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Stimulus Properties of Some Analogues of 4-Methylaminorex

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RUSSELL, B. R., R. A. BERESFORD, D. M. SCHMIERER, N. McNAUGHTON AND C. R. CLARK. *Stimulus properties of some analogues of 4-methylaminorex.* PHARMACOL BIOCHEM BEHAV 51(2/3) 375-378, 1995.—The stimulus properties of aminorex and analogues of 4-methylaminorex, namely (4*S*,5*S*)-4-methylaminorex, *N*-methyl-(4*S*,5*S*)-4-methylaminorex, and the regioisomeric (*R*)- and (*S*)-2-amino-4-phenyl-2-oxazoline (rexamino) were compared in rats trained to distinguish (*S*)-amphetamine (1 mg/kg) from saline.The first three compounds, aminorex, (4*S*,5*S*)-4-methylaminorex, and *N*-methyl-(4*S*,5*S*)-4-methylaminorex shared discriminative stimulus effects with amphetamine, although the stimulus properties for racemic aminorex were less than those of the other two compounds. The two regioisomers, (*R*)-and (*S*)-rexamino, produced only partial generalisation to the amphetamine.

4-Methylaminorex analogues Drug discrimination study Dimethylaminorex isomers Drug abuse

IN RECENT years racemic *cis*-methylaminorex has appeared among the growing number of designer drugs available on the clandestine market, and this compound has been classified as a Schedule I substance. The original reports (11) on 2-amino-5-phenyl-2-oxazoline (aminorex) described it as a potent anorectic agent with interesting CNS stimulant properties. The anorectic properties were initially examined in rats and suggested potency equal to that of (*S*)-amphetamine.

Glennon and Meisenheimer (5) reported the stimulus-generalization properties of the four individual stereoisomers of 4-methylaminorex compared to (*S*)-(+)-amphetamine. These studies showed the *trans*-(4*S*,5*S*)-isomer to be more potent than either *cis*-isomer (4*S*,5*R* and 4*R*,5*S*), which in turn were more potent than the *trans*-(4*R*,5*R*)-isomer. The more potent *trans*-(4*S*,5*S*)-isomer was found to be similar in potency to (*S*)-amphetamine. These stimulant and euphoriant effects, as well as blood pressure elevation, are likely to be the result of a sympathomimetic mechanism.

The stereoisomers of 4-methylaminorex have the potential to become significant problems in the clandestine drug market. These compounds can be prepared in a one-step synthesis from readily available starting materials, norephedrine, norpseudoephedrine, and cyanogen bromide (8). Aminorex is prepared by an analogous synthesis from commercially available 2-amino-1-phenylethanol. Aminorex and 4-methylaminorex have already appeared on the clandestine street market (8,9) and the potential exists for the 3,4-dimethylaminorex isomers to appear as a further designer modification of the aminorex molecule. The dimethylaminorex isomers can be prepared via the same synthetic route using cyanogen bromide and commercially available ephedrine or pseudoephedrine starting materials (9).

In this study, we report the behavioural effects of *N*-methyl-(4*S*,5*S*)-4-methylaminorex, (4*S*,5*S*)-4-methylaminorex, aminorex, and its regioisomers (*R*)- and (*S*)-2-amino-4-phenyl-2-oxazoline (rexamino) compared to that of (*S*)-amphetamine (Fig. 1). All drugs, including amphetamine, were used in the form of their sulfate salt.

METHOD

The animals used in this study were experimentally naive female Sprague–Dawley rats initially 4 months old and weighing 210–230 g. The testing facility was kept at 21–23°C; animals had free access to water at all times but were food deprived for 23 out of 24 h. The animals were fed a commercial rat chow and at the end of the study the mean weight was 267 g.

Behavioural training and testing were carried out in 14 standard operant chambers. These were Skinner boxes (i.e., Rodent Testing Chambers from Campden Instruments Ltd,

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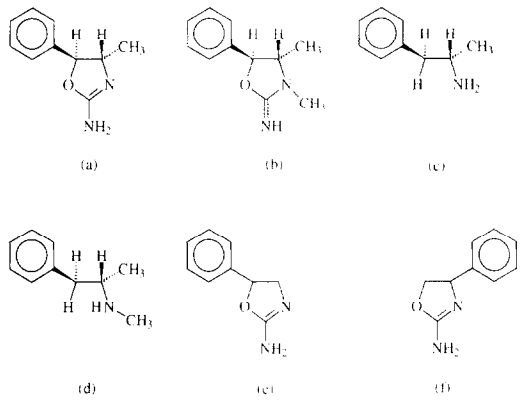

FIG. 1. (a) (4*S*,5*S*)-4 Methylaminorex, (b) (4*S*,5*S*)-4-*N*-dimethylaminorex, (c) (*S*)-amphetamine, (d) methamphetamine, (e) (±)-aminorex, and (f) (±)-rexamino.

London, England). The test chambers were housed inside a light- and sound-attenuated outer chamber with fans providing ventilation and background noise. One wall of the operant chamber contained an intelligence panel with two retractable levers and a food hopper recessed into the wall between them. All operant chambers were interfaced with a British Broadcasting Acorn Computer that controlled the programmed schedule and recorded all events in the training and testing of animals using SPIDER (Paul Fray Ltd) interfacing and software. The animals first received three sessions of an autoshaping schedule of free food delivered with the levers retracted and were then placed on a continuous reinforcement (CR), with box levers extended into the box. The rats quickly learned to press the levers that immediately supplied a 45-mg pellet of food (Noyes) as reinforcement.

In the discrimination training rats were given an intraperitoneal (IP) injection of either 1 mg/kg (*S*)-amphetamine or 1 ml/kg 0.9% saline 15 min prior to the training session. This was to introduce a predictive cue, before the animals had sufficient experience in responding on either lever for a food reward.

For discrimination training the animals were placed on a random-interval (RI) two-lever discrimination schedule. Each rat was assigned a "home" operant chamber so that it would become accustomed to the smell and lever pressures of that particular chamber. Individual rats were administered saline or drug on a pseudorandom basis with neither saline nor drug being given for more than 3 consecutive days. In addition, each group of 14 rats was tested at random as the first group each day. These measures ensured the response given was based on either a drug or nondrug state by removing animal preference for a particular lever and olfactory cues left by the preceding rat in the chamber (2). Initially, one group of rats was trained so that if they pressed the right lever after an IP injection of amphetamine they received a pellet as reinforcement, but if they pressed the left lever they did not. Conversely, if the rats were given an IP injection of saline they would have to push the left lever to receive a pellet for reinforcement. Response accuracy was measured as the number of correct lever presses/total number of lever presses in 1 min of the training session. The RI was initially set at 0 s, thus delivering continuous reinforcement contingent on pressing the correct lever. When an animal produced response accuracy greater than 80% correct in the final 5 min of a 15-min training session, the random interval was increased in the subsequent session by an increment of 3 s up to a final total of 15 s (i.e., on the final schedule, availability of the next reinforcement after the correct lever was pressed and reinforced varied between 0 and 30 s, and all intervals between 0 and 30 s had an equal probability of occurrence). Training continued at a RI of 15 s until the animal reached a criterion of greater than 80% correct responses in the first minute of a session for 10 consecutive sessions. It took an average of 40 sessions for the animals to reach a stable response rate following saline or amphetamine treatment. Eventually 25 rats out of the original 30 reached criterion.

As the animals reached criterion they were used in drug discrimination trials with the newly synthesised compounds under investigation. Drugs were administered as in the training sessions (i.e., by IP injection given 15 min before testing). Lever pressing responses were measured during the first minute after placement in the Skinner boxes, after which rats were removed from their boxes. All results were calculated on the percentage of correct amphetamine-appropriate responding.

Between drug discrimination trials, the rats were given a minimum of three training sessions with either amphetamine or saline, at random. Animals not discriminating amphetamine from saline (having less than 80% appropriate responding) were not used in the subsequent drug trial but were returned for training until they were once again able to reach criterion.

Each drug was tested on four rats, with each dose being tested four times on each rat. Drugs were randomised among the rats that had reached criterion, but the dose-reponse curve for amphetamine was generated from all 25 rats.

All drugs used in this study, including amphetamine, were synthesised in our laboratory by the literature methods (8,9,11). The purity of all compounds was determined by gas chromatography, mass spectrometry, thin-layer chromatography, infrared spectroscopy, and nuclear magnetic resonance spectroscopy (^{13}C and ^{1}H). The solution of (*S*)-amphetamine sulfate was made up daily by dissolution in sterile saline. All other drug solutions were made up daily by dissolving them in one equivalent of sulfuric acid in sterile 0.9% saline and diluted with sterile saline to the desired concentration. The solutions were prepared at concentrations that allowed the appropriate dose to be given in a volume of 1 ml/kg.

The regioisomeric 4-phenyl derivative, rexamino, was prepared by cyanogen bromide treatment of phenylglycinol.

RESULTS

The discrimination of (*S*)-amphetamine from saline was successfully learned by most rats, and all animals used in the drug discrimination trials showed a consistently high accuracy of 96% amphetamine-appropriate responding after administration of amphetamine and a 13% amphetamine-appropriate rate after saline administration.

Treatment with the aminorex isomers, with the exception of the rexamino regioisomers, resulted in successful amphetamine generalization. Both *N*-methyl-(4*S*,5*S*)-4-methylaminorex (ED_{50} 2 μmol/kg) and (4*S*,5*S*)-4-methylaminorex (ED_{50} 1.7 μmol/kg) isomers have a potency similar to (*S*)-amphetamine (ED_{50} 1.7 μmol/kg). The racemate (±)-aminorex (ED_{50} 3 μmol/kg) had a potency similar to (±)-amphetamine. All results showing the doses administered and corresponding percentage response obtained are given in Table 1. The individual enantiomers of rexamino, the regioisomer of aminorex, did not show substitution for amphetamine, although a value of 30–40% amphetamine-appropriate responding was achieved. This does not satisfy any criteria for generalization and the

TABLE 1

GENERALIZATION STUDIES FOR 4-METHYLAMINOREX ANALOGUES

Drug/Dose (μmol/kg)	N	Amphetamine-Appropriate Response per Minute (± SEM)	Mean Response Minute (± SEM)	ED_{50} Dose (95% Confidence Limits)
(±)-Aminorex				
1.9	3/3	19% (3)	49 (9)	
2.8	4/4	34% (11)	27 (5.0)	
3.1	4/4	63% (12)	26 (2.2)	
3.7	4/4	62% (16)	24 (4.4)	
4.9	4/4	74% (3)	19 (2.8)	
6.2	2/2	85% (3)	19 (3.9)	
8.7	2/2	94% (8)	22 (2.5)	3.0 (2.8-3.3)μmol /kg
N-Methyl-(4S,5S)-4-Methylaminorex				
0.5	2/2	21% (12)	32 (9.5)	
1.2	2/2	19% (3)	22 (9.5)	
1.3	6/6	37% (1)	41 (5.2)	
2.1	3/3	51% (10)	37 (6.6)	
2.6	5/5	51% (13)	28 (6.5)	
4.2	3/3	76% (21)	35 (1.7)	
5.3	3/3	90% (3)	28 (2.2)	
7.4	5/5	84% (5)	30 (2.7)	2.0 (1.7-2.5) μmol/kg)
(4S,5S)-4-Methylaminorex				
0.6	3/3	16% (9)	43 (11.2)	
1.1	5/5	26% (4)	34 (8.6)	
2.3	5/5	56% (7)	25 (202)	
3.4	5/5	91% (3)	19 (4.7)	
5.7	4/4	99% (1)	20 (4.5)	1.7(1.4-2.0) μmol/kg)
(S)-Rexamino				
24.6	5/5	26% (10)	32 (5.1)	
37	5/5	32% (4)	40 (2.6)	
49.3	4/4	39% (9)	32 (6.0)	No Stimulus
61.7	4/4	39% (7)	33 (2.5)	Generalization
(R)-Rexamino				
24.6	5/5	30% (5)	33 (4.9)	
30.8	2/2	33% (11)	37 (19)	
37	5/5	35% (9)	28 (6.3)	
49.3	5/5	38% (6)	42 (6.6)	No Stimulus
61.7	6/6	41% (6)	35 (6.6)	Generalization
(S)-Amphetamine				
0.6	4/4	23% (10)	39 (8.4)	
1.2	4/4	28% (5)	39 (1.1)	
2.4	8/8	52% (6)	34 (5.4)	
2.9	4/4	80% (11)	25 (2.5)	
3.1	4/4	86% (5)	24 (1.5)	
4.7	4/4	93% (4)	30 (7.0)	
5.9	9/9	96% (3)	27 (1.0)	1.9 (1.5-2.3) μmol/kg
Saline				
0.9%	9/9	13% (4)	34 (3.0)	

Studies were conducted using rats trained to discriminate 1 mg/kg of (*S*)-amphetamine from saline. All data was collected during a 1-min session. *N*: number of animals responding/number of animals receiving drug.

rexamino isomers can be considered to have little or no amphetamine-type central effects. There were no obvious signs of hyperactivity for up to 1 h after administration of (*S*)- or (*R*)-rexamino and the animals were not tested at later time periods. Where stimulus generalization occurred the ED_{50} values were calculated by probit analysis according to the method of Finney (3). The values given are the approximate dose at which the animals would be expected to make 50% of their responses on the amphetamine-appropriate lever.

DISCUSSION

The amphetamine-like stimulus generalization properties of (4*S*,5*S*)-4-methylaminorex was reported by Glennon and Meisenheimer (5), who obtained an ED_{50} of 1.4 μmol/kg compared to the ED_{50} of 1.7 μmol/kg obtained in this study. Our studies suggest that (±)-aminorex, *N*-methyl-(4*S*,5*S*)-4-methylaminorex (3,4-dimethylaminorex), and (4*S*,5*S*)-4-methylaminorex share discriminative stimulus effects with amphetamine and all have a similar potency. The two regioisomers, (*R*)-rexamino and (*S*)-rexamino, produced only partial generalization and clearly do not share the stimulant properties of amphetamine and many of its structural analogues.

The method used for discrimination testing in this study was a random-interval paradigm in which, unlike other variable interval paradigms, all intervals have an equal probability of reinforcement, and female rats were used instead of the more commonly used males. Female rats have shown a much greater behavioural response to acute injections of amphetamine as measured by locomotor stereotyped (1) or rotational behaviour (12). However, sex difference is not a factor in the metabolism of amphetamine (13). Thus, there should be little difference in the ED_{50} found for these drugs in female vs. male rats. The ED_{50} determined for (*S*)-amphetamine in this study compares well with that found by Oberlender and Nichols (10) of 1.7 μmol/kg, Glennon and Young (6) of 2.3 μmol/kg, and

Glennon (4) of 1.8 μmol/kg. These values help demonstrate the consistency of the results between laboratories and that there is good correlation between the different types of schedules. The (4*S*,5*S*)-enantiomer was the most potent of the four isomers of 4-methylaminorex studied by Glennon and Meisenheimer (5).

Using established structure-activity relationships (SAR) for central stimulant and discriminative stimulus properties, predictions could be made concerning the potency of the aminorex isomers. The 4-and 5-positions of the aminorex isomers correspond to the alpha and benzylic positions of amphetamine, respectively. Because the potency associated with amphetamines that have an α-methyl group in the (*S*) configuration is greater than the (*R*) configuration, the (4*S*)-isomers would be expected to be more potent than the (4*R*)-isomers of methylaminorex or dimethyaminorex. Glennon and Meisenheimer (5) showed this prediction to be true for the 4-methylaminorex isomers and this was the basis for selecting the (4*S*,5*S*)-dimethylaminorex isomer for testing. Additionally, on this basis (*S*)-aminorex would be expected to be more potent than (*R*)-aminorex.

The ED_{50} of 1.7 μmol/kg that was determined in this study for (4*S*,5*S*)-4-methylaminorex correlates well with the value of 1.4 μmol/kg that was determined for amphetamine by Glennon and Meisenheimer (5). Similarly, the comparable potencies found for dimethylaminorex vs. methylaminorex (2.0 μmol/kg and 1.7 μmol/kg, respectively) can be related to the similar potencies found for methamphetamine and amphetamine (2.2 μmol/kg and 2.3 μmol/kg, respectively) by Glennon and Young (6).

Both rexamino isomers showed a maximum amphetamine-like response of approximately 40%. Despite the fact that these animals clearly did not identify these drugs as saline, this result can be interpreted only as partial generalization, using the criterion that the animals need to show a greater than 80% response rate for amphetamine generalization. The partial generalization was achieved after testing both isomers at concentrations 30 times greater than the ED_{50} for amphetamine. Higher doses of rexamino were not tested for discrimination ability, partly because the results were reasonably consistent between 24.6 μmol/kg and 61.7 μmol/kg and partly because behaviour was disrupted too much at higher doses. It is therefore unclear whether these compounds are specifically less potent than amphetamine or are merely producing some non-specific stimulant effect.

An ED_{50} of 3 μmol/kg was determined in this study for (±)-aminorex. This value is similar to that of 2.6 μmol/kg reported for racemic amphetamine (5) and is a value typical of what would be expected on the basis of SAR for amphetamine analogues. If the enantiomers of aminorex had been used, the (*S*)-isomer should have been the more potent based on the SA and could possibly have given a value similar to (*S*)-amphetamine.

When the SAR of amphetamine analogues are considered in relation to aminorex, it may be seen that although the α-methyl group of amphetamine is absent from the aminorex molecule, the drug is still equipotent with amphetamine. Huang and Ho (7) demonstrated that pretreatment of animals with the monamine oxidase inhibitor, iproniazid, before administration of phenethylamine produced amphetamine-appropriate responding. From this they concluded that the α-desmethyl analogues of amphetamine lacked protection from metabolism. The oxazoline ring system may provide this protection from metabolic inactivation by deamination, resulting in similar potency between racemic aminorex and racemic amphetamine.

All of the amphetamine analogues appear to have peripheral effects but, apart from aminorex, none of the other isomers have been examined for this type of activity. Aminorex at toxic doses has been shown in humans to produce psychomotor stimulation. There have been reports, in severe cases, of convulsions and respiratory depression combined with mydriasis, tachycardia, flushing of the skin, acute hypertension, and hyperpnoea produced by aminorex. Considering the SAR and similar discriminative responses caused by the isomers of aminorex, it would be reasonable to assume that they also would produce similar pharmacological responses.

In summary, racemic aminorex, (4*S*,5*S*)-4-methylaminorex, and *N*-methyl-(4*S*,5*S*)-4-methylaminorex (3,4-dimethylaminorex) possess amphetamine-like stimulus properties. The stimulus properties for racemic aminorex (ED_{50} = 3 μmol/kg) were slightly less potent than those for (4*S*,5*S*)-4-methylaminorex (ED_{50} = 1.7 μmol/kg) and *N*-methyl-(4*S*,5*S*)-4-methylaminorex (ED_{50} = 2 μmol/kg).

REFERENCES

1. Beatty, W. W.; Holzer, G. A. Sex differences in stereotyped behaviour in the rat. Pharmacol. Biochem. Behav. 9:777–783; 1978.
2. Extance, K.; Gouidie, A. J. Inter-animal olfactory cues in operant drug discrimination procedures in rats. Psychopharmacology (Berlin) 73:363–371; 1981.
3. Finney, D. J. Probit analysis, 3rd ed. Cambridge, MA: Cambridge University Press; 1971.
4. Glennon, R. A. Stimulus properties of hallucinogenic phenalkylamines and related designer drugs: Formulation of structure-activity relationships. In: Asher, K.; Wesouza, E.; eds. Pharmacology and toxicology of amphetamine and related designer drugs. NIDA Research Monograph Series, vol. 94. Ashgar, K.; De Souza, E. eds. Washington, D.C.: U.S. Government Printing Office; 1989:43–647.
5. Glennon, R. A.; Meisenheimer, B. Stimulus properties of a new designer drug: 4-Methylaminorex ("U4Euh"). Pharmacol. Biochem. Behav. 35:517–521; 1990.
6. Glennon, R. A.; Young, R. Further investigations of the stimulus properties of MDA. Pharmacol. Biochem. Behav. 20:501–505; 1983.
7. Huang, J. T.; Ho, B. T. The effect of pretreatment with iproniazid on the behavioural activities of β-phenethylamine in rats. Psychopharmacology (Berlin) 35:77–81; 1974.
8. Noggle, F. T.; Clark, C. R.; DeRuiter, J. Comparative analytical profiles for 4,5-dihydro-5-phenyl-2-oxazolamine (aminorex) and 4,5-dihydro-4-phenyl-2-oxazolamine (rexamino). Microgram 23: 281–287; 1990.
9. Noggle, F. T.; Clark, C. R.; DeRuiter, J. Liquid chromatographic and spectral analysis of the stereoisomers of dimethylaminorex. Assoc. Off. Anal. Chem. J. 75:423–427; 1992.
10. Oberlender, R.; Nichols, D. E. Drug discrimination studies with MDMA and amphetamine. Psychopharmacology (Berlin) 95:71–76; 1988.
11. 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. Med. Chem. 6:266–272; 1963.
12. Robinson, T. E.; Becker, J. B. Enduring changes in brain behaviour produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. 11:157–198; 1986.
13. Robinson, T. E.; Becker, J. B.; Ramirez, V. D. Sex differences in amphetamine elicited rotational behaviour and the lateralisation of striatal dopamine in rats. Brain Res. Bull. 5:539–545; 1980.