Pharmacology Biochemistry & Behavior, Vol. 35, pp. 517–521. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Stimulus Properties of a New Designer Drug: 4-Methylaminorex ("U4Euh")

RICHARD A. GLENNON AND BRYAN MISENHEIMER

Department of Medicinal Chemistry, School of Pharmacy
Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0581

Received 24 July 1989

GLENNON, R. A. AND B. MISENHEIMER. *Stimulus properties of a new designer drug: 4-Methylaminorex ("U4Euh")*. PHARMACOL BIOCHEM BEHAV **35**(3) 517–521, 1990.—Like other phenylisopropylamine derivatives, 4-methylaminorex is a central stimulant. The *cis* isomer of 4-methylaminorex ("U4Euh"; "ICE") has appeared on the clandestine market as a novel designer drug and was recently classified as a Schedule I substance. In the present investigation, the stimulus properties of racemic *cis*, racemic *trans*, and all four individual optical isomers of 4-methylaminorex were examined in rats trained to discriminate 1 mg/kg of *S*(+)amphetamine sulfate from saline. The *S*(+)amphetamine stimulus generalized to all of the agents investigated and the relative potencies of the optical isomers (followed by ED_{50} values) were as follows: *trans*(4*S*,5*S*) (0.25 mg/kg) > *cis*(4*S*,5*R*) (1.2 mg/kg) = *cis*(4*R*,5*S*) (1.5 mg/kg) > *trans*(4*R*,5*R*). The *trans*(4*R*,5*R*) isomer did not completely substitute for *S*(+)amphetamine unless a longer (i.e., 60-min) presession injection interval was used, suggesting that it has a longer duration of onset than the other isomers of 4-methylaminorex. The results, which are consistent with established structure-activity relationships, suggest that the *trans*(4*S*,5*S*) isomer (which has not been scheduled) is similar in potency to (+)amphetamine (ED_{50} = 0.4 mg/kg) and is more potent than either of the *cis* isomers.

Amphetamine 4-Methylaminorex Stimulants Discrimination Drug abuse

AMONGST the numerous phenylisopropylamines that are capable of producing central stimulant effects, one of the best known examples is amphetamine. Amphetamine is an interesting agent from the standpoint that, although many analogs have been prepared and evaluated over the past 50 years, few structural modifications of amphetamine retain its activity and potency (5). N-Monomethylation (e.g., methamphetamine) and introduction of a benzylic keto group (e.g., cathinone, methcathinone) are two of the few alterations that result in potent agents (5,7). Stereochemistry also plays a role, albeit a relatively small one, in determining the potency of these agents. For example, *S*(+)amphetamine (see Fig. 1 for structures) is about 3 to 5 times more potent than *R*(−)amphetamine as a central stimulant (5).

The 2-amino-4-methyl-5-phenyloxazoline 4-methylaminorex, which possesses a phenylisopropylamine moiety embedded within its cyclic structure, recently appeared on the clandestine market as a new designer drug. *Cis*(±)-4-methylaminorex ("U4Euh," "ICE") has been misrepresented by drug dealers as cocaine and methamphetamine, and has already been responsible for at least one fatality (1). As of this year, *cis* 4-methylaminorex is classified as a Schedule I substance (3). The *cis* racemate had been earlier examined as a potential anorectic agent (McN 822) (10,11); it is an indirect acting sympathomimetic agent (9,14) and has been reported to possess central stimulant properties qualitatively similar to those of amphetamine (11,13). 4-Methylaminorex exists as a pair of geometric isomers (i.e., *cis* and *trans*) and each isomer consists of a pair of optical isomers. That is, there exists four optical isomers of this agent (Fig. 2). Although there is relatively little information on the *cis* and *trans* racemates, even less is known about the individual optical isomers. Klein and co-workers (8) recently synthesized all four isomers and we saw this as a unique opportunity (a) to compare the activity of the four isomers in the same study, and (b) to apply established structure-activity relationships (SARs) to make predictions regarding the relative potencies of these isomers. Using rats trained to discriminate *S*(+)amphetamine sulfate from saline, we examined the stimulus effects of the *cis* and *trans* racemates as well as the individual optical isomers of 4-methylaminorex.

METHOD

Nine male Sprague-Dawley rats (200–300 g) were trained to discriminate intraperitoneal (IP) injections of 1.0 mg/kg of (+)amphetamine sulfate from 1.0 ml/kg of sterile 0.9% saline in a manner exactly as previously described (6). In brief, the animals were first trained to respond on the levers of a standard two-lever operant chamber (Coulbourn Instruments model E10-10) for food (sweetened powdered milk) reward and were then trained to discriminate (+)amphetamine from saline using a variable interval 15-sec schedule of reinforcement. For five of the animals, responses on the right lever were reinforced after administration of amphetamine; for the remaining animals, responses on the left lever were reinforced after administration of amphetamine. All drugs were administered via the IP route 15 min prior to testing (i.e., a 15-min presession injection interval was used, except where noted). During the stimulus generalization studies, maintenance of the drug/saline discrimination was insured by continuing the training sessions throughout this period. Training sessions were conducted with drug or saline on the four days prior to a

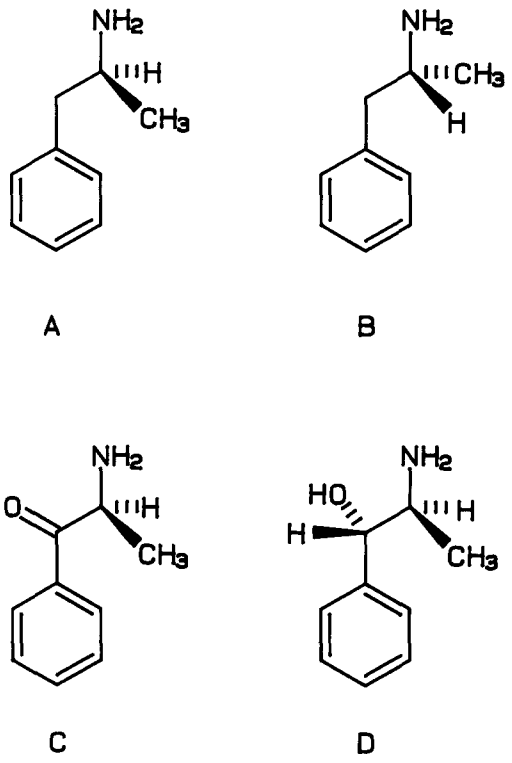

FIG. 1. Chemical structures of *S*(+)amphetamine (A), *R*(−)amphetamine (B), *S*-cathinone (C), and (+)norpseudoephedrine (1*S*,2*S*) (D) showing stereochemical relationships.

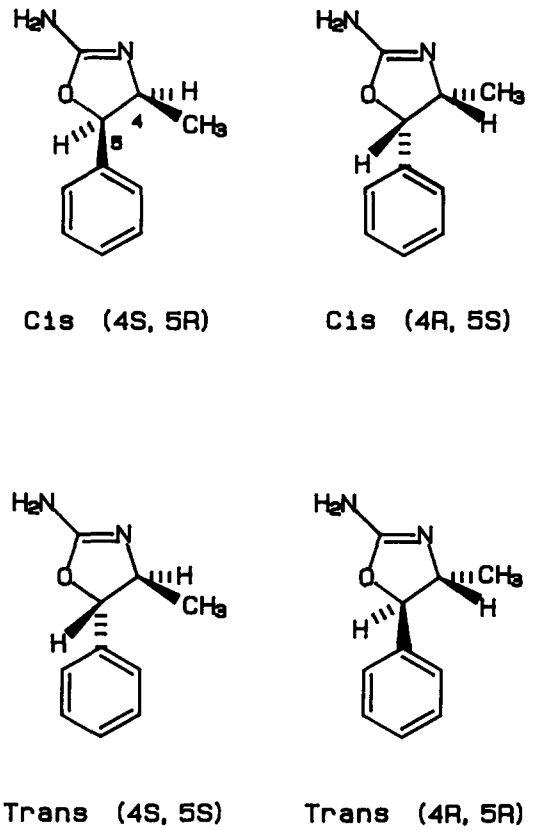

FIG. 2. Chemical structures of the four optical isomers of 4-methylaminorex.

generalization test; that is, animals would be administered either training drug or saline and the proper responses were reinforced during a 15-min training period. Once per week, the animals' learning would be assessed by allowing the animals to respond under each of the two conditions during a nonreinforced 2.5-min extinction session, followed by a reinforced 12.5-min training session. Animals not making 80% or greater of their responses on the drug-appropriate lever after administration of the training dose of the training drug, or making more than 20% of their responses on the same lever after administration of 1 ml/kg of saline, during the 2.5-min portion of the session, were not used in that particular week's generalization test session. In the generalization test sessions, animals administered doses of 4-methylaminorex isomers were allowed to respond under extinction conditions for 2.5 min and were then returned to their individual home cages. Where longer pressession injection intervals were employed, animals were administered drug and then returned to their home cages until just prior to testing. No animal received more than a single dose of challenge drug per week. Doses of agents were normally administered in a random sequence with the proviso that only lower doses of a given agent would be examined once a dose had been found to result in stimulus generalization. Criterion for stimulus generalization was ≥80% of total responses on the drug-appropriate lever; disruption of behavior was considered to have occurred if an animal made less than 5 responses during the 2.5-min extinction session. Where stimulus generalization occurred, ED_{50} values were calculated by the method of Finney (4) and represent the approximate dose at which the animals would be expected to make 50% of their responses on the drug-appropriate lever.

S(+)Amphetamine sulfate was purchased from Sigma (St. Louis, MO) and the isomers of 4-methylaminorex were a gift from Dr. Robert Klein of the DEA Special Testing and Research Laboratories. With the exception of the *cis* racemate (hydrochloride salt), all of the isomers were used as their free bases; these bases were dissolved in one equivalent of 0.01 N or 0.1 N hydrochloric acid solution before dilution to the proper concentrations with sterile 0.9% saline. All solutions were made fresh daily, and all drugs were administered via the IP route.

RESULTS

Both the *cis* and *trans* racemic mixtures resulted in *S*(+)amphetamine-stimulus generalization, with the *trans* racemate (ED_{50} dose = 0.41 mg/kg; 2.3 μmoles/kg) being three times more potent than the *cis* racemate (ED_{50} dose = 1.56 mg/kg; 7.3 μmoles/kg) (Table 1). Both agents are somewhat less potent than (+)amphetamine (ED_{50} value = 0.42 mg/kg; 1.7 μmoles/kg). Of the individual isomers of 4-methylaminorex, three resulted in stimulus generalization (using the standard 15-min presession injection interval) with the *trans*(4*S*,5*S*) isomer being the most potent (ED_{50} dose = 0.25 mg/kg; 1.4 μmoles/kg) (Table 1). The two *cis* isomers [*cis*(4*S*,5*R*) ED_{50} dose = 1.22 mg/kg; 6.9 μmoles/kg, and *cis*(4*R*,5*S*) ED_{50} dose = 1.52 mg/kg; 8.6 μmoles/kg] were similar in potency, and were several times less potent than the *trans*(4*S*,5*S*) isomer. The *trans*(4*R*,5*R*) isomer did not substitute for *S*(+)amphetamine at a dose of more than ten times the ED_{50} dose of the *trans*(4*S*,5*S*) isomer. Approximately 45 to 50 min after injection, it was noticed that the animals receiving the 3 mg/kg

TABLE 1

RESULTS OF STIMULUS GENERALIZATION STUDY WITH 4-METHYLAMINOREX ISOMERS IN RATS TRAINED TO DISCRIMINATE *S*(+)AMPHETAMINE (1 mg/kg) FROM SALINE

Agent	Dose (mg/kg)	N*	% Amph-Appropriate Responding†	Mean Resp/Minute†	ED_{50} Dose (95% Confidence Limits)
(±)*Cis*	1.2	3/4	26% (13)	17.7 (5.1)	
	1.5	4/4	31% (8)	24.9 (7.3)	
	1.8	3/4	63% (11)	18.4 (6.5)	
	2.2	3/3	97% (3)	18.7 (8.5)	
	3.0	2/3	100%	12.2 (9.4)	1.56 (1.21–1.98) mg/kg
Cis(4*S*,5*R*)	1.0	3/5	29% (11)	12.4 (4.9)	
	1.2	4/4	46% (25)	12.7 (4.9)	
	1.8	4/6	88% (7)	4.9 (2.5)	
	2.2	3/4	97% (3)	14.4 (5.0)	1.22 (0.86–2.22) mg/kg
Cis(4*R*,5*S*)	1.2	3/3	34% (9)	12.0 (8.8)	
	1.8	4/4	46% (16)	20.6 (1.7)	
	2.2	3/4	81% (11)	7.3 (2.2)	
	2.5	3/4	99% (1)	25.1 (7.7)	1.52 (0.90–2.14) mg/kg
(±)*Trans*	0.1	4/5	4% (3)	10.9 (1.1)	
	0.3	7/9	32% (15)	9.8 (2.5)	
	0.38	4/4	49% (14)	14.2 (2.8)	
	0.5	9/9	74% (8)	7.1 (1.8)	
	1.2	4/5	89% (11)	6.3 (2.3)	
	2.2	4/5	97% (2)	13.5 (5.1)	0.41 (0.24–0.70) mg/kg
Trans(4*S*,5*S*)	0.15	4/4	20% (10)	14.0 (3.4)	
	0.25	4/4	21% (13)	14.6 (4.3)	
	0.30	3/4	59% (13)	14.0 (5.5)	
	0.38	3/4	98% (1)	16.1 (8.6)	0.25 (0.17–0.36) mg/kg
Trans(4*R*,5*R*)	0.38	4/4	15% (7)	17.9 (5.1)	
	1.0	3/3	3% (3)	8.2 (6.2)	
	1.8	4/5	7% (5)	10.4 (5.9)	
	2.5	4/6	40% (24)	8.8 (6.7)	
	3.0	4/4	35% (18)	13.2 (3.1)	
	5.0	2/4	43% (1)	7.8 (2.2)	
	3.0‡	3/4	42% (7)	11.3 (7.0)	
	3.0§	4/4	73% (17)	15.5 (6.1)	
	4.0§	6/7¶	85% (9)	14.7 (5.6)	
S(+)Amph	1.0	9/9	93% (5)	14.6 (4.1)	0.42** mg/kg
S(+)Amph§	1.0	4/4	95% (2)	21.8 (6.1)	
Saline (1.0 ml/kg)		9/9	12% (3)	14.1 (3.1)	

*N = Number of animals responding/number of animals receiving drug.

†Data obtained during 2.5-min extinction session.

‡Although a 15-min presession injection interval was routinely employed, a 40-min presession injection interval was used for this dose.

§A 60-min presession injection interval was used for this dose.

¶One additional animal (not included in the count) made only 16% of its responses on the amphetamine-appropriate lever; tested the following week, this same animal made 23% of its responses on the amphetamine-appropriate lever. With one exception, all other animals made more than 80% of their responses on the amphetamine-appropriate lever.

**An ED_{50} value was previously published (6).

dose of the *trans*(4*R*,5*R*) isomer appeared hyperactive. For this reason, during subsequent weeks, the presession injection interval was lengthened from 15 to either 40 or 60 min. Although there was little difference in amphetamine-appropriate responding for 3 mg/kg of the *trans*(4*R*,5*R*) isomer when the presession injection interval was 15 min (35%) or 40 min (42%) (Table 1), amphetamine-appropriate responding increased to 73% when the 60-min interval was used. Using the 60-min interval, amphetamine-

appropriate responding increased to 85% when 4 mg/kg of the *trans(4R,5R)* isomer was administered. The training drug, 1 mg/kg of *S*(+)amphetamine, produced 93% and 95% amphetamine-appropriate responding at 15 and 60 minutes, respectively, postinjection.

DISCUSSION

Benzylic hydroxylation is known to significantly reduce the central stimulant potency of phenylisopropylamines (5). This is due, most probably, to the increased polarity of the resulting agent (and, consequently, to its reduced ability to penetrate the blood-brain barrier) (5), and not simply to the intrinsic nature (i.e., the presence) of a benzylic oxygen atom. Cathinone, for example, which possesses this oxygen atom in the form of a keto group (Fig. 1), is similar in action and potency to amphetamine itself [e.g., (7)]. N-Alkylation of amphetamine with small alkyl groups results in retention of amphetamine-like stimulus properties; for example, both N-monomethylamphetamine and N-monoethylamphetamine substitute for amphetamine (6). [N,N-*Di*methylation of *S*(+)amphetamine results in an agent that produces saline-appropriate responding at doses (e.g., 18 mg/kg) of more than forty times the ED_{50} dose of *S*(+)amphetamine (Glennon, unpublished findings).] Thus, it is not surprising that 4-methylaminorex, a cyclic, conformationally restrained N-monoalkyl phenylisopropylamine, is a central stimulant.

On the basis of established structure-activity relationships for the central stimulant and discriminative stimulus properties of phenylisopropylamines (5,15), predictions can be (and were) made as to the relative potency of each of the optical isomers of 4-methylaminorex. Structurally, the 4- and 5-positions of 4-methylaminorex correspond to the alpha- and benzylic-positions, respectively, of the phenylisopropylamines. Because greater potency is associated with phenylisopropylamines that have an alpha methyl group in the *S* absolute configuration than in the *R* configuration (5), the 4*S*-isomer of 4-methylaminorex would be expected to be more potent than the corresponding 4*R*-isomer. Benzylic hydroxylation of *S*(+)amphetamine affords two optical isomers: (+)norpseudoephedrine (i.e., cathine; 1*S*,2*S*) and (−)-norephedrine (1*S*,2*R*). Although (+)norpseudoephedrine is only one-tenth as potent as (+)amphetamine as a locomotor stimulant (2) and one-seventh as potent as *S*-cathinone in drug discrimination studies using rats trained to discriminate cathinone from saline (5), it is, nonetheless, more potent than (−)norephedrine (2). The absolute configuration of the hydroxyl group in (+)norpseudoephedrine is *S* (i.e., 2*S*). (12). Thus, a greater potency is associated with this *S* hydroxyl group, and a greater potency might be expected for the isomers of 4-methylaminorex where the 5-position possesses an absolute configuration of *S*. On this basis, the *trans*(4*S*,5*S*) isomer of 4-methylaminorex would be expected to be the most potent, and the *trans*(4*R*,5*R*) isomer the least potent, isomer. The *cis*(4*S*,5*R*) and *cis*(4*R*,5*S*) isomers each possess one substituent in the "correct" configuration and one in the "incorrect" configuration. It might be anticipated that these agents would be roughly equipotent and fall somewhere between the *trans*(4*S*,5*S*) and *trans*(4*R*,5*R*) isomers in terms of potency. Indeed, this is found to be the case. The relative rank-order of potencies is found to be: *trans*(4*S*,5*S*) > *cis*(4*S*,5*R*) = *cis*(4*R*,5*S*) > *trans*(4*R*,5*R*).

The *trans*(4*R*,5*R*) isomer failed to result in stimulus generalization (using the standard 15-min presession injection interval); doses of 2.5 to 5 mg/kg resulted only in partial generalization (i.e., 35–43% amphetamine-appropriate responding). Unlike the other isomers, however, the *trans*(4*R*,5*R*) isomer appeared to produce locomotor stimulation and hyperactivity 45 to 50 min postadministration. With the possibility that this isomer may have a delayed onset, several doses were evaluated using a longer presession injection interval. With a 60-min interval, a 4 mg/kg dose of the *trans*(4*R*,5*R*) isomer substituted for *S*(+)amphetamine. *S*(+)Amphetamine produced greater than 80% amphetamine appropriate responding both at 15 and 60 minutes postadministration. Although limited supplies of *trans*(4*R*,5*R*) 4-methylaminorex precluded a more detailed evaluation, it would seem that (at 60 min) this isomer is approximately ten times less potent (on a molar basis) than *S*(+)amphetamine (at 15 min).

In summary, this study reports the first pharmacological investigation of all four optical isomers of 4-methylaminorex. This agent appears to possess an amphetamine-like discriminative profile, and consistent with established SAR, the *trans*(4*S*,5*S*) isomer is more potent than the *cis* isomers, which are, in turn, more potent than the *trans*(4*R*,5*R*) isomer. Although the *trans*(4*R*,5*R*) isomer did not completely substitute for *S*(+)amphetamine when a 15-min presession injection interval was used, stimulus generalization occurred with a 60-min interval. Evidently, this isomer possesses a longer duration of onset, and may conceivably contribute to the overall pharmacology of the *trans* racemate. Interestingly, only the *cis* isomers (i.e., "U4Euh") and not the *trans* isomers (i.e., *trans* "U4Euh") are included in the recent control (Schedule I) of this agent (3). The present results demonstrate that the *trans*(4*S*,5*S*) isomer of 4-methylaminorex is similar in potency to *S*(+)amphetamine (ED_{50} values = 1.4 and 1.7 μmoles/kg, respectively) in drug discrimination studies and suggest that the *trans* racemate might also be an amphetamine-like agent subject to abuse. However, at this time, there is insufficient evidence to conclude that 4-methylaminorex produces only amphetamine-like effects; its pharmacology, particularly its human pharmacology, needs to be examined in greater detail.

ACKNOWLEDGEMENTS

This work was supported in part by USPHS grant DA01642. We also wish to thank Dr. Robert Klein and the DEA for providing us with the isomers of 4-methylaminorex.

REFERENCES

1. Davis, F. T.; Brewster, M. E. A fatality involving U4Euh, a cyclic derivative of phenylpropanolamine. J. Forensic Sci. 33:549–553; 1988.
2. Fairchild, M. D.; Alles, G. A. The central locomotor stimulatory activity and acute toxicity of the ephedrine and norephedrine isomers in mice. J. Pharmacol. Exp. Ther. 158:135–139; 1967.
3. Federal Register, Schedules of controlled substances; Placement of (±)*cis*-4-methylaminorex into Schedule I: April 13, 1989. 54:14799–14800; 1989.
4. Finney, D. Probit analysis. London: Cambridge University Press; 1952.
5. Glennon, R. A. Psychoactive phenylisopropylamines. In: Meltzer, H. Y., ed. Psychopharmacology. The third generation of progress. New York: Raven Press; 1987:1627–1634.
6. Glennon, R. A.; Yousif, M.; Patrick, G. Stimulus properties of 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) analogs. Pharmacol. Biochem. Behav. 29:443–449; 1988.
7. Glennon, R. A.; Yousif, M.; Naiman, N.; Kalix, P. Methcathinone: A new and potent amphetamine-like agent. Pharmacol. Biochem. Behav. 26:547–551; 1987.
8. Klein, R. F. X.; Sperling, A. R.; Cooper, D.; Kram, T. C. The stereoisomers of 4-methylaminorex. J. Forensic Sci.; in press.
9. Patil, P. N.; Yamauchi, D. Influence of the optical isomers of some centrally acting drugs on norepinephrine responses. Eur. J. Pharmacol. 12:132–135; 1970.
10. Poos, G. I.; Carson, J. R.; Rosenau, J. D.; Roszkowski, A. P.;

Kelley, N. M.; McGowin, J. 2-Amino-5-aryl-2-oxazolines. Potent new anorectic agents. J. Med. Chem. 6:266–272; 1963.
11. Roszkowski, A. P.; Kelley, N. M. A rapid method for assessing drug inhibition of feeding behavior. J. Pharmacol. Exp. Ther. 140:367–374; 1963.
12. Schorno, X.; Steinegger, E. CNS-Active phenylpropylamines of *Catha edulis* FORSK. (Celastraceae) of Kenyan origin. Experientia 357:572–574; 1979.
13. Wollweber, H.; Hiltmann, R.; Stoepel, K.; Kroneberg, H.-G. 1-Phenyl-3-iminoperhydro-3H-oxazolo[3,4-a]pyridine, mit blutdrucksteigernder Wirksamkeit. Eur. J. Med. Chem. 15:111–117; 1980.
14. Yelnosky, J.; Katz, R. Sympathomimetic actions of *cis*-2-amino-4-methyl-5-phenyl-2-oxazoline. J. Pharmacol. Exp. Ther. 141:180–184; 1963.
15. Young, R.; Glennon, R. A. Discriminative stimulus properties of amphetamine and structurally related phenalkylamines. Med. Res. Rev. 6:99–130; 1986.