Pharmacology Biochemistry & Behavior, Vol. 30, pp. 613–616. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

A Comparison of the Discriminative Stimulus Properties of *l*-5-Hydroxytryptophan in the Presence of Either Citalopram or Ro 4-4602

J. C. WINTER[1] AND R. A. RABIN

Department of Pharmacology and Therapeutics, School of Medicine
State University of New York at Buffalo, Buffalo, NY 14214

Received 5 October 1987

WINTER, J. C. AND R. A. RABIN. *A comparison of the discriminative stimulus properties of l-5-hydroxytryptophan in the presence of either citalopram or Ro 4-4602.* PHARMACOL BIOCHEM BEHAV **30**(3) 613–616, 1988.—The establishment of stimulus control by 5-HTP, the amino acid precursor for serotonin (5-HT), has been reported previously [1–3]. In the present investigation, two groups of rats were trained with 5-HTP versus saline in a 2-lever discrimination procedure. Prior to the administration of 5-HTP, subjects were pretreated with either Ro 4-4602, an inhibitor of peripheral decarboxylase (R-HTP), or citalopram, a specific 5-HT reuptake inhibitor (C-HTP). Neither C-HTP nor R-HTP was antagonized completely by either pirenperone or pizotyline. When C-HTP and R-HTP were tested in a third group of rats trained with LSD, complete generalization was not observed. The results of cross tests in the R-HTP and C-HTP groups with LSD, TFMPP, 8-OH-DPAT, C-HTP, and R-HTP indicate that the stimuli induced by R-HTP and C-HTP are similar but not identical. Taken together, these data suggest that 5-HTP produces a compound stimulus that is not readily explained in terms of either 5-HT_1 or 5-HT_2 receptors alone.

Stimulus control LSD 5-HTP Citalopram Ro 4-4602 TFMPP 8-OH-DPAT

IN 1982, Barrett and his colleagues [1] reported the successful training of L-5-HTP, the amino acid precursor of 5-hydroxytryptamine (serotonin, 5-HT), as a discriminative stimulus in rats. They avoided in large measure the peripheral effects of the drug by the use of a peripherally acting decarboxylase inhibitor, Ro 4-4902. In their work, 5-HTP generalized to fenfluramine and was potentiated by fluoxitine. The 5-HTP cue was not blocked by methysergide, cyproheptadine, metergoline, or methiothepin but a later study by the same group [3] found pizotyline (BC-105) to be effective. The latter report postulates a unique 5-HT receptor subserving the 5-HTP cue. The ability of 5-HTP in the presence of Ro 4-4602 to function as a discriminative stimulus was confirmed by Cunningham and her associates [2]. However, Cunningham *et al.* did not observe antagonism of the 5-HTP cue by pizotyline as had been reported earlier [3]. In addition, results of tests of generalization with LSD and with quipazine were not in agreement. Friedman *et al.* (unpublished results cited in [4]) observed no generalization while complete generalization was reported by Cunningham *et al.* [2].

In the present investigation, rats were trained to discriminate the effects of 5-HTP versus saline as described by the previous workers. In addition, a second group was trained with 5-HTP in the presence of citalopram, a specific inhibitor of 5-HT uptake (Hytell [5]). In both 5-HTP-trained groups, tests of generalization were conducted with LSD and with the 5-HT_1-selective agonists, TFMPP and 8-OH-DPAT. The ability of LSD to generalize to 5-HTP was tested in a third group of rats trained with the former drug versus saline.

METHOD

Animals

Male Fischer 344 rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. They were housed in pairs under a natural light-dark cycle and allowed free access to water in the home cage. Subjects were main-

[1]Request for reprints should be addressed to J. C. Winter, 127 Farber Hall, SUNY, Buffalo, NY 14214.

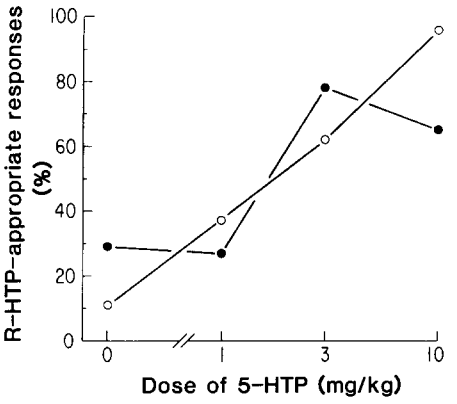

FIG. 1. The effects of 5-HTP/Ro 4-4602 alone (open circles) and in the presence of pizotyline (closed circles) in eight rats trained with 5-HTP/Ro 4-4602 as a discriminative stimulus. Ro 4-4602 (20 mg/kg), pizotyline (10 mg/kg), and 5-HTP (training dose=10 mg/kg) were injected IP 90, 60, and 30 min, respectively, before testing. Each point represents the mean of one determination in each subject. *Ordinate:* Mean percentage of responses on the 5-HTP/Ro 4-4602-appropriate lever. *Abscissa:* Dose plotted on a log scale.

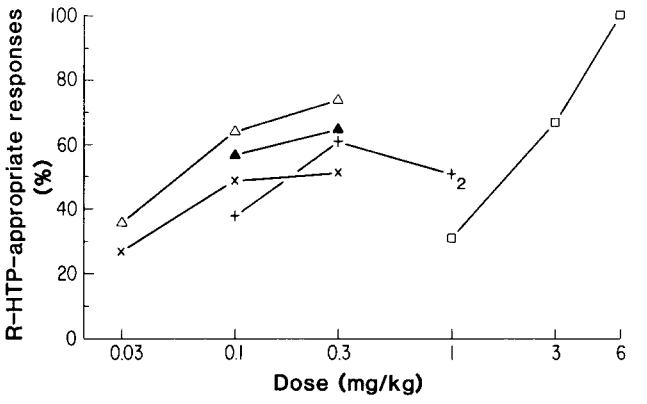

FIG. 2. Tests of generalization in eight rats trained with 5-HTP/Ro 4-4602 as a discriminative stimulus. Open triangles: LSD; closed triangles: LSD in the presence of pirenperone (0.16 mg/kg; 60 min before testing); ×: 8-OH-DPAT; +: TFMPP; □: 5-HTP/citalopram (1 mg/kg; 45 min before testing). LSD, 8-OH-DPAT, and TFMPP were injected IP 15 min before testing. A number adjacent to a point indicates the number of animals that completed the session. All other details are as in Fig. 1.

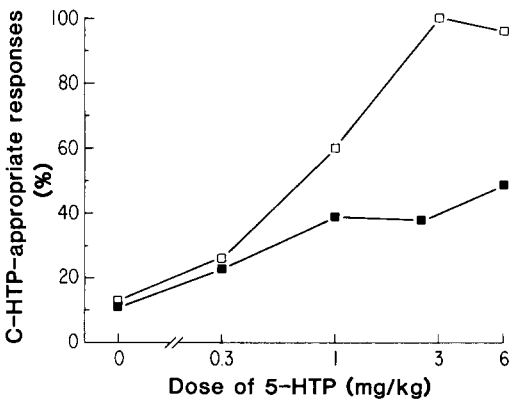

FIG. 3. The effects of 5-HTP/citalopram alone (open squares) and in the presence of pizotyline (closed squares) in eight rats trained with 5-HTP/citalopram as a discriminative stimulus. Pizotyline (10 mg/kg), citalopram (1 mg/kg), and 5-HTP (training dose=6 mg/kg) were injected IP 60, 45, and 30 min, respectively, before testing. Each point represents the mean of one determination in each subject. *Ordinate:* Mean percentage of responses on the 5-HTP/citalopram-appropriate lever. *Abscissa:* Dose plotted on a log scale.

tained at 75–80% of their expected free-feeding weight by limiting access to food for 2 hours per day.

Apparatus

Three small animal test chambers (Coulbourn Instruments model E10-10) housed in larger light-proof sound-insulated boxes were used for all experiments. Each box had a house light and exhaust fan. The chamber contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Procedure

After learning to drink from the dipper, subjects were trained to depress first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from one to ten and all subsequent training and testing employed a fixed ratio (FR10) schedule of reinforcement. Discrimination training was then begun. Two groups of ten rats each were trained with 5-HTP versus saline using a 30 min pretreatment time. In the first group, designated R-HTP, Ro 4-4602 was administered 60 min before 5-HTP, i.e., 90 min before training. In the second group, designated C-HTP, citalopram was injected 30 min before 5-HTP. A third group of rats (N=10) was trained with 0.1 mg/kg of LSD versus saline using a 15-min pretreatment time.

Following the administration of drugs, every tenth response on the drug-appropriate lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced following the injection of saline. For half of the subjects, the left lever was designated as the drug-appropriate lever. During discrimination training, drug and saline were alternated on a daily basis. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to delivery of the first reinforcer were on the appropriate lever.

After 5-HTP-induced stimulus control was well established in Groups I and II, cross tests (tests of generalization) were conducted with a range of doses of 5-HTP in the presence of either Ro 4-4602 or citalopram. In this way a dose-response relationship was obtained for each drug. The same range of doses was then examined in the presence of pizotyline. Additional tests were conducted with LSD, 8-OH-DPAT, and TFMPP. Cross tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion of 83%

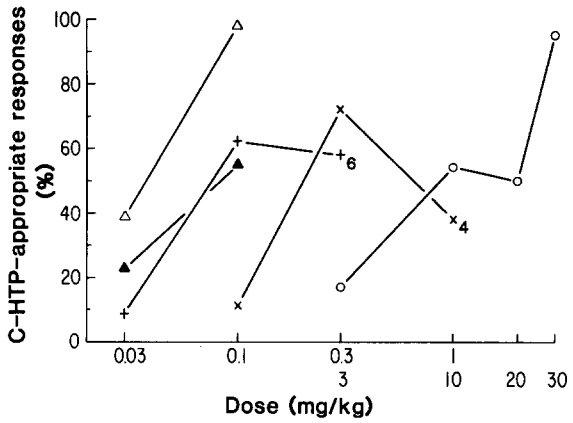

FIG. 4. Tests of generalization in eight rats trained with 5-HTP/citalopram as a discriminative stimulus. Open triangles: LSD; closed triangles: LSD in the presence of pirenperone (0.16 mg/kg; 60 min before testing); ×: 8-OH-DPAT; +: TFMPP; ○: 5-HTP/Ro 4-4602. LSD, 8-OH-DPAT, and TFMPP were injected IP 15 min before testing. A number adjacent to a point indicates the number of animals that completed the session. All other details are as in Fig. 2.

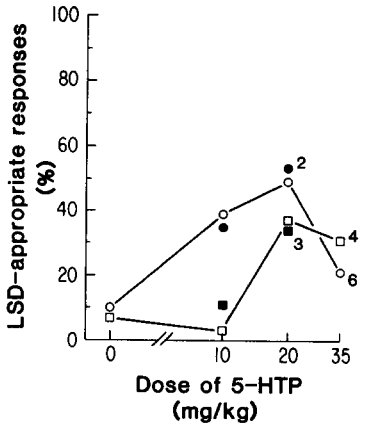

FIG. 5. Tests of generalization in ten rats trained with LSD (0.1 mg/kg) as a discriminative stimulus. Open circles: 5-HTP/Ro 4-4602; open squares: 5-HTP/citalopram. Closed symbols indicate pretreatment with pizotyline (10 mg/kg; 60 min before testing). Each point represents the mean of one determination in each subject. A number adjacent to a point indicates the number of animals that completed the session. *Ordinate:* Mean percentage of responses on the LSD lever. *Abscissa:* Dose plotted on a log scale.

correct responding. In general, tests were equally divided between Thursday and Friday sessions. During cross tests, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total reponses emitted on the drug-appropriate lever. Agonists (LSD, 8-OH-DPAT, and TFMPP) and antagonists (pizotyline and pirenperone) were administered 15 minutes and 60 minutes, respectively, before testing. All comparisons of data were by means of individual applications of Wilcoxon's signed ranks test. Differences were considered to be significant if they would be expected to arise by random sampling alone with a probability <0.05.

Drugs

(+)-Lysergic acid diethylamide (+)−tartrate (LSD) was provided by the National Institute on Drug Abuse, Rockville, MD. L-5-HTP and *m*-trifluoromethylphenylpiperazine (TFMPP) were purchased from Aldrich Chemical Co., Milwaukee, WI. Racemic 8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) was purchased from Research Biochemicals Inc., Wayland, MA. Pizotyline maleate (BC-105, pizotifen) and pirenperone (R 47 465) were gifts from Sandoz Pharmaceuticals, East Hanover, NJ, and Janssen Pharmaceutica Research Laboratories, Beerse, Belgium, respectively. Citalopram (Lu 10-171 HBr) and Ro 4-4602/1 (DL-serine-2-(2,3,4,-trihydroxybenzyl)hydrazide HCl) were generously provided by H. Lunbeck & Co. A/S, Copenhagen, Denmark, and Hoffmann-LaRoche Inc., Nutley, NJ, respectively. All drugs were dissolved in saline and injected IP in a constant volume of 1 ml/kg of body weight.

RESULTS

Figures 1 and 2 show the results of tests of generalization in rats trained with 5-HTP/Ro 4-4602. In Fig. 1 we see the dose-response relationship and the effects of pizotyline upon it. No statistically significant effects were produced by pizotyline at doses of 1 and 3 mg/kg of 5-HTP but antagonism was significant at the training dose ($p<0.05$). In Fig. 2, it is seen that of the drugs tested only 5-HTP/citalopram was followed by complete generalization. LSD, 8-OH-DPAT, and TFMPP all yielded intermediate results. In tests of LSD following pretreatment with pirenperone, no significant antagonism was observed.

The results of tests of generalization in subjects trained with 5-HTP/citalopram are shown in Figs. 3 and 4. An orderly dose-response relationship is seen in Fig. 3. When subjects were pretreated with pizotyline, significant antagonism ($p<0.05$) was observed at doses of 5-HTP of 3 and 6 mg/kg. The results of tests of generalization shown in Fig. 4 indicate that only LSD and 5-HTP/Ro 4-4602 substitute completely. Results of tests with 8-OH-DPAT and TFMPP were intermediate in nature. The dose of LSD which substituted completely for 5-HTP/citalopram was significantly but incompletely antagonized by pirenperone.

When subjects trained with LSD as a discriminative stimulus were tested with either 5-HTP/Ro 4-4602 or 5-HTP/citalopram, complete generalization was not observed (Fig. 5). This was true despite the fact that doses of 5-HTP were employed which established stimulus control when trained versus saline. Pretreatment with pizotyline produced no significant antagonism of either 5-HTP/Ro 4-4602 or 5-HTP/citalopram. The combination did however result in more disruption of behavior as indicated by a smaller number of subjects completing the sessions.

DISCUSSION

The observation that R-HTP establishes stimulus control (Fig. 1) confirms the reports of earlier workers [1–3]. However, it should be noted that we were unable to maintain responding at the doses previously employed. Whereas

others have used 40 to 50 mg/kg Ro 4-4602 together with 30 to 50 mg 5-HTP, our maximum combination compatible with reliable responding was 20 mg Ro 4-4602 and 10 mg 5-HTP. The observation that pizotyline antagonizes R-HTP only at the training dose and then only to a limited extent holds a middle ground between Friedman *et al.* [3],who observed antagonism, and Cunningham *et al.* [2] who did not. The latter group rejected as unlikely the possibility that differences in routes of administration explained their differences. We agree; the dose of pizotyline (10 mg/kg) given IP as indicated in Fig. 1 completely antagonizes LSD and DOM in rats trained with 0.1 mg/kg LSD versus saline (Winter and Rabin [7]). However, our observation that a statistically significant antagonism did occur at the training dose of R-HTP indicates that a modest degree of antagonism may be expected with various combinations R-HTP and pizotyline.

The results of cross tests with LSD, 8-OH-DPAT, and TFMPP (Fig. 2) suggest that the R-HTP cue is not a simple matter of agonistic activity at 5-HT_1 or 5-HT_2 receptors. Indeed, the failure of pirenperone to antagonize the intermediate results produced by LSD argue against a significant role for 5-HT_2 receptors. Once again our results occupy a middle ground between the report that LSD does not substitute for R-HTP (unpublished results cited in [4]) and that LSD substitutes completely and is blocked completely by ketanserin [2]. The complete substitution in R-HTP-trained rats by C-HTP indicates a functionally significant overlap between the effects of these two treatments.

Stimulus control by combination of citalopram and 5-HTP (C-HTP) has previously been reported. The data of Fig. 3 indicate an orderly dose-effect relationship and a significant but incomplete antagonism by pizotyline. In Fig. 4 it is seen that TFMPP and 8-OH-DPAT produce intermediate results while LSD and R-HTP substitute completely.

Despite the fact that C-HTP substitutes completely for R-HTP (Fig. 2) and vice versa (Fig. 4) and the fact that TFMPP and 8-OH-DPAT yield similar results, it seems likely that R-HTP and C-HTP do not induce an identical stimulus complex. Visual inspection and comparison of Figs. 1 and 3 indicate that differences exist between the interactions of pizotyline with R-HTP (Fig. 1) and C-HTP (Fig. 3) with the latter training condition being much more reliably antagonized. Furthermore, comparison of Figs. 2 and 4 reveals that LSD substitutes more completely and is antagonized to a greater extent by pirenperone in subjects trained with C-HTP. However, even in the C-HTP group, antagonism was incomplete (Fig. 4).

We may rationalize the disparate results obtained with R-HTP and C-HTP (Figs. 1–4) by hypothesizing a more prominent 5-HT_2 component for the C-HTP stimulus complex. Thus, R-HTP would be antagonized by the 5-HT_2-selective antagonist, pizotyline, to a lesser extent than is C-HTP (Figs. 1 and 3). Likewise, LSD, whose stimulus properties are predominantly mediated by 5-HT_2 receptors, would substitute to a greater degree and would be antagonized to a greater degree by the 5-HT_2-selective antagonist, pirenperone, in subjects trained with C-HTP (Figs. 2 and 4). However, this hypothesis is not supported by the results of tests of generalization of LSD to R-HTP and C-HTP (Fig. 5) in that C-HTP did not substitute for LSD to a greater degree than did R-HTP.

The observation that the maximum degree of substitution of R-HTP in LSD-trained subjects was about 50% (Fig. 5) is in complete agreement with the results of Cunningham *et al.* [2]. Similar results with R-HTP and the failure of either R-HTP or C-HTP to be antagonized by pizotyline in the LSD-trained subjects again argues against a prominent role for a 5-HT_2-mediated component.

Although the use of the stimulus properties of 5-HTP as a reference point for the evaluation of a wide variety of serotonergically-active drugs is intuitively attractive, there are now a number of reasons to question this approach. On a biochemical level, there are significant uncertainties regarding the specificity with which peripherally administered 5-HTP increases 5-HT levels at physiologically meaningful sites (a recent review is provided in [6]). It is perhaps a reflection of these complexities that the two previous groups which examined the stimulus properties of 5-HTP are in substantial disagreement [1–4] and that the present results are in complete agreement with neither. While the study of the stimulus complex produced by 5-HTP is certainly worthy in its own right, we conclude that it does not provide a suitable reference standard at this time.

ACKNOWLEDGEMENTS

We thank Mrs. Deborah T. Petti for technical assistance. The present investigation was supported in part by grant No. 03385 from the National Institute on Drug Abuse.

REFERENCES

1. Barrett, R. J.; Blackshear, M. A.; Sanders-Bush, E. Discriminative stimulus properties of L-5-hydroxytryptophan: Behavioral evidence for multiple serotonin receptors. Psychopharmacology (Berlin) 76:29–35; 1982.
2. Cunningham, K. A.; Callahan, P. M.; Appel, J. B. Differentiation between the stimulus effects of 1-5-hydroxytryptophan and LSD. Eur. J. Pharmacol. 108:179–186; 1985.
3. Friedman, R.; Barrett, R. J.; Sanders-Bush, E. Additional evidence that l-5-hydroxytryptophan discrimination models a unique serotonin receptor. Psychopharmacology (Berlin) 80:209–213; 1983.
4. Friedman, R. L.; Barrett, R. J.; Sanders-Bush, E. Discriminative stimulus properties of quipazine: Mediation by serotonin$_2$ binding sites. J. Pharmacol. Exp. Ther. 228:628–635; 1984.
5. Hyttel, J. Effect of a specific 5-HT uptake inhibitor, citalopram (LU 10-171), on ^{3}H-5-HT uptake in rat brain synaptosomes in vitro. Psychopharmacology (Berlin) 83:20–27; 1978.
6. Kuhn, D. M.; Wolf, W. A.; Youdim, M. B. H. Serotonin neurochemistry revisited: A new look at some old axioms. Neurochem. Int. 8:141–154; 1986.
7. Winter, J. C.; Rabin, R. A. Interactions between serotonergic agonists and antagonists in rats trained with LSD as a discriminative stimulus. Pharmacol. Biochem. Behav. 30:in press; 1988.