 4-Ethoxy-amphetamine, 4-methoxy-amphetamine and (+)-amphetamine were tested on groups of ten, 11 and nine animals, respectively.

Uptake experiments

This protocol was based on the procedure established by Martin et al (1978). Rats were stunned and killed by decapitation, and their brains were removed and placed on an ice-cooled plate and both striata dissected out. Tissue prisms of 0.1 × 0.1 × 2 mm approximately were obtained with a McIlwain chopper and dispersed in cold incubation medium containing 123 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20 mM Tris-HCl buffer, pH 7.4, 10 mM glucose, 12.5 μM nialamide (inhibitor of monoamine

Table 1

IC_{50} values for the inhibition of uptake of neurotransmitter amines in the striatum by amphetamines[a]

	Neurotransmitter[b]		
Drug	^{3}H-dopamine	^{3}H-5-HT	IC_{50} inhibition 5-HT uptake/ IC_{50} inhibition DA uptake
(+)-amphetamine	0.19 ± 0.03 (10)	11.3 ± 1.4 (12)	59.5
4-methoxyamphetamine	15.0 ± 1.4 (9)	1.9 ± 0.3 (7)	0.12
4-ethoxyamphetamine	13.0 ± 0.8 (7)	1.7 ± 0.2 (8)	0.13

[a]The results are expressed in μM (means ± SEM) and were determined by plotting the percentage of inhibition of uptake versus drug concentrations (on a log scale) for a series of concentrations of each drug; [b]For each drug, the number of experiments conducted in duplicate is indicated in parentheses.

oxidase) and 1 mM ascorbic acid. The tissue suspension at a concentration of 1 mg/mL was then equilibrated at 37°C in a shaking water bath for 15 minutes. [^{3}H]-labelled dopamine or 5-HT (final concentration = 0.02 μM) was then added simultaneously with various concentrations of drug and the incubation continued for a further five minutes. The tissue was subsequently separated from the incubation medium by rapid filtration using a Millipore filtration device and was washed twice with a warm (37°C) incubation medium. The filter containing the tissue was then placed in a scintillation vial, and a liquid scintillation cocktail was added.

A series of concentrations of each drug was studied, and the percentage of inhibition was plotted against drug concentrations (on a log scale) to determine the mean concentration giving 50% inhibition of uptake (IC_{50}).

Release experiments

The procedure described here is taken from the study by Baker et al (1980) and is a modification of the procedure developed by Raiteri et al (1974; 1975). The initial part of the experiment was carried out as described in the uptake experiments above, except that the drug was omitted during the incubation period. The tissue was subsequently separated from the incubation medium by rapid filtration through a filter contained in a superfusion chamber thermostatically maintained at 37°C (Brandel Superfusion 6 apparatus). The tissue was washed twice with incubation medium at 37°C. Subsequently, more incubation medium was added to the chamber containing the filter and tissue. The outflow from the chamber was then attached to the pumping mechanism and the incubation medium drawn over the tissue at a rate of 0.5 mL/minutes; one-minute fractions were collected, and at the end of fraction 4, the incubation medium in the chamber was replaced by medium containing the drug of interest (or by more control medium in the case of the control chambers), and the superfusion was continued for a further 12 minutes. Scintillation fluid was added to each of the collection vials, and the radioactivity in each vial was counted. The amount of radioactivity present in fractions 10 to 14 inclusive was compared for the controls and the drugs. The system had a dead volume (volume contained in collection tubes) of five fractions, so any release became evident in fraction 10 and subsequent fractions.

Statistical analyses

The data from the ICSS and release experiments were subjected to ANOVAs, followed, when necessary, by the Newman-Keuls test. The conventional $p < 0.05$ was the criterion for statistical significance.

RESULTS AND DISCUSSION

(+)-Amphetamine decreased both M_0 and M_{50} for ICSS from electrodes implanted in the medial forebrain bundle in the region of the lateral hypothalamus (Fig. 1A) with doses from 0.25 mg/kg to 4 mg/kg. This replicates the findings of

Table 2

Effects of amphetamine analogues (10 μM) on basal release of ^{3}H-dopamine and ^{3}H-5-HT from striatal prisms[a]

	Mean release of ^{3}H-dopamine	Mean release of ^{3}H-5-HT	
Drug	(% of controls)	(% of controls)	N
(+)-amphetamine	194 ± 6.7[b]	121 ± 4.3[b]	12
4-ethoxyamphetamine	107 ± 3.2	145 ± 8.4[b]	10
4-methoxyamphetamine	108 ± 3.4	155 ± 8.7[b]	9

[a]The values represent comparisons of amounts of radioactivity released in fractions 10 to 14 inclusive and are expressed as means ± SEM; [b]$p < 0.05$ compared with control values. In the case of ^{3}H-5-HT release, the value for (+)-amphetamine is significantly less than those for 4-ethoxyamphetamine and 4-methoxyamphetamine.

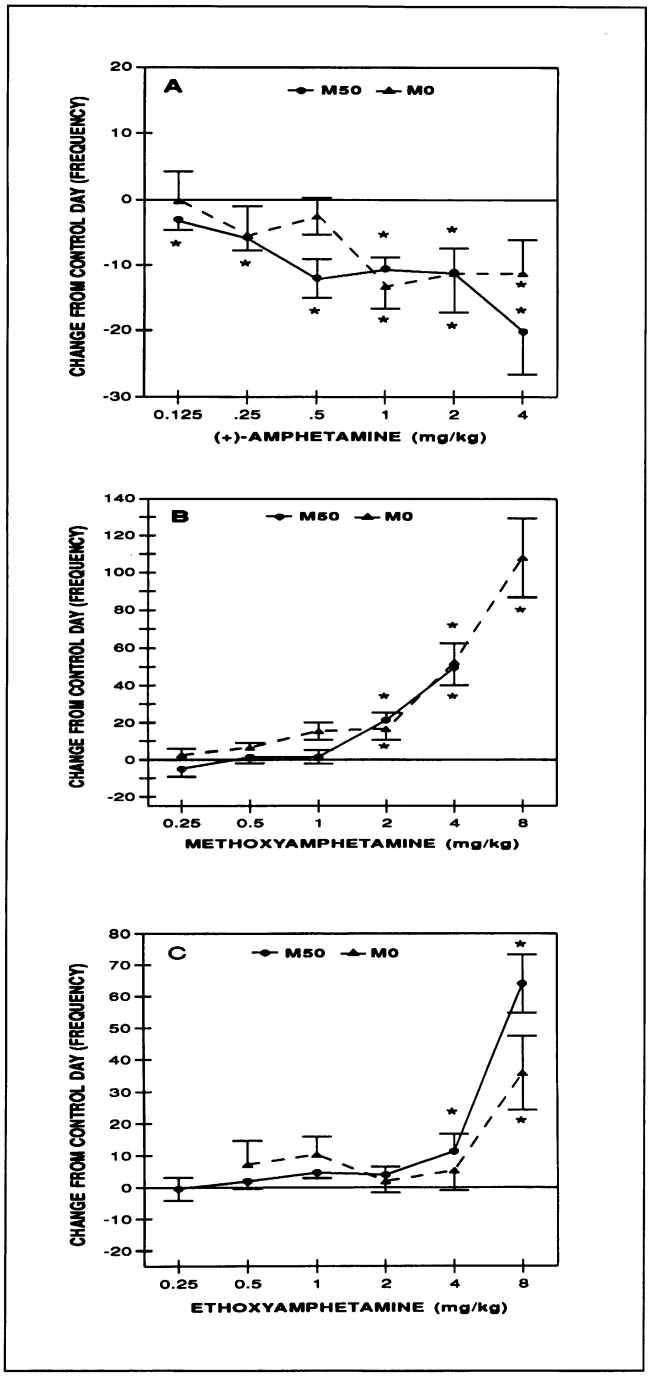

Fig. 1: Effects of (+)-amphetamine (A), 4-methoxyamphetamine (B) and 4-ethoxyamphetamine (C) on medial forebrain bundle stimulation frequency required to sustain responses on a lever at 50% of maximal response rate (M_{50}) and on the minimum frequency necessary to elicit responding (M_0). The value for each dose was compared with the value of the previous day on which the animals received a vehicle injection. The results represent means ± SEM (n = 9 to 11). Significantly different from controls, *$p < 0.05$.

well established reinforcement-enhancing effects of (+)-amphetamine at this site (Gallistel and Karras 1984). However, 4-methoxyamphetamine (Fig. 1B) was without effect at doses between 0.25 mg/kg and 1 mg/kg or 2 mg/kg, but increased the thresholds at higher doses. 4-Ethoxyamphetamine had effects similar to those of 4-methoxyamphetamine, but higher doses were required to increase thresholds (Fig. 1C). The threshold-increasing effects of both 4-methoxyamphetamine and 4-ethoxyamphetamine paralleled the drug-induced decreases in total responses. The apparent decrease in reinforcement efficacy may be the result of attentional effects (for example, hallucinogenic actions) or disruptions of motor performance induced by the drugs.

These results indicate that 4-ethoxyamphetamine does not increase the reinforcing efficacy of electrical stimulation of the medial forebrain bundle in the lateral hypothalamus, as does (+)-amphetamine or 3,4-methylenedioxymethamphetamine (MDMA, or "ecstasy"), a designer amphetamine with mood-altering properties (Hubner et al 1988). Indeed, administration of 4-ethoxyamphetamine appears to result in a decrease in the reinforcement efficacy in a fashion similar to 4-methoxyamphetamine but with a higher dose requirement.

As shown in Tables 1 and 2, 4-ethoxyamphetamine has effects on the uptake and release of 5-HT and dopamine similar to those of 4-methoxyamphetamine. Both drugs had different profiles from that of (+)-amphetamine, with stronger effects on the uptake and release of 5-HT than of dopamine. These results on uptake and release are compatible with the behavioral effects of 4-methoxyamphetamine and 4-ethoxyamphetamine. For the release experiments, a dose of 10 μM was chosen for comparison of the drugs. (At 1 μM, (+)-amphetamine produced no effect on 5-HT release.) The differential behavioral effects of 4-ethoxyamphetamine and 4-methoxyamphetamine from those of (+)-amphetamine, observed particularly at high doses, parallel previous findings. Martin-Iverson et al (1991) demonstrated similar differential effects of these drugs on locomotor activity. They also showed that at similar doses (8 μmol/kg to 32 μmol/kg) the drugs reached levels in brain in the 10 μM range (assuming that 1 g of tissue equals 1 mL). Martin-Iverson and Lodge (1991), in a study in which the three drugs were administered with osmotic minipumps at a dose of 40 μmol per day for one, three, seven or 14 days, reported striatal drug levels ranging from 5 μM to 11 μM.

The effects of psychomotor stimulants such as (+)-amphetamine on reinforcement are apparently the result of actions on dopamine neurons, most likely by increasing dopamine release, in the case of (+)-amphetamine-like stimulants, or the blockade of dopamine uptake, in the case of methylphenidate-type stimulants (Wise and Rompre 1989). Neither 4-methoxyamphetamine nor 4-ethoxyamphetamine had (+)-amphetamine-like effects on ICSS, and neither compound had a marked effect on the release or

uptake of dopamine. Both 4-methoxyamphetamine and 4-ethoxyamphetamine had stronger effects on the uptake and release of 5-HT than of dopamine. 4-Methoxyamphetamine is a potent hallucinogen, assessed as being five times more potent than mescaline (Shulgin 1978). Hallucinogenic activity has been related to effects on 5-HT systems (Jacobs and Trulson 1981), although its exact mechanism or mechanisms of action are not known. The preferential effect of 4-methoxyamphetamine on the uptake and release of 5-HT is consistent with this view. Furthermore, since its effects so closely parallel those of 4-methoxyamphetamine, it is likely that 4-ethoxyamphetamine is also hallucinogenic.

In summary, the results of a series of experiments studying ICSS and amine uptake and release in rats suggest that the neuropsychopharmacological profile of 4-ethoxyamphetamine is more similar to that of the hallucinogen 4-methoxyamphet amine than to that of (+)-amphetamine.

Further studies, which include experiments on the effects of 4-ethoxyamphetamine on electrically or high K^+-induced release of ^{3}H-dopamine and ^{3}H-5-HT, seem warranted. Another aspect of 4-ethoxyamphetamine and 4-methoxyamphetamine which should be considered is the presence of a chiral centre in these drugs. They are currently available as racemic mixtures of (+) and (-) enantiomers, and individual enantiomers of psychotropic drugs often differ in their pharmacological and pharmacokinetic properties (Coutts and Baker 1989; Jamali et al 1989). Future studies of levels in the brains of rats and extension to plasma levels in humans as well as uptake and release studies should involve a comparison of the individual enantiomers.

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