Pharmacology Biochemistry & Behavior, Vol. 9, pp. 249–253. Printed in the U.S.A.

Role of Peripheral Mechanisms in the Behavioral Effects of 5-Hydroxytryptophan[1,2]

RICHARD B. CARTER, LINDA A. DYKSTRA, J. DAVID LEANDER

Departments of Psychology and Pharmacology, University of North Carolina Chapel Hill, NC 27514

AND

JAMES B. APPEL

Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina Columbia, SC 29208

(Received 22 February 1978)

CARTER, R. B., L. A. DYKSTRA, J. D. LEANDER AND J. B. APPEL. *Role of peripheral mechanisms in the behavioral effects of 5-hydroxytryptophan.* PHARMAC. BIOCHEM. BEHAV. 9(2) 249–253, 1978.—The effects of 5-hydroxytryptophan (5-HTP) were studied in rats trained to press a lever under a fixed-ratio (FR-32) schedule of water presentation. *d*-, *l*- and *d,l*-5-HTP all decreased responding in a dose-related manner. The levo isomer (12.5–25 mg/kg) was twice as potent as the racemic form (25–50 mg/kg) in this respect. Moderate doses of *d*-5-HTP (<100 mg/kg) did not affect responding, whereas 200 mg/kg produced almost complete suppression. The response decrement produced by 25 mg/kg *l*-5-HTP was completely antagonized by pretreatment with either 50 mg/kg or 400 mg/kg of the decarboxylase inhibitor, benserazide (Ro4-4602). The specific peripheral decarboxylase inhibitor, carbidopa (MK-486) (50 mg/kg) and the peripheral serotonergic antagonist, xylamidine tosylate (1 mg/kg) also antagonized the effects of 25 mg/kg *l*-5-HTP. These results suggest that at least some of the behavioral effects of 5-HTP are due to increases in levels or turnover of 5-HTP in peripheral serotonergic neuronal systems.

5-HTP Serotonin (5-HT) Benserazide (Ro4-4602) Carbidopa (MK-486) Xylamidine tosylate Decarboxylase inhibitors Peripheral serotonergic antagonists Schedule-controlled responding

RESEARCHERS investigating behavioral changes resulting from alterations in the functional activity of the neurotransmitter, serotonin (5-HT), have often utilized its immediate amino acid precursor, *d,l*-5-hydroxytryptophan (*d,l*-5-HTP) to increase the concentration of 5-HT in brain. Aprison and Ferster were the first to observe a quantitative relationship between the dose of *d,l*-5-HTP and the extent of disruption of responding maintained under a multiple FR 50, FI 10 min schedule of food presentation in pigeons [1,2]. In subsequent studies, this disruption was found to be correlated most closely with changes in 5-HT concentrations in the brain, more specifically the telencephalon and diencephalon [5], and was not correlated with changes in norepinephrine or dopamine [3]. Similar results were obtained in studies with rats [4]. Thus, it was hypothesized that the behavioral effects of *d,l*-5-HTP were the result of changes in concentrations of 5-HT in central neuronal systems [1–6]. Furthermore, it was assumed that since only the *l* isomer of *d,l*-5-HTP readily crosses the blood-brain barrier, it alone was responsible for the behavioral changes observed and that the *d* isomer, isolated outside the blood-brain barrier, was not behaviorally active [5].

Recent data, however, have suggested that serotonergic mechanisms which lie outside the blood-brain barrier (hereafter referred to as peripheral systems) may play an important role in the mediation of the behavioral effects of *d,l*-5-HTP. It has been found that xylamidine tosylate, a peripheral serotonergic antagonist [9], partially blocks the disruption in responding produced by *d,l*-5-HTP in rats under a FR 20 schedule of milk presentation, whereas cinanserin, a potent serotonergic antagonist both centrally and peripherally, almost entirely blocks such effects [16]. Similarly, it has been shown that *d,l*-5-HTP-induced disruption of responding is antagonized when rats are pretreated with both low and high doses of the decarboxylase inhibitor, benserazide (Ro4-4602) [8]. Low doses (e.g. 50 mg/kg) of benserazide inhibit peripheral but not central *l*-aromatic amino decarboxylase, whereas high doses (e.g. 400 mg/kg) inhibit both central and peripheral decarboxylase [7,13].

These findings suggest that peripheral mechanisms may be involved in the behavioral effects of *d,l*-5-HTP. For example, the *d* isomer might be behaviorally active or the *l* isomer might be exerting effects in the periphery as well as in central neuronal systems. If peripheral mechanisms are in-

[1]A preliminary account of this work was presented at the 62nd Annual Meeting of the Federation of American Societies for Experimental Biology held in Atlantic City, NJ, April 9–14, 1978.

[2]Send reprint requests to: Richard B. Carter, Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC 27514.

volved in the behavioral effects of *d,l*-5-HTP, then they should be blocked by a peripherally acting decarboxylase inhibitor and/or a peripheral serotonergic antagonist.

The present experiments were performed to determine whether the behavioral effects of *l*-5-HTP could be antagonized peripherally. Responding in rats was maintained under a FR 32 schedule of water presentation and the effects of *d*-, *l*- and *d,l*-5-HTP were compared. Then, the ability to antagonize the disruption in responding produced by *l*-5-HTP was examined with two doses of the central and peripheral decarboxylase inhibitor, benserazide, the peripheral decarboxylase inhibitor, carbidopa (MK-486) [14], and the peripheral serotonergic antagonist xylamidine tosylate [9].

METHOD

Animals

Seven experimentally naive male rats weighing between 290–300 g, were obtained from ARS Sprague-Dawley, Madison, WI. They were housed in a room of constant temperature (75°F) with a 12 hr (600–1800 hr) day-night cycle. Food was freely available but water was given only in the experimental situation and for approximately 40 hr on weekends. Experiments were conducted at approximately the same time every day, usually 5 days per week.

Apparatus

A commercially available experimental chamber (BRS/LVE, Model No. 143-24) contained in a sound and light attenuating, ventilated enclosure was used for training and testing. The chamber contained one lever on the left side of the front panel with a liquid dipper in the center of that panel. The liquid dipper was electrically programmed to deliver 0.01 ml of tap water. Sessions were 30 min in duration and were conducted in the presence of a house light. Electromechanical programming and recording equipment were located in an adjacent room.

Procedure

After a week of adaptation to their home cages, all animals were deprived of water for a period of 36 hr. They were then placed into the experimental chamber and a shaping procedure was instituted to establish the lever-pressing response. On succeeding days, the schedule of water presentation was gradually raised from a fixed-ratio of 1 to a fixed-ratio of 32 (FR 32), under which the rat was required to make 32 responses in order to obtain water. When a stable rate of bar-pressing was obtained ($< \pm 5\%$ change in rate), daily intraperitoneal (IP) injections of 1.0 ml/kg of isotonic saline solution (NaCl) were begun.

Pharmacological Procedure

Doses of *d*-, *l*- and *d,l*-5-HTP were administered in random order. Each dose of each drug was given to at least three rats. No rat received the same dosage more than once. Drugs were generally given on Thursdays or Fridays with the previous 2 days serving as NaCl injection control days. Following the completion of the *d*-, *l*- and *d,l*-5-HTP dose-effect curves, the effects of pretreatment with benserazide, xylamidine tosylate or carbidopa when combined with 25 mg/kg of *l*-5-HTP were determined. Doses of these drugs were also given in a random order. Each dose of each drug was given to at least three rats. No rat received the same dosage more than once.

Control and drug injections were administered IP. Injections of 5-HTP were given 15 min prior to the start of the experimental session. Pretreatments with benserazide, xylamidine tosylate and appropriate vehicle controls were administered 4 hr prior to the start of the experimental sessions. Pretreatments with carbidopa and appropriate vehicle controls were administered 1 hr prior to the beginning of the session.

Benserazide (Ro4-4602) was dissolved to concentrations such that injections were administered in a volume of 2 ml/kg in 0.9% NaCl solution acidified in 0.001 N HCl. Carbidopa (MK-486) (50 mg/kg) was administered as a volume of 2 ml/kg in distilled-deionized water adjusted to a pH of 2.0 with dilute HCl and NaOH. Xylamidine tosylate (N-(2-*m*-methoxyphenoxypropyl)-*m*-methylphenylacetamidine *p*-toluenesulfonate monohydrate) (1 mg/kg) was dissolved to a concentration of 1 mg/ml in 0.9% NaCl solution. *d*-, *l*-, and *d,l*-5-Hydroxytryptophan were dissolved in distilled deionized water adjusted to a pH of 2.0 with dilute HCl and NaOH. All doses of 5-HTP were administered at volumes of 1.0 ml/kg body wt. except for the 100.0 and 200.0 mg/kg doses of *d*-5-HTP which were administered at volumes of 2.0 ml/kg of body wt.

Benserazide, carbidopa, and xylamidine tosylate were gifts of Hoffmann-LaRoche Co., Nutley, N.J.; Merck, Sharp and Dohme Co., West Point, PA; and the Wellcome Research Laboratories, Beckenham, Kent, England, respectively. The *d*-, *l*- and *d,l*-5-HTP were purchased from Sigma Chemical Co., St. Louis, MO.

Data Analysis

Total number of responses for each session was recorded on digital counters. The data from the 2 days immediately preceding a drug day (NaCl) were used as controls. The total number of responses on test days was divided by the average number of responses during the 2 control days and multiplied by 100. This percent of control responses was determined for each rat and a group mean percent control responses was determined. Tests for contrast [15] (MANOVA) were used to determine the significance of the data.

RESULTS

Figure 1 shows dose-response curves for the effects of *d*-, *l*-, and *d,l*-5-HTP on responding maintained by a FR 32 schedule of water presentation. All compounds decreased the total number of responses per session in a dose-related fashion. The *l* isomer was twice as potent as the racemic form in its ability to disrupt bar-pressing. No effect was seen at moderate doses (25–50 mg/kg) of *d*-5-HTP. The 100 mg/kg dose produced a marked behavioral disruption, whereas the 200 mg/kg dose almost completely suppressed responding. Animals which received 200 mg/kg of *d*-5-HTP exhibited piloerection, tremors and ataxia. One of these animals subsequently died.

The pattern of disruption following the high doses of *l*-and *d,l*-5-HTP generally consisted of a single period of nonresponding followed by almost complete recovery within several fixed-ratio performances. At lower doses decreases in responding were due largely to decreases in overall response rates. Following the 200 mg/kg dose of *d*-5-HTP, re-

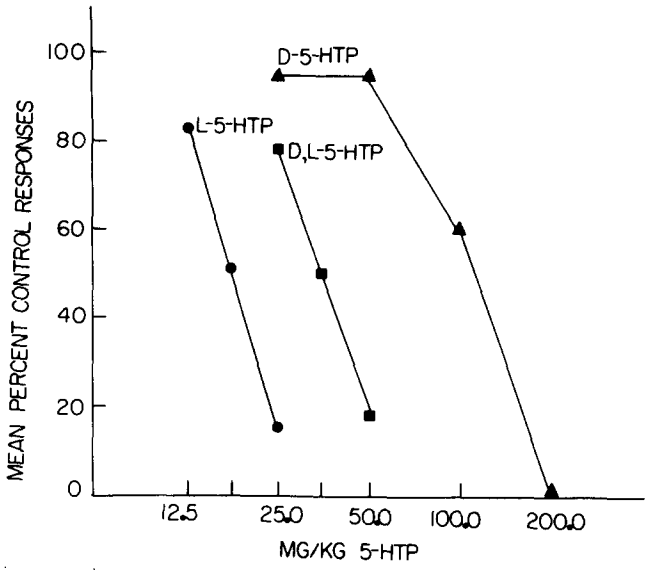

FIG. 1. Dose effect of *d*- (▲, *l*- (●) and *d,l*-5-HTP (■) on mean percent control responding under a FR 32 schedule. Abscissa: dose of 5-HTP, log scale. Each point is the mean of at least three animals. The standard error never exceeded 14% for any point. Standard errors have been omitted for clarity. Absolute rates of responding for all animals under control conditions in all experiments ranged from 1.21–2.94 responses/second with a mean of 1.97 ± 0.32 responses/second.

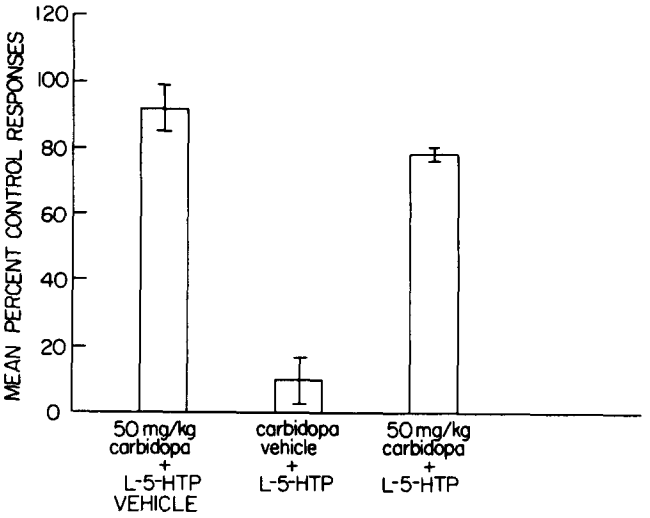

FIG. 3. Effects of pretreatment with carbidopa on *l*-5-HTP (25 mg/kg) induced decrement of responding under a FR 32 schedule. Abscissa: pretreatment conditions. Each bar represents the mean ± SEM of three animals.

sponding consisted of a few responses distributed throughout the session.

Figure 2 shows the effects of pretreatment with benserazide upon response suppression by 5-HTP. Responding was not altered (97% of control) in animals receiving 400 mg/kg of benserazide. On the other hand, responding was decreased markedly (15% of control) in animals receiving 25 mg/kg of *l*-5-HTP plus vehicle for benserazide. Pretreatment with either a small (50 mg/kg) or a large (400 mg/kg) dose of benserazide completely blocked the rate-decreasing effect of 25 mg/kg of *l*-5-HTP (93 and 95% of control, respectively).

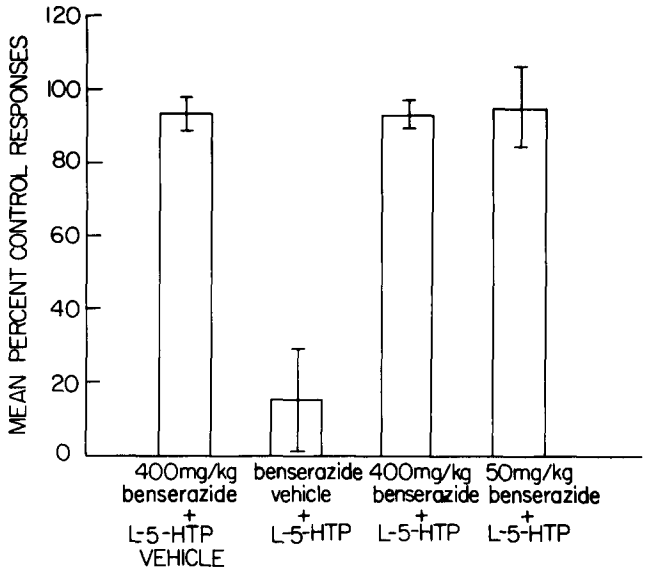

FIG. 2. Effects of pretreatment with benserazide on *l*-5-HTP (25 mg/kg) induced decrement of responding under a FR 32 schedule. Abscissa: pretreatment and treatment conditions. Each bar represents the mean ± SEM of three animals.

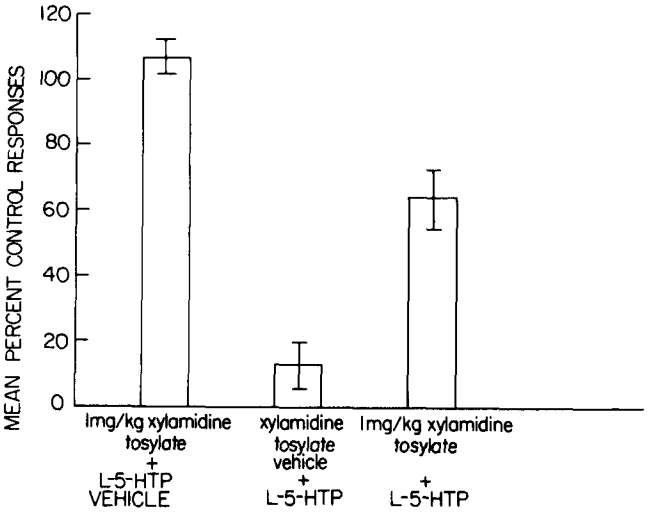

FIG. 4. Effects of pretreatment with xylamidine tosylate on *l*-5-HTP (25 mg/kg) induced decrement of responding under a FR 32 schedule. Abscissa: pretreatment and treatment conditions. Each bar represents the mean ± SEM of three animals.

Tests for contrast showed that there were no significant differences between animals that received 400 mg/kg of benserazide plus 5-HTP vehicle, 50 mg/kg of benserazide plus 25 mg/kg of *l*-5-HTP or 400 mg/kg of benserazide plus 25 mg/kg of *l*-5-HTP; however, differences between these animals and those which received benserazide vehicle plus 25 mg/kg *l*-5-HTP ($p<0.001$) were significant.

The effects of pretreatment with 50 mg/kg of carbidopa are shown in Fig. 3. Responding was only slightly decreased in animals that received 50 mg/kg of carbidopa plus 5-HTP vehicle (91% of control), whereas responding was decreased markedly (9% of control) in animals which received the vehicle for carbidopa plus 25 mg/kg of *l*-5-HTP. Animals that received 50 mg/kg of carbidopa in combination with 25 mg/kg

of *l*-5-HTP showed some (76% of control) decrease in responding, but it was not statistically different from the animals which received the carbidopa plus 5-HTP vehicle. The responding of both of these groups were significantly different from those animals which received the vehicle for carbidopa plus 25 mg/kg of *l*-5-HTP ($p<0.001$).

The effects of xylamidine tosylate pretreatment are shown in Fig. 4. Responding was increased slightly in animals given 1 mg/kg of xylamidine tosylate plus 5-HTP vehicle (107% of control), whereas responding was decreased markedly (12% of control) in animals who received xylamidine tosylate vehicle plus 25 mg/kg of *l*-5-HTP. A dose of 1 mg/kg of xylamidine tosylate partially blocked the decreases in responding produced by *l*-5-HTP (63% of control). Animals which received xylamidine tosylate vehicle plus 25 mg/kg of *l*-5-HTP had significantly decreased responding compared to those animals which received 1 mg/kg of xylamidine tosylate plus 5-HTP vehicle ($p<0.001$) and to those which received 1 mg/kg of xylamidine tosylate plus 25 mg/kg of *l*-5-HTP ($p<0.01$). The responding of animals which received 1 mg/kg of xylamidine tosylate plus 25 mg/kg of *l*-5-HTP were decreased significantly compared to the responding of those animals that received 1 mg/kg of xylamidine tosylate plus 5-HTP vehicle ($p<0.01$).

DISCUSSION

The results show that *d*-, *l*- and *d,l*-5-HTP decrease responding maintained under an FR schedule of water presentation in the rat in a dose-related manner. The *l* isomer is approximately twice as potent as the racemic form in this respect. Doses of *d*-5-HTP up to 50 mg/kg (twice the amount of the *d* isomer contained in a 50 mg/kg dose of *d,l*-5-HTP) do not produce decreases in responding. Decreases in responding following administration of *d,l*-5-HTP, therefore, are completely explicable in terms of the *l* isomer. This finding is in basic agreement with the data of Penn, *et al.* [12]. High doses of the *d* isomer do produce dose-related decreases in responding although the manner in which such an effect occurs is not at all clear. It has been demonstrated that at moderate doses (25 mg/kg), *d*-5-HTP does not cross the blood-brain barrier or lead to increases in 5-HT in the CNS to any appreciable degree [12]. It is not known whether *d*-5-HTP leads to increases in 5-HT outside the CNS. It appears, however, that the behavioral disruption observed at 200 mg/kg of *d*-5-HTP might be due to nonspecific toxic effects, since this dose of *d*-5-HTP produced piloerection, tremors, ataxia and death which did not occur after any dose of *l*-5-HTP. Further studies of the biochemical effects of *d*-5-HTP would be necessary in order to hypothesize a mechanism for its behavioral effects.

Antagonism of the *l*-5-HTP induced decreases in responding by both high and low doses of the decarboxylase inhibitor, benserazide agrees with data we previously reported for *d,l*-5-HTP [8]. Taken together, these data suggest that the decreases in responding produced by both *l*-5-HTP and *d,l*-5-HTP are a function of increased levels or turnover of 5-HT or metabolites in the periphery. This viewpoint is strengthened by the fact that the peripheral decarboxylase inhibitor, carbidopa, and the peripheral serotonergic antagonist, xylamidine tosylate also antagonize the decreases in responding produced by *l*-5-HTP. Neither carbidopa nor xylamidine tosylate produced complete antagonism as did benserazide, but lack of complete dose-effect curves preclude any statement as to the relative efficacy of these drugs or to the relative involvement of 5-HT neuronal systems. For example, catecholaminergic neuronal systems have been implicated in the behavioral effects of 5-HTP [10,17]. 5-HTP may be taken up and decarboxylated in dopamine and norepinephrine containing neurons producing effects even at moderate (behaviorally active) doses [17]. If this is the case, a decarboxylase inhibitor would be expected to produce a more complete blockade than a serotonergic antagonist.

Central and peripheral mechanisms have been implicated in a variety of behavioral effects produced by 5-HTP. Yunger and Harvey [17] report that 50 mg/kg of benserazide did not diminish the ability of 37.5 mg/kg *l*-5-HTP to restore the jump threshold of medial forebrain bundle lesioned rats to normal levels. This would indicate that such an effect was mediated centrally. Conversely, Modigh [11] has observed that decreases in motor activity which occur in mice given large doses (100–800 mg/kg) of *d,l*-5-HTP are not only blocked by 75 mg/kg of carbidopa, but that the activity is actually increased markedly above control values. These results suggest that decreases in motor activity following large doses of 5-HTP are mediated peripherally. In contrast, it has been assumed on the basis of correlational data that decreases in responding maintained by food presentation in pigeons and rats following administration of 5-HTP are the result of increases in levels of 5-HT in the telencephalon and diencephalon [6].

The data presented in this paper suggest that the rate-decreasing effects of 5-HTP on operant responding are not due to central serotonergic mechanisms. The administration of a peripheral decarboxylase inhibitor in combination with 5-HTP would seem to be a necessary control in studies that utilize 5-HTP as a pharmacologic tool in order to elucidate the central/peripheral locus of effect. These results also emphasize the need for detailed neurochemical analyses of the effects of 5-HTP on 5-HT metabolism both centrally and peripherally following decarboxylase inhibition.

In conclusion, we find that *d*-, *l*- and *d,l*-5-HTP produces dose-related decreases in FR responding in the rat. The levo isomer would appear to be largely responsible for the behavioral effects observed following administration of the racemic form, although specific serotonergic effects by the dextro isomer have not been ruled out. These rate decreasing effects would appear to be largely, if not entirely, the result of the activation of serotonergic neuronal systems outside the blood-brain barrier.

ACKNOWLEDGEMENTS

We wish to thank Dr. Marcus B. Waller for the loan of valuable equipment, Ms. Peggy Hansen for typing the manuscript and Ms. Cedonia Edwards for drawing the figures. This research was supported by USPHS Grants MH-24333 and MH-24593 from the National Institute of Mental Health, DA-01711 from the National Institute on Drug Abuse and ES-01104 from the National Institute of Environmental Health Sciences.

REFERENCES

1. Aprison, M. H. and C. B. Ferster. Neurochemical correlates of behavior. I. Quantitative measurements of the behavioral effects of the serotonin precursor, 5-hydroxytryptophan. *J. Pharmac. exp. Ther.* **131:** 100–107, 1961.
2. Aprison, M. H. and C. B. Ferster. Neurochemical correlates of behavior. II. Correlation of brain monoamine oxidase activity with behavioral changes after iproniazid and 5-hydroxytryptophan administration. *J. Neurochem.* **6:** 350–357, 1961.
3. Aprison, M. H. and J. N. Hingtgen. Neurochemical correlates of behavior. IV. Norepinephrine and dopamine in four brain parts of the pigeon during the period of atypical behavior following the injection of 5-hydroxytryptophan. *J. Neurochem.* **12:** 959–968, 1965.
4. Aprison, M. H. and J. N. Hingtgen. Neurochemical correlates of behavior. V. Differential effects of drugs on approach and avoidance behavior in rats with related changes in brain serotonin and norepinephrine. *Recent Adv. Biol. Psychiat.* **8:** 87–100, 1966.
5. Aprison, M. H. and J. N. Hingtgen. Serotonin and behavior: A brief summary. *Fedn Proc.* **31:** 121–129, 1972.
6. Aprison, M. H., M. A. Wolf, G. L. Poulos and T. L. Folkerth. Neurochemical correlates of behavior. III. Variation of serotonin content in several brain areas and peripheral tissues of the pigeon following 5-hydroxytryptophan administration. *J. Neurochem.* **9:** 575–584, 1962.
7. Bartholini, G. and A. Pletscher. Decarboxylase inhibitors. *Pharmac. Therap.* **1:** 407–584, 1962.
8. Carter, R. B. and J. B. Appel. Blockade of the behavioral effects of 5-HTP by the decarboxylase inhibitor Ro4-4602. *Pharmac. Biochem. Behav.* **4:** 407–409, 1976.
9. Copp, F. C., A. F. Green, H. F. Hodson, A. W. Randall and M. F. Sim. New peripheral antagonists of 5-hydroxytryptamine. *Nature* **214:** 200–201, 1967.
10. Fuxe, K., L. L. Butcher and J. Engel. dl-5-hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition. *J. Pharm. Pharmac.* **23:** 420–424, 1971.
11. Modigh, K. Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice. *Psychopharmacologia* **23:** 48–54, 1972.
12. Penn, P. E., W. J. McBride, J. N. Hingtgen and M. H. Aprison. Differential uptake, metabolism and behavioral effects of the *d* and *l* isomers of 5-hydroxytryptophan. *Pharmac. Biochem. Behav.* **7:** 515–518, 1977.
13. Pletscher, A. and K. F. Gey. The effect of a new decarboxylase inhibitor on endogenous and exogenous monoamines. *Biochem. Pharmac.* **12:** 223–228, 1963.
14. Warsh, J. J. and H. C. Stancer. Brain and peripheral metabolism of 5-hydroxytryptophan—^{14}C following peripheral decarboxylase inhibition. *J. Pharmac. exp. Ther.* **197:** 545–555, 1976.
15. Winer, B. J. *Statistical Principles in Experiental Design.* New York: McGraw-Hill, 1971, pp. 170–177.
16. Winter J. C. Behavioral effects of N,N-diethyltryptamine: Absence of antagonism by xylamidine tosylate. *J. Pharmac. exp. Ther.* **169:** 7–16, 1969.
17. Yunger, L. M. and J. A. Harvey. Behavioral effects of *l*-5-hydroxytryptophan after destruction of ascending serotonergic pathways in the rat: the role of catecholaminergic neurons. *J. Pharmac. exp. Ther.* **196:** 307–315, 1976.