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Methcathinone: A New and Potent Amphetamine-Like Agent

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GLENNON, R A , M YOUSIF, N NAIMAN AND P KALIX *Methcathinone A new and potent amphetamine-like agent* PHARMACOL BIOCHEM BEHAV **26**(3) 547–551, 1987 —The purpose of the present investigation was to examine the effect of N-monomethylation of phenylisopropylamine derivatives on amphetamine-like activity In tests of stimulus generalization using rats trained to discriminate 1 0 mg/kg of (+)-amphetamine from saline, the N-monomethyl derivatives of 1-(X-phenyl)-2-aminopropane, where X=2,4-dimethoxy (2,4-DMA), 3,4-dimethoxy (3,4-DMA), 2,4,5-trimethoxy (2,4,5,-TMA), and 2-methoxy-4,5-methylenedioxy (MMDA-2), did not produce amphetamine-appropriate responding at the doses evaluated However, the N-monomethyl derivative of cathinone (i e , methcathinone), like cathinone, resulted in stimulus generalization Further studies with this agent revealed that (a) in the amphetamine-trained animals, methcathinone (ED50=0 37 mg/kg) is more potent than racemic cathinone or racemic amphetamine (ED50=0 71 mg/kg in both cases), (b) methcathinone is capable of inducing release of radioactivity from [^{3}H]dopamine-prelabeled tissue of rat caudate nucleus in a manner similar to that observed with cathinone, amphetamine, and methamphetamine, and (c) methcathinone is more potent than cathinone as a locomotor stimulant in mice as determined by their effect on spontaneous activity The results of the present study provide evidence for a structural analogy between the prototypic psychostimulants amphetamine/methamphetamine and cathinone/methcathinone, and lend further support to the concept that amphetamine and cathinone correspond in their pharmacological effects

Cathinone Methcathinone Drug discrimination Central stimulants MDA Amphetamine Methamphetamine Release Spontaneous activity MDMA

PHENYLISOPROPYLAMINES (PIAs) can produce a variety of pharmacological effects depending upon the presence and location of pendant substituent groups [5,14]. The parent compound of this family of agents, phenylisopropylamine itself (i.e , amphetamine), is a psychostimulant, whereas certain methoxy-substituted derivatives, for example 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), are hallucinogenic in humans [14] Using a drug discrimination paradigm with rats trained to discriminate either (+)-amphetamine (AMPH) or DOM from saline, we have examined a large series of PIAs in tests of stimulus generalization in order to determine which agents produce AMPH-like, and which produce DOM-like, effects (e.g., [5, 9, 16]). In the course of our studies, we have noted that N-monomethylation of those PIAs that produce DOM-like effects reduces their potency, whereas N-monomethylation of amphetamine (to afford methamphetamine) has little effect on AMPH-like potency. To confirm and extend these results, it was of interest to investigate the activity of several additional N-monomethyl PIA analogs in amphetamine-trained rats

Because the stimulant effects of amphetamine and related PIA stimulants are generally explained by an induction of release at CNS catecholamine storage sites [10,12], comparisons were planned, for any N-methyl derivatives that produced AMPH-stimulus generalization, with regard to their capacity to induce release at dopaminergic nerve terminals

Such studies should provide us with a better understanding of the effect of N-methylation on amphetamine-like activity and might have a direct bearing on the action of structurally-related drugs of abuse such as MDA and its N-monomethyl derivative MDMA (see below). More importantly, information will be obtained with regard to the relationship between molecular structure, amphetamine-like activity, and mechanism of action.

METHOD

Rationale for Drugs Employed

Cathinone was selected for evaluation because it is one of the few PIA derivatives that retains potent amphetamine-like character [16], furthermore, its N-monomethyl analog has not been previously pharmacologically characterized. In addition, we also wanted to examine the effect (in amphetamine-trained animals) of N-monomethylation of examples of two other types of agents: (1) PIAs that produce DOM-like effects [i e., 1(2,4-dimethoxyphenyl)-2-aminopropane (2,4-DMA), 1-(2,4,5-trimethoxyphenyl)-2-aminopropane (2,4,5-TMA), and 1-(2-methoxy-4,5-methyl-

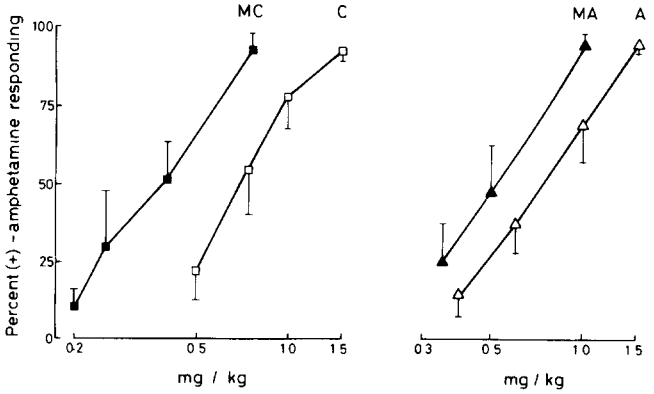

FIG 1 Effect of methcathinone (MC), cathinone (C), methamphetamine (MA), and amphetamine (A) in rats trained to discriminate 1 0 mg/kg of (+)-amphetamine from saline All four agents were racemates, each point reflects the results obtained with four animals The Y axis represents the number of responses (as a percent of total responses) made on the amphetamine-appropriate lever

enedioxyphenyl)-2-aminopropane (MMDA-2)] and (2) a structurally-related PIA that produces neither DOM- nor AMPH-like effects (i e , 3,4 -DMA) [5,9]

The purpose of examining 2,4-DMA and 2,4,5-TMA is that while these agents produce DOM-like effects (i.e., result in DOM-stimulus generalization), they produce partial generalization in AMPH-trained animals, suggesting that they possess a stimulant component of action [9]. This is consistent with the results of studies in humans, though hallucinogenic, these agents produce considerable central stimulation [14] MMDA-2, the 2-methoxy analog of 1-(3,4-methylenedioxyphenyl)-2-aminopropane (3,4-MDA, "MDA"), reportedly produces subjective effects in humans similar to those of MDA [14], in humans, MDA produces both hallucinogenic and central stimulant effects [14] Thus, N-monomethylation of these agents might enhance their AMPH-like character in the same manner as N-monomethylation of MDA, resulting in MDMA ("Ecstasy"), enhancing its AMPH-like character [5, 7, 8]

3,4-DMA was examined for several reasons although it does not result in AMPH-stimulus generalization [5] and is not a locomotor stimulant in mice [15] it appears to produce some amphetamine-like effects in rodents [1], it constitutes an "opened" methylenedioxy bridge analog of MDA, and it results in stimulus generalization in MDA-trained animals [8] Here too, N-monomethylation might enhance AMPH-like character

Drug Discrimination Studies

Six male Sprague-Dawley rats (ca 250–300 g) were used in these studies. The animals were trained (15-min training session) to discriminate intraperitoneal injections (15-min pre-session injection interval) of 1 0 mg/kg of (+)-amphetamine sulfate from vehicle (sterile 0.9% saline) under a variable interval 15-sec schedule of reinforcement for food (sweetened powdered milk) reward. Standard two-lever operant chambers (Coulbourn Instruments model E10-10) were used The exact training procedure followed that which we have already described in detail elsewhere [9]. Daily training sessions were conducted with (+)-amphetamine (1 0 mg/kg)

TABLE 1

RESULTS OF STIMULUS GENERALIZATION STUDIES USING ANIMALS TRAINED TO DISCRIMINATE 1 0 mg/kg OF (+)-AMPHETAMINE FROM SALINE

Agent	Dose*	N†	Responding‡	Response Rate§
(+)AMPH	1 0	6/6	93% (3)	13 3 (2 2)
Saline	(1 ml/kg)	6/6	10% (5)	13 7 (1 8)
2,4-DMMA	5 0	4/4	16% (10)	12 2 (1 9)
	8 0	3/4	8% (8)	4 7 (1 5)
	10 0	2/4	27% (3)	5 6 (2 8)
3,4-DMMA	4 0	4/4	1% (1)	15 5 (4 1)
	6 0	3/3	2% (1)	5 1 (1 7)
	8 0	0/4	—	
2,4,5-TMMA	2 0	4/4	0%	13 2 (3 7)
	4 0	4/4	1% (1)	18 6 (1 9)
	6 0	3/3	5% (3)	14 9 (4 3)
	10 0	4/4	8% (6)	4 9 (0 9)
MMDMA	2 0	4/4	10% (8)	20 9 (6 1)
	4 0	3/4	0%	11 0 (5 5)
	6 0	4/4	9% (8)	9 9 (4 1)
	7 0	0/4	—	
	8 0	0/4	—	
	10 0	1/4	—	

*Dose in mg/kg †N=Number of rats responding/number to receive drug ‡Number of responses (as a percent of total) made on the amphetamine-appropriate lever, followed by ±SEM Data obtained during 2 5-min extinction session §Mean responses/min followed by ±SEM Data obtained during 2 5-min extinction session — Disruption of behavior (i e , no responding)

or saline (1 0 ml/kg), on every fifth day, learning was assessed during an initial 2 5-min non-reinforced (extinction) period followed by a 12 5-min training session Data collected during the extinction period included responses per min (i e , response rate) and number of responses on the (+)-amphetamine-designated lever (expressed as percent of total responses)

Tests of stimulus generalization were conducted in order to determine if the N-methyl analogs would substitute for the (+)-amphetamine-stimulus, racemic amphetamine, methamphetamine, and cathinone were included for purposes of comparison. During this phase of the study, maintenance of the (+)-amphetamine/saline discrimination was insured by continuation of the training sessions. On one of the two days before a generalization test, half the animals would receive (+)-amphetamine and the other half would receive saline, after a 2 5-min extinction session, training was continued for 12 5 min Animals not discriminating drug from saline (i e , animals not making >80% of their responses on the amphetamine-designated lever after administration of 1 0 mg/kg of (+)-amphetamine, or making >20% of their responses on this same lever after administration of saline) were excluded from the subsequent generalization test session During the investigations of stimulus generalization, test sessions were interposed amongst the training sessions The animals were allowed 2 5 min to respond under non-reinforcement conditions, the animals were then removed from the operant chambers and returned to their individual home cages An odd number of training sessions (usually five, but never less than three) separated any two generalization test sessions Doses of the challenge drugs were

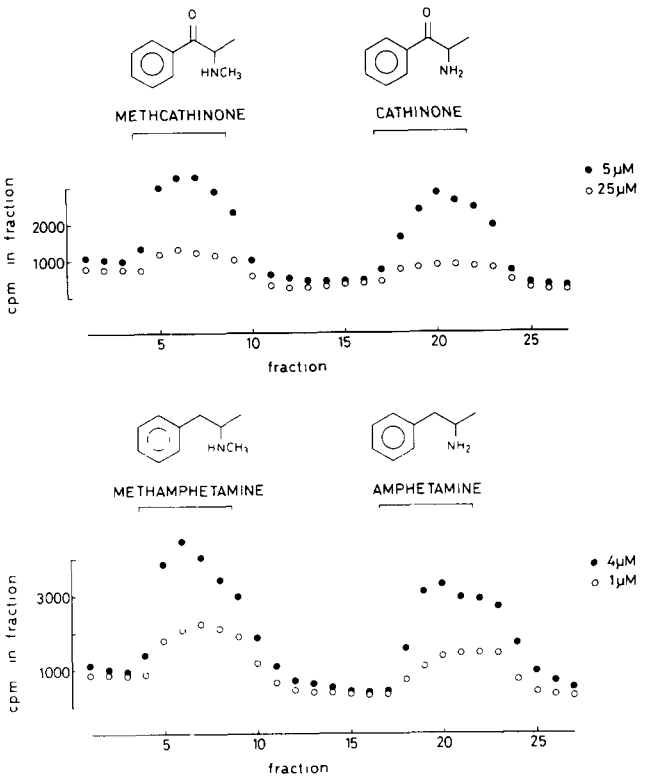

FIG 2 Effect of racemic methcathinone as compared to S(−)-cathinone (upper panel), and of S(+)-methamphetamine as compared to S(+)-amphetamine (lower panel), on the release of radioactivity from rat caudate nucleus prelabeled with [^{3}H]dopamine Tissue samples were exposed during 15 min to a given concentration of the N-methyl compounds and then, after an interval of 24 min, to the same concentration of the corresponding des-methyl compound Each fraction corresponds to 3 min of efflux

administered in a random sequence using a 15-min presession injection interval to, routinely, groups of three or four rats Stimulus generalization was said to have occurred when the animals, after given a dose of challenge drug, made 80% or greater of their responses on the (+)-amphetamine-appropriate lever. Animals making fewer than 5 total responses during the entire 2.5-min extinction session were considered as being disrupted Where generalization occurred, ED50 values were calculated from the dose-response data by method of Finney [3] The ED50 values are doses at which the animals would be expected to make 50% of their responses on the drug-designated lever All solutions were prepared fresh daily in sterile 0 9% saline and all injections were made via the intraperitoneal route A 15-min presession injection interval was used throughout

Locomotor Activity Studies

Male (ICR) mice were acclimated in individual photocell activity chambers for 20 min and were then removed for an intraperitoneal injection of either saline or drug Each mouse was returned to a chamber and, 20 min later, interruptions of the photocell beam were recorded for a period of 10 min The apparatus and the procedure have been previously reported in detail [13]

Release Experiments

Samples of rat striatal tissue, dissected from the head of the caudate nucleus, were cut with a scalpel into cubes measuring less than 1 mm in each dimension. The tissue was incubated for 20 min at 37 degrees C 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, 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 continuously oxygenated with a mixture of 95% oxygen-5% carbon dioxide (pH 7.3) At the end of the labeling period the tissue samples were transferred to a perspex perfusion chamber of approximately 0 3 ml volume. They were then superfused at a rate of 0.5 ml/min with the above solution at 37 degrees C. The spontaneous efflux of radioactivity from the tissue stabilized during an initial washing period of one hour to a steady background which was not altered by the flow stop which was necessary for switching to a modified superfusion medium. The test substances were dissolved in distilled water a few minutes prior to use and they were added at a 400-fold concentration at least to the superfusion medium. During the experimental period, the superfusate was collected in successive 3-min fractions and its tritium content was determined by scintillation counting. The figures show typical experiments with results representative of several analogous observations

Drugs

(+)-Amphetamine was used as its sulfate salt, all other agents were used as their hydrochloride (HCl) salts. S(−)-cathinone HCl and racemic cathinone HCl were obtained as gifts from the United Nations Narcotics Laboratory. Racemic amphetamine HCl, racemic methamphetamine HCl, racemic N-monomethyl-1-(3,4-dimethoxyphenyl)-2-aminopropane HCl (3,4-DMMA), and (+)-methamphetamine had been previously synthesized in our laboratory and were available from an earlier study. With the exception of methcathinone, all of the new N-methyl derivatives were prepared by acylation of the corresponding primary amine with ethyl chloroformate, followed by reduction of the resultant carbamate esters with lithium aluminum hydride, ethereal solutions of the free bases were treated with HCl gas to precipitate the HCl salts which were subsequently recrystallized from absolute ethanol (layering with anhydrous ether when necessary) Infrared and proton nuclear magnetic resonance spectra are consistent with the assigned structures Melting points (degrees C) and results of microanalysis are as follows (calculated/found): N-mono methyl-1-(2,4-dimethoxyphenyl)-2-aminopropane HCl (2,4-DMMA) (mp=128–130), $C_{12}H_{19}NO_2 \cdot HCl \cdot 1/4H_2O$ (C 57 59/57 46%, H 8.25/8.15%, N· 5.60/5 91%); N-monomethyl-1-(2-methoxy-4,5-methylenedioxyphenyl)-2-aminopropane HCl (MMDMA) (mp=166–167), $C_{12}H_{17}NO_3 \cdot HCl$ (C 55.49/55 55%, H 6.99/7.02%, N· 5 39/5.36%); N-monomethyl-1-(2,4,5-trimethoxyphenyl)-2-aminopropane HCl (2,4,5-TMMA) (mp=151–153), $C_{13}H_{21}NO_3 \cdot HCl$ (C 56 62/56 69%, H 8.04/8.07%, N 5.08/5.05%) Racemic N-monomethylcathinone HCl ("methcathinone"), melting point 185–187 degrees C (lit mp 183 [4]), was prepared by the chromic acid oxidation of (−)-norephedrine in a manner similar to that of a published procedure [2] Even though optically active starting material was used, racemization ap-

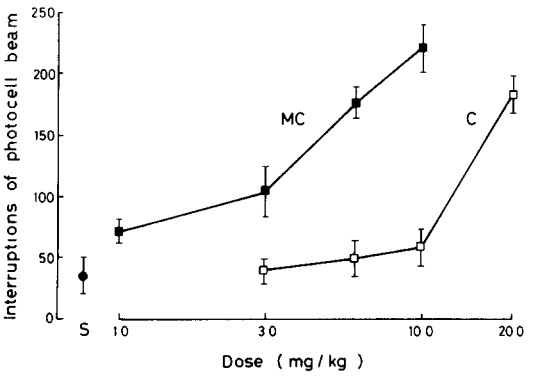

FIG 3 Effect of methcathinone (MC) and racemic cathinone (C) on spontaneous locomotor activity S=saline control (n=54) Each dose of drug was evaluated in 12 mice (except that n=6 for the 10 mg/kg dose of cathinone), results are expressed as means±SEM

pears to have occurred during oxidation in that the product is optically inactive as determined by polarimitry

RESULTS

Drug Discrimination Studies

Tests of stimulus generalization with the challenge drugs were conducted once the animals had been trained to discriminate (+)-amphetamine from saline. The (+)-amphetamine-stimulus generalized to racemic amphetamine [ED50=0 71 (0.43–1.15) mg/kg], cathinone (ED50=0 71 (0.46–1.03) mg/kg], methamphetamine [ED50=0.49 (0 31–0.78) mg/kg], and methcathinone [ED50=0.37 (0.22–0.61) mg/kg] (Fig. 1) The (+)-amphetamine stimulus did not generalize to 2,4-DMMA, 2,4,5-TMMA, nor to MMDMA (Table 1) At 10 mg/kg, i.e., at more than twenty times the ED50 dose of racemic methamphetamine, 2,4-DMMA and 2,4,5-TMMA produced less than 30% amphetamine-appropriate responding, because the animals' response rates were already depressed by more than 50% at the highest doses tested, higher doses of these agents were not evaluated. 3,4-DMMA and MMDMA produced saline-like effects at 6 mg/kg and, at higher doses, produced disruption of behavior (Table 1)

Release Experiments

Because methcathinone was the only new N-methyl derivative that produced (+)-amphetamine-appropriate responding, it was the only derivative examined with respect to a releasing effect on dopaminergic nerve terminals, in these experiments, the effects of racemic methcathinone were compared with those of S(−)-cathinone, S(+)-amphetamine, and S(+)-methamphetamine When slices of rat caudate nucleus that had been prelabeled with [^{3}H]dopamine were superfused with a solution containing low concentrations of methcathinone, a rapid and reversible increase of the release of radioactivity occurred. The reaction was dose-dependent Subsequent superfusion of the preparation with S(−)-cathinone at the same concentration caused an increase of efflux that was of similar amplitude, the two agents were also found to be equipotent when they were tested at a somewhat higher concentration (Fig 2, upper panel) Analogous observations were made when S(+)-amphetamine and S(+)-methamphetamine were compared under the same experimental conditions (Fig 2, lower panel) Similar results were obtained when the agents were tested in inverse order

Locomotor Activity Studies

The effects of methcathinone and racemic cathinone on spontaneous activity in mice are shown in Fig. 3. At doses of 3 to 10 mg/kg, the effect of cathinone was similar to that produced by saline (i e , baseline responding) At comparable doses, methcathinone produced a dose-related increase in locomotor activity (Fig 3)

DISCUSSION

N-Monomethylation of the hallucinogenic PIAs 2,4-DMA, 2,4,5-TMA, and MMDA-2 (to afford 2,4-DMMA, 2,4,5-TMMA, and MMDMA, respectively) did not result in amphetamine-like agents (i e , the amphetamine-stimulus did not generalize to any of the three agents at the doses evaluated) (Table 1) Because 2,4-DMA and 2,4,5-TMA are fairly potent hallucinogenic agents to begin with [14], it is likely that their N-monomethyl derivatives might still possess some residual hallucinogenic activity, and this might account for the decrease in response rates and/or the disruption of behavior noted at the higher doses. Likewise, the amphetamine-stimulus did not generalize to 3,4-DMMA, the N-monomethyl derivative of 3,4-DMA We had earlier demonstrated amphetamine-stimulus generalization to MDA and its monomethyl derivative MDMA, due to the close structural resemblance of 3,4-DMA and 3,4-DMMA with MDA and MDMA, we must conclude that there is something unique about the methylenedioxy bridge regarding its contribution to amphetamine-like stimulus effects. Nevertheless, the presence of the methylenedioxy group does not assure amphetamine-like properties in that MMDMA produces saline appropriate responding even at nearly 10 times the ED50 dose of racemic amphetamine

As is the case with amphetamine, however, N-monomethylation of cathinone does result in an active agent (i.e , methcathinone) both with respect to amphetamine-like stimulus effects (Fig 1) and release from dopamine terminals (Fig 2) Because neither of these experiments provide direct information as to central stimulant properties, methcathinone and cathinone were examined with regard to their effect on locomotor activity of mice Racemic cathinone has previously been shown to be a fairly potent locomotor stimulant [6, 11, 15], in the present study, methcathinone was found to be several times more potent than cathinone itself (Fig 3)

The present observations are of interest for several reasons (1) N-monomethylation enhanced (or at least did not decrease) the behavioral potency only of an agent with demonstrated amphetamine-like effects (i e , cathinone), (2) unlike methcathinone, other derivatives of cathinone are, with regard both to behavioral activity and release from dopamine terminals, far less potent than the parent compound [12], (3) the present findings provide a structural analogy between the prototypic psychostimulants amphetamine/methamphetamine and cathinone/methcathinone Thus, the cathinone molecule shares not only the pharmacological profile of amphetamine [11], but also shares the features that the S-isomer is more potent than the R-enantiomer [5, 6, 10, 16] and that N-monomethylation affects activity in a similar manner

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