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FURTHER EVIDENCE FOR AN AMPHETAMINE-LIKE MECHANISM OF ACTION OF THE ALKALOID CATHINONE

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Abstract—The alkaloid (−)cathinone is a potent stimulant with pharmacological properties closely resembling those of (+)amphetamine. Since (−)cathinone is capable of inducing release at physiological catecholamine storage sites, it has been suggested that (−)cathinone and (+)amphetamine have the same mechanism of action.

In the present study, the potency of (−)cathinone in inducing the release of radioactivity from ^{3}H-dopamine prelabelled tissue of the rat caudate nucleus was compared to that of several structural analogs, i.e. to that of four other aminophenones. (−)Cathinone was found to be the most potent of the compounds under investigation, and among these only demethylcathinone had an effect that was within the same order of magnitude as that of (−)cathinone. Furthermore, (−)cathinone and two of its analogs were evaluated in behavioral experiments with regard to their ability to substitute for (+)amphetamine in rats trained to discriminate between (+)amphetamine and saline. It was found that, unlike the other aminophenones, (−)cathinone is capable of producing (+)amphetamine-like stimulus effects, and these can be antagonized by haloperidol in a dose-related manner.

Taken together, these findings support the hypothesis that (+)amphetamine and (−)cathinone produce their central stimulant effect via the same dopaminergic mechanism.

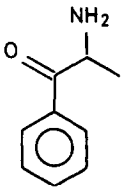
About ten years ago, the alkaloid *S*(−)-α-aminopropiophenone was isolated from the leaves of the khat bush (*Catha edulis*, Celastraceae), a material widely used as a stimulant in East Africa and the Arab Peninsula. The new compound is now known under the name cathinone; it has been shown to be a potent stimulant and to have pharmacological properties closely resembling those of amphetamine [1]. Thus, when administered to animals, cathinone induces a sympathomimetic syndrome, and it causes anorexia, hypermotility and stereotyped oral activities such as licking and gnawing. Moreover, it has been demonstrated in a series of *in vitro* experiments that cathinone, as amphetamine, induces release at physiological catecholamine storage sites [2–4]. Since the CNS stimulation caused by amphetamine is attributed to catecholamine release [5], it has been suggested that cathinone and amphetamine have the same mechanism of action.

With regard to structure, cathinone differs from amphetamine only in that the benzylic methylene group of the latter is replaced by a keto group. Furthermore, (+)amphetamine and (−)cathinone are stereochemical analogs since both possess the same absolute configuration (i.e. *S*). In view of the possibility that other compounds bearing a benzylic keto group, i.e. aminophenones, might have effects similar to those of cathinone, several such compounds were evaluated *in vitro* with regard to their ability to cause release from CNS dopamine storage sites, and *in vivo* with respect to their ability to substitute for (+)amphetamine in a drug discrimination test performed by rats. For the release experiments, the caudate nucleus of the rat was chosen since it has a high density of dopaminergic terminals and is known to play a role in psychostimulant effects. Furthermore, the drug-induced release of neurotransmitter from rat striatum is well documented and, in the case of dopamine-prelabelled tissue, the identity of the released material has been ascertained [6]. In the drug discrimination studies, (+)amphetamine was chosen as the training drug because it has been demonstrated previously that the (+)amphetamine-stimulus generalizes to cathinone [7].

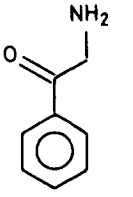
MATERIALS AND METHODS

Release studies. Samples of rat striatal tissue, excised from the head of the caudate nucleus immediately after killing of the animals, were cut with a scalpel into cubes measuring less than 1 mm in each dimension. These were then incubated for 20 min at 37° in 1 ml of a solution containing (mmol/l) NaCl 136; KCl 5.6; $NaHCO_3$ 20.0; NaH_2PO_4 1.2; $CaCl_2$ 2.2; $MgCl_2$ 1.2 and glucose 5.5, and to which 12 μCi ^{3}H-dopamine (0.8 nmol dopamine) had been added. During the incubation (as well as during the subsequent superfusion) the medium was continously oxygenated with a mixture of 95% O_2 and 5% CO_2; its pH was 7.2. At the end of the labeling period, two of the tissue cubes were placed into each of five parallel flow cells and superfused at 37° with dopamine-free medium at a rate of 0.15 ml/min. During an initial washout period of 1 hr, the spontaneous efflux of radioactivity from the preparations stabilized to a steady slow decrease; during the subsequent experimental period, the superfusate was collected in successive 3-min fractions. The test substances were dissolved in the physiological solution described above and added to the superfusion fluid during collection of the fourth fraction. Exposure to the test substances was terminated at the beginning of the collection of the tenth fraction and the exper-

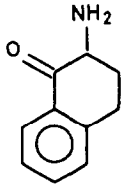
Table 1.



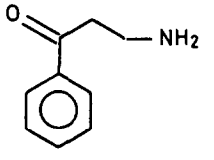
α-Aminopropiophenone (cathinone)



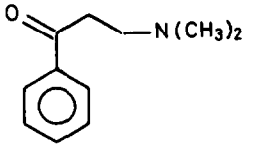
Aminoacetophenone (demethylcathinone)



2-Amino-1-tetralone (ringcathinone)



β-Aminopropiophenone



N,N-Dimethylaminopropiophenone

iment was discontinued after collection of fraction 14. The tritium content of the fractions was then determined by scintillation counting and the ratio between the radioactivity of fractions 8 and 2 was determined. In a series of control experiments addition of 5 μmol/l (+)amphetamine to the medium superfusing the tissue samples resulted in an increase of the efflux of radioactivity to 235.0 ± 9.2% during fraction 8 as compared to that during fraction 2, whereas the efflux decreased to 73.8 ± 2.4% when drug-free solvent was added to the superfusion medium. For each of the substances tested at least three dose-response experiments, carried out on different days, were performed.

Drug discrimination studies. Male Sprague–Dawley rats were trained to discriminate 1.0 mg/kg (+)amphetamine sulfate from saline solvent under a variable-interval 15-sec schedule of reinforcement for sweetened milk reward using standard two-lever operant chambers (Coulbourn Instruments model E 10-10). For the present investigation, animals were used that had been trained for an earlier study; the training procedure has already been described in detail [7]. Persistence of the ability to discriminate (+)amphetamine from saline was ensured by interposing all test sessions between discrimination training sessions; the latter were conducted during the two days preceding any test session, and an odd number of training sessions (at least three) separated any two test sessions. Animals that made either less than 80% amphetamine-appropriate responding when given amphetamine or more than 20% amphetamine-appropriate responding when given saline were considered as not discriminating between the two treatments and were excluded from the immediately following test session. During the test sessions, the animals were allowed 2.5 min (with no reinforcement) for lever responding, and were then removed from the operant chambers.

Generalization tests were run in order to determine if the cathinone analogs would substitute for the (+)amphetamine-stimulus. Several doses of the analogs were administered in a random sequence with a 15 min injection-time interval prior to the extinction test period. Stimulus generalization was defined as occurring when the animals, after adminis-

tration of a given dose of challenge drug, made more than 80% of the responses on the amphetamine-designated lever. Animals making less than a total of five responses during the 2.5 min extinction session were reported as displaying disruption of behavior. For those compounds where generalization occurred, ED_{50} values were determined from the dose–response data by the method of Finney [8]. In the stimulus antagonism studies, groups of rats were injected with varying doses of haloperidol (or, for positive controls, with 1 ml/kg saline), and 45 min later with either (+)amphetamine (1.0 mg/kg), (−)cathinone (0.8 mg/kg), or saline (1 ml/kg). After a further period of 15 min, the animals were placed in the operant chamber and were allowed 2.5 min (with no reinforcement) for responding.

Drugs. S(−)-α-Aminopropiophenone hydrochloride (cathinone HCl) was kindly provided by Dr. O. Braenden, United Nations Narcotics Laboratory. Published procedures were used for synthesizing aminoacetophenone hydrochloride (α-demethylcathinone HCl) [9], racemic 2-amino-1-tetralone hydrochloride (ringcathinone HCl) [10], β-aminopropiophenone hydrochloride [11], and *N,N*-dimethylaminopropiophenone hydrochloride [12]. Haloperidol was donated by McNeil Laboratories, and (+)amphetamine sulfate was purchased from Sigma Chemicals. All drugs were administered by intraperitoneal injection; solutions were prepared fresh daily with 0.9% sterile saline.

RESULTS AND DISCUSSION

When tissue samples of rat caudate nucleus prelabelled with ^{3}H-dopamine were superfused with saline, the release of radioactivity from the preparation decreased slowly with time and the tritium content of fraction 8 of the collection period was 73.8 ± 2.4% of that of fraction 2. On the other hand, when 4.8 μM/l (−)cathinone was added to the superfusion medium, the radioactivity of fraction 8 increased to 187.1 ± 23.7% of that of fraction 2; the increase of release was rapid and reversible. Several other aminophenones (see Table 1) of closely related structure were also found to induce the release of radioactivity; however, much higher concentrations of these were needed for obtaining an effect of similar amplitude. As can be calculated from the curves of Fig. 1, an efflux of radioactivity twice that observed under control conditions was produced by approximately 13.5 μM/l of α-demethylcathinone, 50 μM/l of β-aminopropiophenone, 65 μM/l of ringcathinone, and 150 μM/l of *N,N*-dimethylaminopropiophenone, as compared to only 2.6 μM/l of (−)cathinone. Thus, (−)cathinone was found to be the most potent of the compounds under investigation, and, among its synthetic analogs, only α-demethylcathinone had an effect that was within the same order of magnitude as that of (−)cathinone itself.

It has been demonstrated previously that stimulus generalization occurs between (+)amphetamine and cathinone regardless of which is used as the training drug [7, 13]; for the purpose of the present investigation, this dose-related generalization was replicated (Table 2). The conformationally restricted cathinone analog, ringcathinone, produced saline-appropriate responding both at two and four times the generalization dose of (−)cathinone; at five times this dose (i.e. at 4 mg/kg), ringcathinone produced

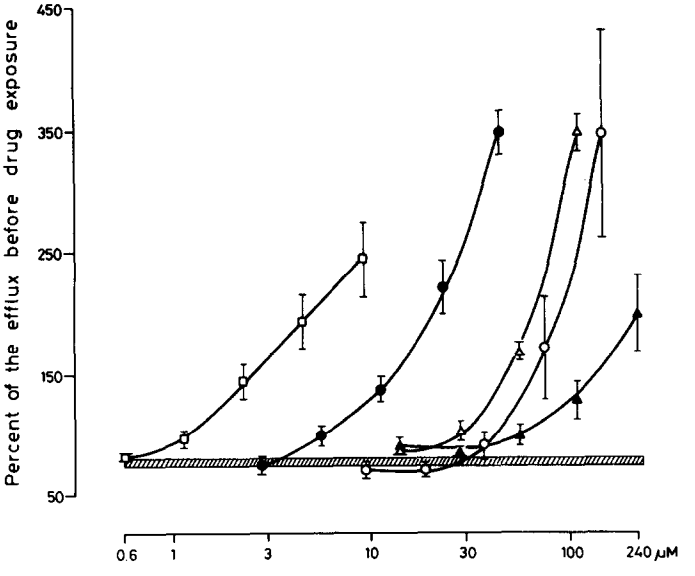


Fig. 1. The effect of (−)cathinone (□) and other aminophenones (see Table 1) on the efflux of radioactivity from rat caudate nucleus tissue prelabelled with ^{3}H-dopamine: ●, α-demethylcathinone (aminoacetophenone); ○, ringcathinone (2-amino-1-tetralone); ▲, *N,N*-dimethylaminopropiophenone; △, β-aminopropiophenone. Results are expressed as the ratio between the efflux during superfusion with a given substance and the efflux before superfusion with the substance. Under control conditions, i.e. addition of drug-free solvent to the superfusion medium, the efflux decreased during the test period to 73.76 ± 2.39% (hatched bar); points are mean ± SEM from 3–6 determinations.

Table 2. Results of stimulus generalization studies with animals trained to discriminate between (+)amphetamine and saline

	Dose (mg/kg)	Number of animals responding vs number receiving drug	Amphetamine-appropriate responding* in % ± SEM	Mean of responses per min* (±SEM)
(−)Cathinone†	0.2	4/4	28 (±6)	12.2 (±1.8)
	0.4	4/4	42 (±16)	12.0 (±1.0)
	0.8	4/4	96 (±3)	11.6 (±1.9)
(±)Ringcathinone	1.6	5/5	2 (±1)	12.3 (±2.0)
	2.0	4/4	0	10.3 (±2.3)
	3.0	4/4	7 (±3)	12.8 (±1.4)
	3.2	3/3	8 (±2)	6.0 (±2.4)
	4.0	1/4	‡	
N,N-Dimethyl-aminopropiophenone	1.0	3/3	5 (±3)	11.7 (±4.9)
	2.5	3/3	8 (±4)	11.2 (±3.8)
	2.8	1/3	‡	
	3.0	0/2	‡	
	5.0	1/3	‡	
(+)Amphetamine§	1.0	5/5	95 (±2)	12.4 (±3.0)
Saline	1 ml/kg	5/5	12 (±4)	11.9 (±2.6)

* Data obtained during 2.5 min extinction session.
† ED_{50} = 0.34 (0.18–0.62) mg/kg.
‡ No responding: disruption of behavior.
§ ED_{50} = 0.42 mg/kg (ref. 7).

disruption of behavior. *N,N*-Dimethylaminopropiophenone produced qualitatively similar results in that 2.5 mg/kg resulted in saline-appropriate responding, whereas 2.8 mg/kg resulted in disruption of behavior (Table 2). α-Demethylcathinone does not produce (+)amphetamine-like effects, probably because it is structurally unprotected toward oxidative deamination and therefore undergoes rapid metabolism *in vivo* [7]. Since β-aminopropiophenone is likewise unprotected, it was not evaluated in the drug discrimination paradigm.

(+)Amphetamine presumably produces its discriminative stimulus effects via a mechanism that involves release of dopamine [14, 15]. To this extent, it has been shown that the stimulus effects of (+)amphetamine can be blocked by pretreatment of the animals with various dopamine antagonists (see Young and Glennon [16] for a review); among these haloperidol has been demonstrated to be particularly effective [14, 17]. Figure 2 shows that haloperidol is capable of antagonizing, in a dose-related manner, the generalization of the (+)amphetamine-stimulus to (−)cathinone; the results for (+)amphetamine are shown for comparison.

Taken together, the results of the present study demonstrate that the potency of (−)cathinone is considerably greater than that of the other aminophenone analogs as far as induction of release from CNS dopamine terminals is concerned. Furthermore, unlike the other aminophenones, (−)cathinone is capable of producing (+)amphetamine-like stimulus-effects, and these are antagonized by halo-

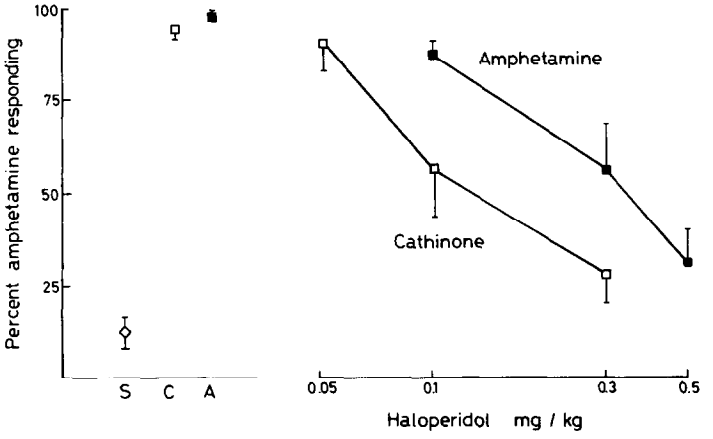


Fig. 2. Antagonism by haloperidol of (+)amphetamine-appropriate responding produced by 1.0 mg/kg of (+) amphetamine and 0.8 mg/kg of (−)cathinone. S, C and A indicate the effect of saline (1 ml/kg), (−)cathinone (0.8 mg/kg), and (+)amphetamine (1.0 mg/kg) in animals pretreated with 1 ml/kg of saline. For each point N is 5.

peridol in a dose-related manner. Thus, the results of the present study support the hypothesis that (+)amphetamine and (−)cathinone produce their central stimulant effects via the same dopaminergic mechanism [18].

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