Pharmacology Biochemistry & Behavior, Vol. 17, pp. 291–295, 1982. Printed in the U.S.A.

Discriminative Stimulus Properties of Ketamine Stereoisomers in Phencyclidine-Trained Rats

KATHLEEN T. BRADY[2] AND ROBERT L. BALSTER[3]

Pharmacology Department, Medical College of Virginia, Virginia Commonwealth University Richmond, VA 23298

Received 11 January 1982

BRADY, K. T. AND R. L. Balster. *Discriminative stimulus properties of ketamine stereoisomers in phencyclidine-trained rats*. PHARMAC. BIOCHEM. BEHAV. 17(2) 291–295, 1982.—Rats were trained to discriminate phencyclidine (PCP) from saline in a two-lever drug discrimination task on a fixed-ratio 32 schedule of food presentation. The subjects were given IP injections of 3.0 mg/kg PCP or saline daily on a double alternation schedule. After reliable discriminative control of lever choice was established, dose-response determinations for generalization to the training dose of PCP were made with several doses of PCP, a racemic mixture of ketamine and the pure levo (−) and dextro (+) salts of ketamine. All three forms of ketamine produced dose-dependent PCP-appropriate responding. ED_{50} values were determined for each drug for percent drug-lever appropriate responding and for suppression of operant responding during test sessions. There was a greater difference between doses which produced drug-lever appropriate responding and doses which suppressed response rates for PCP than for any of the forms of ketamine. (±)- and (+)-ketamine were about 2 times more potent than (−)-ketamine for producing drug-lever appropriate responding but were roughly equipotent for response rate suppression. Thus there is no qualitative and little quantitative stereospecificity for the PCP-like discriminative stimulus effects of ketamine in rats.

Katamine Phencyclidine Stereospecificity Drug discrimination Schedule-controlled behavior Rats

KETAMINE, 2-(0-chlorophenyl)-2-methyl aminocyclohexanone, is a dissociative anesthetic which is very similar to phencyclidine (PCP) in its spectra of pharmacological effects [8]. It is a chiral compound which is used clinically as the racemic mixture of two optical isomers. While most studies of the pharmacological activitiy of ketamine have involved the use of the racemic mixture, several recent reports indicate differences in the activity of the enantiomers.

In reviewing the literature concerned with the pharmacological activity of ketamine stereoisomers, it is important to note that salts of the isomers of ketamine change their rotational signs on conversion to their respective free bases. Because most of the studies cited used the hydrochloride (HCl) salt forms of the ketamine isomers, we have assumed that the optical rotation of the isomers refers to the salt form when this is not specified by the authors. It seems important, however, to specify free base or salt when referring to the optical rotation of this group of compounds.

In rodents, the (+) salt has been reported to be more potent than the (−) salt in hypnotic activity [7] and as an analgesic [13]. Meliska *et al.* [9] reported that while high doses of both the (−) and the (+) salts decreased rates of schedule-controlled responding in rats, low doses of the (−) salt, but not the (+) salt, increased response rates. They concluded that there was a qualitative difference in the effect of the enantiomers on schedule-controlled behavior. In a recent clinical study, White *et al.* [20] reported that (+)-ketamine HCl produced more effective anesthesia with less incidence of emergence reactions than (−)-ketamine HCl. Opiate binding studies [16] indicate that (+)-ketamine HCl is 3 to 4 times more potent than (−)-ketamine HCl in displacing ^{3}H-naloxone binding. In addition, Smith *et al.* [15] recently reported that (+)-ketamine HCl was 3–4 times more potent in inhibiting catecholamine transport than the (−) salt, while (−)-ketamine HCl was slightly more potent against serotonin uptake. Thus, while there is some evidence for qualitative differences in the effects of ketamine stereoisomers, most studies report similar effects, with the (+)-isomer being generally found to be more potent.

Studies of the discriminative stimulus properties of ketamine and PCP have shown that these two drugs cross-generalize in rats [5, 6, 11, 12], pigeons [3], squirrel monkeys

[1]Research supported by N.I.D.A. grant DA-01442.
[2]Predoctoral trainee supported by N.I.D.A. training grant DA-07027.
[3]Send reprint requests to R. L. Balster, Box 613, MCV Station, Richmond, VA 23298.

[1] and rhesus monkeys [21]. All of these studies have been done with racemic ketamine. Several recent studies [2, 4, 17] have shown stereoselectivity in the discriminative stimulus effects of several psychotomimetic opioids which cross-generalize to PCP or ketamine in drug discrimination studies. The present study was carried out to investigate the possibility of stereoselectivity in the PCP-like discriminative stimulus properties of ketamine in rats.

METHOD

Subjects

The subjects were ten experimentally naive male Sprague-Dawley rats (Flow Laboratories, Dublin, VA). The animals were maintained at 80% of their free-feeding weights (280–340 grams) throughout the experiment by adjusted post-session feedings. They were individually housed in wire mesh cages where they had unlimited access to water.

Apparatus

A standard two-lever operant rat chamber (Model E10-10, Coulbourn Instruments, Inc., Lehigh Valley) housed inside a light and sound attenuating cubicle (Model E10-20, Coulbourn Instruments, Inc.) was used. The levers were 10 cm from the floor of the chamber and 13 cm from each other. A food cup into which 45 mg Noyes food pellets (Formula A) could be delivered via a Gerbrand model D1 automatic feeder was in the center of the wall equidistant from and 2 cm below the two response levers. A house light was located 20 cm directly above the food cup. Solid state programming equipment and recording devices were located in an adjacent room.

Procedure

Responding was shaped on a fixed-ratio 32 (FR 32) schedule of food presentation. Thirty-min sessions were conducted seven days a week. Initially, the subjects were trained to respond to either lever on a FR 1 schedule. Once lever pressing on both levers was established (2–10 sessions) drug injections were begun. Each animal received either saline or 3.0 mg/kg PCP IP 10 min pre-session on a double alternation schedule (two days saline, two days PCP, etc.). From this point on, responding on only one lever was reinforced during the session. The animal was placed in the darkened chamber immediately after the injection for the duration of the 10-min presession interval, and the house light was illuminated at the start of the session. For five animals, responses on the left lever resulted in reinforcement on PCP days and responses on the right lever resulted in reinforcement on saline days. The lever conditions were reversed for the other five subjects. Responses on the incorrect lever reset the FR contingency for reinforced responding on the correct lever. Response requirements were gradually increased until all animals reliably responded under a FR 32 schedule (28–58 sessions).

When reliable FR 32 responding was established, test probes were initiated. Every third session began with a 2-min test probe during which responding on either lever was reinforced, after which the session was continued as usual with only one lever correct. Discrimination training was continued until the subject had four consecutive test periods with 85 percent or more responding on the appropriate lever.

Following discrimination training, the effects of substituting 0.3, 1.0 and 10.0 mg/kg doses of PCP for the 3.0 mg/kg training dose were determined. Five animals received the doses in ascending order and five animals received the doses in descending order. All drug doses were tested twice in each animal; one determination was preceded by a PCP training day and the second determination was preceded by a saline training day. Test sessions were conducted only if the animal completed the first FR on the appropriate lever on the control day preceding the test day. Test sessions consisted of a 2-min period during which responding on either lever was reinforced, after which the session was terminated and the animals were returned to their cages. The double alternation continued on control days with the test sessions interspersed such that one PCP and one saline training day preceded each successive test day.

Following the PCP dose-response determination, stimulus generalization testing was conducted in a similar manner with four doses (1.0–30.0 mg/kg) of (±)-ketamine HCl, (−)-ketamine HCl and (+)-ketamine tartrate. These doses were chosen to cover a range from no effect to nearly complete suppression of response rates during the 2-min test session. Each dose was tested twice in each animal; one determination was preceded by a PCP training day and the second determination was preceded by a saline training day. The order in which the different drugs were given to each animal was randomized. All doses of a given drug were administered to a given subject before testing the next drug. Five animals received each drug in ascending dose order and five animals received each drug in descending dose order. A vehicle test session was conducted following the completion of the dose series for each drug.

Data Analysis

Both overall response rates on both levers and proportion of responses on the drug-appropriate lever were analyzed for test days. The saline test session conducted with each drug dose-response determination was used to calculate control response rate and percent drug-lever responding after vehicle administration for that drug. Drug-lever responding was determined by dividing the number of drug-lever appropriate responses made during the period by the total number of responses made during that time. Effects of test drug doses on overall response rates on both levers were calculated as the percent of the response rate on the vehicle test days conducted for each drug. For the doses that were tested twice, the average of the two values was used in the ED_{50} determination. The effective dose 50 percent (ED_{50}) for each drug was determined by least squares linear regression analysis using the dose-response data for percent drug-lever responding and for percent of vehicle response rates for the linear portions of the dose-response curves. These calculations were made after a log 10 transformation of the dose expressed as μg/kg of the base. For percent drug-lever responding all the doses were used in the regression analyses for all the drugs except (−)-ketamine where only the highest doses were included. For percent of vehicle response rates the three highest doses were used in the regression analyses for all drugs except PCP where only the two highest doses were included.

Drugs

PCP was supplies by Bio-Ceutic Laboratories (Sernylan). Racemic ketamine HCl was supplied by Parke-Davis and Co. (Ketalar). (−)-Ketamine HCl and the *d*-tartaric acid salt

TABLE 1

POTENCY OF PCP AND KETAMINE ISOMERS FOR STIMULUS GENERALIZATION AND SUPPRESSION OF OVERALL RESPONSE RATES IN RATS

Drug	ED_{50}* Drug-Lever Responding	Ratio to PCP	Slope†	ED_{50}‡ Response Rate Suppression	Ratio to PCP	Slope†	ED_{50} for Response Rate Suppression/ED_{50} for Drug-Lever Responding
PCP HCl	1.0 (0.10– 1.3)	1.0	58	4.3 (3.8– 4.7)	1.0	−160	4.3
(±) KET HCl	4.4 (3.5– 6.2)	4.4	61	9.7 (7.5–13.5)	2.3	−86	2.2
(+) KET tartrate	3.9 (2.8– 4.9)	3.9	54	7.3 (4.7–14.8)	1.7	−95	1.9
(−) KET HCl	8.3 (6.3 ± 11.2)	8.3	71	8.9 6.9–13.8)	2.1	−102	1.1

*Dose (mg/kg of base) resulting in 50% drug-lever appropriate responding as determined by linear regression and 95% confidence limits in parentheses.

†In log dose (μg/kg) − percent effect units.

‡Dose (mg/kg of base) resulting in a 50% decrease in response rates as determined by linear regression and 95% confidence limits in parentheses.

of (+)-ketamine were supplied by Bristol Laboratories. The estimated purity of each isomer was greater than 95%. The drugs were diluted with or dissolved in sterile saline to a concentration that resulted in an injection volume of 1.0 ml/kg. All injections were given IP 10 min presession. Dose and isomeric designations refer to the salts. Vehicle injections were 1.0 ml/kg of 0.9 percent saline.

RESULTS

Overall response rates for each animal remained fairly stable throughout the experiment. Average values ±S.E.M. in responses per sec for the response rates on vehicle test days for each animal were: 1.91±0.31, 1.03±0.21, 1.45±0.4, 1.1±0.3, 1.01±0.25, 1.56±0.31, 0.98±0.12, 0.86±0.1, 1.33±0.2, 1.28±0.31. The training dose of PCP (3.0 mg/kg) produced approximately 85 percent drug-lever appropriate responding (Fig. 1, upper left panel). This dose had no effect on response rate. All three forms of ketamine produced a dose-related increase in the percent of responses made on the PCP-appropriate lever (Fig. 1). While each of the three forms produced over 80 percent drug-lever appropriate responding at some dose, only the racemic mixture produced this level of drug-lever appropriate responding at doses which did not decrease response rates. For both stereoisomers, doses which produced greater than 50 percent drug-lever appropriate responding (10.0 mg/kg) also decreased response rates to approximately 50 percent of control. Test days for which the response rates were less than 0.05 response per sec were not included in the percent drug-lever appropriate responding tabulation.

Table 1 shows the ED_{50} values for suppression of operant responding and for percent drug-lever responding for each of the four compounds tested. PCP was more potent than any of the forms of ketamine on both measures. Although the isomers of ketamine showed qualitatively similar effects there was a small potency difference. For the production of PCP appropriate responding the (+)-isomer was over twice as potent as the (−)-isomer. This potency separation was not as large for overall response rate suppression. For this latter measure, the confidence limits for the ED_{50}'s for all three forms of ketamine overlapped. On the other hand, the greater potency of the (+)-isomer relative to the (−)-isomer for drug-lever responding appears to be reliable since the confidence limits for the ED_{50}'s of the stereoisomers do not overlap.

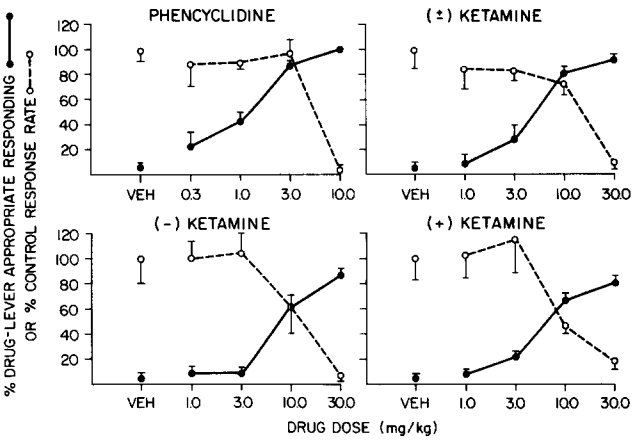

FIG. 1. Dose-response curves for phencyclidine HCl, (±)-ketamine HCl, (+)-ketamine tartrate and (−)-ketamine HCl for percent drug-lever responding (solid lines) and for effects on overall response rates (dashed lines) in rats (N=10). The mean percent drug lever responding ±S.E.M. and the mean percent of control response rate ±S.E.M. are on the ordinate with drug dose (log scale) on the abscissae. For the latter measure, control rates were obtained from the response rates on both levers during the 2-min test sessions with vehicle pretreatment.

For all four drugs the estimated dose necessary for the production of 50 percent drug-lever appropriate responding was less than the dose estimated to suppress overall response rates by 50 percent (Table 1). The ratios of these doses are shown in the table. The greatest separation of ef-

fects was seen with PCP. Racemic ketamine and the (+)-isomer were both approximately twice as potent for producing the PCP-cue as they were for response rate suppression. On the other hand, the (−)-isomer showed less specificity for PCP-like effects since it was roughly equipotent for producing generalization and for disruption of responding.

DISCUSSION

The results indicate that both stereoisomers of ketamine have discriminative stimulus properties similar to those of PCP. All three compounds produced PCP-appropriate responding in a dose-dependent fashion. The slopes of all of the dose-response curves were similar, also suggestive of qualitatively similar effects.

While the higher doses of all four compounds suppressed operant responding in a dose-dependent fashion, there was a greater difference between doses which produced drug-lever appropriate responding and doses which suppressed response rates for PCP (over 4-fold) than for any of the forms of ketamine. In contrast, using squirrel monkeys trained to discriminate PCP from saline we [1] recently found that for (±)-ketamine the ED_{50} dose for response rate suppression was approximately 3 times larger than the ED_{50} dose for drug-lever appropriate responding, comparable to the separation of effects seen with PCP in that study. Perhaps species differences play a role in this discrepancy.

Previous research is consistent in showing that (±)-ketamine is less potent than PCP in drug discrimination studies. The 4.4 fold potency difference seen in the present study is among the smallest reported in rats for this effect [5, 11, 12, 14]. In the squirrel monkey, we [1] found roughly a 9-fold potency difference between ketamine and PCP for producing PCP-lever responding.

There is some inconsistency in the literature concerning the relative potencies of racemic ketamine and the (+) and (−) salts. In both rats [7] and mice [13], (+)-ketamine HCl is 3 times more potent than (−)-ketamine HCl in producing analgesia and the racemic mixture falls approximately halfway between these two in potency. This is consistent with the finding by Smith *et al.* [16] that (+)-ketamine HCl is 3 to 4 times more potent than (−)-ketamine HCl in displacing ^{3}H-naloxone binding at the opiate receptor and the report of White *et al.* [20] indicating that (+)-ketamine HCl is approximately 3 times more potent than (−)-ketamine HCl as an anesthetic in humans. In contrast, other studies indicate that the stereoisomers and racemic mixture of ketamine are approximately equipotent in increasing non-specific locomotor activity in rodents [7, 10, 13] and in causing loss of righting reflex and lethality in rats [7]. Our data indicate no large molar potency difference between racemic ketamine and the (+) and (−) salts for production of PCP-appropriate responding or for suppression of operant responding. However, there was some separation in the potency of the stereoisomers for PCP-like effects with the (+)-isomer about twice as potent as the (−)-isomer. The (+)-isomer and the racemic mixture also appeared to have more specificity for PCP-like effects since about 2-fold higher doses were required to result in overall response-rate disruption than were needed to produce the PCP cue. The (−)-isomer was roughly equipotent for these effects.

Several investigations have indicated that low doses of racemic ketamine produced response rate increases in pigeons [18], mice [19] and rats [10] trained under a FI schedule of food presentation. Meliska *et al.* [9] recently reported increases in response rates to as high as 3 times control rates after the administration of (−)-ketamine HCl to rats trained under a FI schedule but only response rate decreases were seen after (+)-ketamine HCl administration. In the present study, low doses of all three compounds produced small response rate increases in individual animals, but when the data was averaged across animals no response rate increasing doses were found for any of the compounds. This possible discrepancy between this study and those reporting larger, more consistent response rate increases in the literature could be due to a difference in baseline rates of performance between these studies. The effects of (±)-ketamine on operant performance have been shown to be dependent on the baseline rate of the responding [18,19] such that low rate responding is increased and high rate responding in decreased. Response rates after vehicle administration averaged 1.32±0.16 responses per sec in this study. These relatively high rates may not be sensitive to rate-increasing effects.

In conclusion, (±)-ketamine, and the (+)- and (−)-isomers of ketamine produced dose-dependent drug-lever appropriate responding in rats trained to discriminate PCP from saline, indicating qualitatively similar stimulus properties of these compounds. While (+)-ketamine and (±)-ketamine were approximately 2-fold more potent than (−)-ketamine in producing PCP-appropriate responding, all three forms of ketamine were approximately equipotent for response rate suppression. While this may indicate some quantitative stereospecificity in the PCP-like effects of the isomers, the difference is not very dramatic. This isomeric pair would appear to be a poor candidate for studies of the stereospecificity of the discriminative stimulus effects of arylcycloalkylamines.

ACKNOWLEDGEMENTS

We wish to thank Dr. Maxwell Gordon of Bristol Laboratories for supplying the ketamine isomers. Drs. Everette L. May and William H. Soine provide helpful instruction on the stereochemistry of ketamine. Bonny T. Hopkins prepared the manuscript for publication.

REFERENCES

1. Brady, K. T. and R. L. Balster. Discriminative stimulus properties of phencyclidine and five analogues in the squirrel monkey. *Pharmac. Biochem. Behav.* **14:** 213–218, 1981.
2. Brady, K. T., R. L. Balster and E. L. May. Stereoisomers of N-allylnormetazocine: phencyclidine-like behavioral effects in squirrel monkeys and rats. *Science* **215:** 178–180, 1982.
3. Herling, S., E. H. Coale, Jr., D. W. Hein, G. Winger and J. H. Woods. Similarity of the discriminative stimulus effects of ketamine, cyclazocine and dextrorphan in the pigeon. *Psychopharmacology* **73:** 286–291, 1981.
4. Herling, S. and J. H. Woods. Discriminative stimulus effects of narcotics: Evidence for multiple receptor-mediated actions. *Life Sci.* **28:** 1571–1584, 1981.

5. Holtzman, S. G. Phencyclidine (PCP)-like discriminative effects of opioids in the rat. *J. Pharmac. exp. Ther.* **214:** 614–619, 1980.
6. Järbe, T. U. C., J. O. Johansson and B. G. Henriksson. Drug discrimination in rats: the effects of phencyclidine and ditran. *Psychopharmacologia* **42:** 33–39, 1975.
7. Marietta, M. P., W. L. Way, N. Castagnoli and A. J. Trevor. On the pharmacology of ketamine enantiomorphs in the rat. *J. Pharmac. exp. Ther.* **202:** 157–165, 1977.
8. McCarthy, D. A., G. Chen, D. H. Kaump and C. Ensor. General anesthetic and other pharmacological properties of 2-(0-chlorophenyl)-2-methylamino cyclohexanone HCl (CI-581). *J. New Drugs* **5:** 21–33, 1965.
9. Meliska, C. J., A. J. Greenberg and A. J. Trevor. The effects of ketamine enantiomers on schedule-controlled behavior in the rat. *J. Pharmac. exp. Ther.* **212:** 198–202, 1979.
10. Meliska, C. J. and A. J. Trevor. Differential effects of ketamine on schedule-controlled responding and motility. *Pharmac. Biochem. Behav.* **8:** 679–683, 1978.
11. Overton, D. A. A comparison of the discriminable effects of ketamine, phencyclidine and pentobarbital. *Archs int. Pharmacodyn. Thér.* **215:** 180–189, 1975.
12. Poling, A. D., F. J. White and J. B. Appel. Discriminative stimulus properties of phencyclidine. *Neuropharmacology* **18:** 459–463, 1979.
13. Ryder, S., W. L. Way and A. J. Trevor. Comparative pharmacology of the optical isomers of ketamine in mice. *Eur. J. Pharmac.* **49:** 15–23, 1978.
14. Shannon, H. E. Evaluation of phencyclidine analogs on the basis of their discriminative stimulus properties in the rat. *J. Pharmac. exp. Ther.* **216:** 543–551, 1981.
15. Smith, D. J., A. J. Azzaro, S. B. Zaldivar, S. Palmer and H. S. Lee. Properties of the optical isomers and metabolites of ketamine on the high affinity transport and catabolism of monoamines. *Neuropharmacology* **20:** 391–396, 1981.
16. Smith, D. J., G. M. PeKoe, L. L. Martin and B. Coalgate. The interaction of ketamine with the opiate receptor. *Life Sci.* **26:** 789–795, 1980.
17. Teal, J. J. and S. G. Holtzman. Stereoselectivity of the stimulus effects of morphine and cyclazocine in the squirrel monkey. *J. Pharmac. exp. Ther.* **215:** 369–376, 1980.
18. Wenger, G. R. The effect of phencyclidine and ketamine on schedule-controlled behavior in the pigeon. *J. Pharmac. exp. Ther.* **196:** 172–179, 1976.
19. Wenger, G. R. and P. B. Dews. The effects of phencyclidine, ketamine, *d*-amphetamine and pentobarbital on schedule-controlled behavior in the mouse. *J. Pharmac. exp. Ther.* **196:** 616–624, 1976.
20. White, P. F., J. Ham, W. L. Way and A. J. Trevor. Pharmacology of ketamine isomers in surgical patients. *Anesthesiology* **52:** 231–239, 1980.
21. Young, A. M., S. Herling, G. D. Wenger and J. H. Woods. Comparison of discriminative and reinforcing effects of ketamine and related compounds in the rheses monkey. In: *Problems of Drug Dependence, 1980*, NIDA Research Monograph 34, edited by L. S. Harris. Washington, DC: U. S. Government Printing Office, 1981, pp. 173–179.