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NEUROCHEMICAL SIMILARITIES BETWEEN *d,l*-CATHINONE AND *d*-AMPHETAMINE

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Summary

Cathinone, the principal alkaloid of Khat, was compared to the psychomotor stimulant *d*-amphetamine on a number of neurochemical measures. Like *d*-amphetamine, *d,l*-cathinone released and blocked the uptake of tritiated dopamine (DA) in synaptosomal preparations. In addition, repeated high doses of *d,l*-cathinone produced long-lasting DA depletions in various rat brain regions and decreased the number of synaptosomal DA uptake sites in a manner similar to that seen after repeated *d*-amphetamine administration. Importantly, this DA neurotoxic effect of *d,l*-cathinone, like that of *d*-amphetamine, is selective since regional brain levels of norepinephrine (NE) or serotonin (5-HT) are not altered on a long-term basis by repeated administration of *d,l*-cathinone. These findings are discussed with reference to the current practice of Khat leaf chewing by people in north-eastern Africa.

Introduction

Cathinone is the principal psychoactive constituent of the Khat plant *(Catha edulis)* which is native to eastern Africa and the Arab peninsula. Its leaves have been chewed since ancient times to alleviate fatigue, reduce hunger and induce euphoria. Khat use was initially restricted by local custom to certain segments of the population. However, increased availability of the fresh plant has led to extensive use by the people of Yemen and neighboring countries. Consequently, the World Health Organization and the United Nations have initiated socioeconomic and biological investigations into the

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use of Khat [1 - 3]. Many problems are engendered by the repeated self-administration of Khat. Physiological problems include decreased food intake resulting in malnutrition, gastrointestinal disorders (e.g., gingivitis, gastritis, constipation), increased respiration and blood pressure, headaches, and myocardial insufficiency. Socioeconomic problems arise from expenditure on a nonessential commodity and loss of foreign currency due to decreased cultivation of exportable crops in favor of the non-exported Khat plant [1, 4].

The structure of *d,l*-cathinone (1-α-aminopropiophenone) was first described in 1978 [5]. Subsequent behavioral pharmacological studies have indicated that this drug is *d*-amphetamine-like in its ability to maintain self-administration [6, 7], increase activity [8, 9], decrease food intake [9], and reduce food-reinforced responding [7]. Furthermore, cross-tolerance to the food intake decreasing effects of *d*-amphetamine and *d,l*-cathinone has been demonstrated [7, 9, 10]. In a drug discrimination paradigm, *d,l*-cathinone administration also results in responding on a lever previously reinforced following *d*-amphetamine administration [11]. The present study was undertaken to compare the neurochemical effects of *d,l*-cathinone with those of *d*-amphetamine. We now report that *d,l*-cathinone is similar to *d*-amphetamine in blocking dopamine uptake, inducing dopamine release and producing selective long-lasting toxic effects on dopamine neurons in the central nervous system.

Methods

Male Sprague-Dawley rats weighing approximately 250g were housed individually with free access to food and water. The influence of *d,l*-cathinone and *d*-amphetamine on the in vitro uptake of ^{3}H-DA was determined using rat neostriatal synaptosomes as described in detail elsewhere [12]. Briefly, drugs were dissolved in various concentrations in Krebs Ringer phosphate (KRP) buffer (pH 7.4 at 37 °C) and added in 0.1 ml aliquots to tubes containing 3.7 ml of buffer with ^{3}H-dopamine at a concentration of 0.2 μM. After adding 0.2 ml of the crude neostriatal synaptosomal suspension, tubes were vortexed, incubated for 5 min at 37 °C and then ^{3}H-dopamine accumulation was measured in the presence of various drug concentrations.

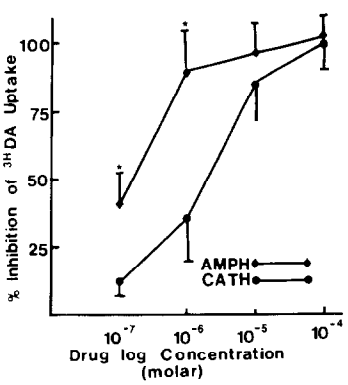
Release experiments were conducted using neostriatal synaptosomes preloaded with ^{3}H-DA during a 10 min incubation at 37 °C in KRP buffer containing 0.2 μm ^{3}H-DA. Loaded synaptosomes were then returned to 0 - 4 °C. Aliquots (0.4 ml) of the preloaded synaptosomal suspension were then added to tubes containing 3.5 ml KRP buffer and 0.1 ml of the test drug (dissolved in buffer) or buffer alone. Tubes were vortexed, incubated for 10 min at 37 °C and returned to ice. Remaining synaptosomal ^{3}H-dopamine was separated by rapid filtration under vacuum through GF/A filters. Filters were rinsed with 5 ml 0.9% saline 3 times and transferred to scintilla-

tion vials containing 10 ml of Quantaflor (Mallinckrodt). Filter-bound radioactivity was measured by liquid scintillation spectroscopy at an efficiency of 15 - 23% as determined by the external standard method. Release was defined as the decrease in filter-bound radioactivity resulting from the presence of drug in the incubation mixture.

The possibility of long-lasting toxic changes in in vivo neuronal monoamine storage or uptake was determined in groups of rats that received 100 mg/kg of *d,l*-cathinone hydrochloride on the first day and 200 mg/kg per day on the next three days. The total daily dose was divided into two equal doses administered subcutaneously approximately 12 hours apart. Control rats received an equal volume of vehicle. All rats were allowed to survive at least two weeks after the last injection. Monoamine levels, (dopamine (DA), norepinephrine (NE) and serotonin (5-HT)) in various brain regions were determined by high-performance liquid chromatography with electrochemical detection as described elsewhere [13]. The uptake of ^{3}H-DA by neostriatal synaptosomes prepared from *d,l*-cathinone treated rats was determined two weeks after the last drug injection as detailed above. All dissections were performed as previously described [12].

Results

Both *d*-amphetamine and *d,l*-cathinone inhibited uptake of ^{3}H-DA by neostriatal synaptosomes (Fig. 1). The fact that the dose-effect function of *d*-amphetamine is to the left of that of *d,l*-cathinone suggests that *d*-amphetamine is more potent than *d,l*-cathinone. At very high concentrations (10^{-4}M), both *d*-amphetamine and *d,l*-cathinone completely inhibited ^{3}H-DA accumulation. *d*-Amphetamine was also more potent and effective in releasing ^{3}H-DA than *d,l*-cathinone (Fig. 2). At the highest concentration



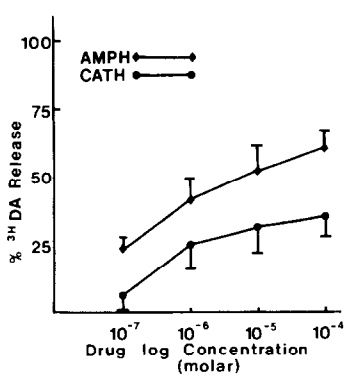


Fig. 1. ^{3}H-DA uptake inhibition induced by *d*-amphetamine (AMPH) and *d*-cathinone (CATH). *$p < 0.05$; Mann-Whitney U-test.

Fig. 2. ^{3}H-DA release induced by *d, l*-amphetamine (AMPH) and *d, l*-cathinone (CATH).

tested, *d*-amphetamine induced efflux of approximately 50% of synaptosomal radioactivity, whereas *d,l*-cathinone released only approximately 30%.

Rats treated with *d,l*-cathinone for four days and killed 2 weeks later displayed significant depletions of DA in the caudate nucleus, midbrain and telencephalon (Table 1). No changes in DA levels in the hypothalamus and

TABLE 1

Monoamine levels of brain regions of rats treated with cathinone[1]

	Caudate	Midbrain	Telencephalon	Hypothalamus	Pons-Medulla
Control	10.86	0.17	0.30	0.41	0.04
	(0.69)	(0.02)	(0.04)	(0.07)	(0.41)
DA					
Cathinone	5.41*	0.11*	0.17*	0.50	0.04
	(0.96)	(0.01)	(0.04)	(0.05)	(0.01)
Control	X	0.96	0.22	2.59	0.78
NE		(0.16)	(0.01)	(0.36)	(0.04)
Cathinone	X	1.02	0.23	2.42	0.81
		(0.19)	(0.03)	(0.41)	(0.05)
Control	0.56	0.22	0.10	0.32	0.15
	(0.11)	(0.02)	(0.01)	(0.03)	(0.01)
5-HT					
Cathinone	0.42	0.16	0.08	0.29	0.11
	(0.03)	(0.02)	(0.01)	(0.03)	(0.01)

[1] Rats were treated with cathinone or saline (control) for 4 days and allowed to survive for 2 weeks before they were killed for assay. Monoamine values are in μg/g. *$p < 0.05$; Mann Whitney U-test.

DA = dopamine; NE = norepinephrine; 5-HT = serotonin.

pons-medulla nor in the serotonin or norepinephrine levels in any brain region were found in *d,l*-cathinone-treated rats (Table 1). *d,l*-Cathinone-treated rats also exhibited a significant decrease in the V_{max} of ^{3}H-DA uptake, with no alteration in the apparent K_m (Table 2).

Discussion

The present study has shown that *d,l*-cathinone resembles *d*-amphetamine both in terms of its acute effect on in vitro DA uptake and release and its long-lasting toxic action on neostriatal DA terminals. Like *d*-amphetamine, *d,l*-cathinone inhibits DA uptake and stimulates DA release in synaptosomal preparations. Although the present results do not allow for potency comparisons between *d,l*-cathinone and *d*-amphetamine (since *d,l*-cathinone and *d*-amphetamine were tested) Zelger and Carlini [14], using a rat neostriatal slice preparation, have recently reported that racemic cathinone is

TABLE 2

K_m and V_{max} for control and cathinone treated rats

	K_m (μM)	V_{max} (DPM ^{3}H-DA/mg tissue)
Control	0.20 (0.03)	5.75×10^4 (0.39)
Cathinone	0.14 (0.03)	$1.84 \times 10^{4**}$ (0.38)

Rats were treated with cathinone or saline (control) for 4 days and allowed to survive for 2 weeks before they were killed for assay.
**$p < 0.05$, Student's t-test.

less potent than racemic amphetamine in blocking DA uptake. These same investigators also reported that *d,l*-cathinone is less potent and efficacious than *d,l*-amphetamine in releasing DA. Based on these and the present findings, it seems reasonable to conclude that cathinone's effects on DA systems are weaker than those of amphetamine and that cathinone augments DA synaptic transmission by retarding DA reuptake inactivation and/or enhancing DA release.

The foregoing observations prompt one to speculate that the similar behavioral effects of cathinone and amphetamine (see Introduction) may stem from a common neurochemical mechanism of action of these two drugs. That is, both drugs may exert their characteristic behavioral effects through brain DA neurons. In this regard, it will be of interest to determine whether 6-hydroxydopamine (6HDA) lesions which destroy DA neurons and which abolish many of the behavioral effects of *d*-amphetamine [15, 16], also abolish the behavioral effects of *d,l*-cathinone.

This study has also demonstrated that *d,l*-cathinone mimics *d*-amphetamine's long-term toxic effect on brain DA terminals. Specifically, it has been shown that repeated high doses of *d,l*-cathinone produce a long-term loss of neostriatal DA and its synaptosomal uptake sites (Tables 1 and 2). Previous results from this [17] and other [18] laboratories have indicated that these DA neurochemical deficits are most likely reflective of DA nerve terminal destruction. The regional distribution of DA terminal damage following *d,l*-cathinone is similar to that observed after *d*-amphetamine. The most affected DA terminal projection appears to be the nigrostriatal, whereas the least affected DA terminals belong to the tubero-infundibular DA system (Table 1). Importantly, the DA neurotoxic effect of both *d,l*-cathinone and *d*-amphetamine is selective, as evidenced by the lack of long-term effect on regional NE and 5-HT levels (Table 1 and ref. 19). Thus, the neurotoxic action of *d,l*-cathinone also parallels that of *d*-amphetamine.

In summary, the present study has demonstrated that *d,l*-cathinone resembles *d*-amphetamine both in terms of its in vitro effects on DA uptake and release and its selective toxic effects on central DA nerve terminals.

The relevance of the latter finding to the problem of Khat abuse remains to be determined. In this study high doses of *d,l*-cathinone were needed to induce DA neurotoxicity when the dosing interval was 12 hours. While it is doubtful that humans ever ingest such high doses, it is possible that when the dosing interval is shortened, lower *d,l*-cathinone doses induce DA neurotoxicity. This appears to be the case with *d*-amphetamine [20]. Also, differences in the half-life of cathinone in the rat and man may render man more sensitive to the toxic effects of cathinone. Therefore, the present results should at least raise the possibility of DA nerve terminal damage following cathinone abuse.

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