2665

THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Vol. 193, No. 3
*Printed in U.S.A.*

# EFFECTS OF CHLORIMIPRAMINE AND LYSERGIC ACID DIETHYLAMIDE ON EFFLUX OF PRECURSOR-FORMED $^3$H-SEROTONIN: CORRELATIONS WITH SEROTONERGIC IMPULSE FLOW[1]

DOROTHY W. GALLAGER AND GEORGE K. AGHAJANIAN

*Departments of Psychiatry and Pharmacology, Yale University School of Medicine and the Connecticut Mental Health Center, New Haven, Connecticut*

Accepted for publication February 3, 1975

## ABSTRACT

GALLAGER, DOROTHY W. AND GEORGE K. AGHAJANIAN: Effects of chlorimipramine and lysergic acid diethylamide on efflux of precursor-formed $^3$H-serotonin: Correlations with serotonergic impulse flow. J. Pharmacol. Exp. Ther. **193:** 785–795, 1975.

The effects of *d*-lysergic acid diethylamide (LSD) and chlorimipramine (CIMI) on the firing rate of serotonergic (5-HT) neurons and on the *in vivo* efflux of 5-HT were investigated in parallel. A cerebroventricular perfusing technique was used to measure the efflux of $^3$H-5-HT formed *in vivo* from $^3$H-tryptophan. Impulse flow in serotonergic neurons was monitored by single unit recording from raphe (5-HT) neurons. Doses of LSD and CIMI, which caused a similar degree of inhibition of raphe cell firing, were found to affect 5-HT efflux differently. LSD, at both the 75, and 150 μg/kg doses, produced a similar decrease in $^3$H-5-HT efflux. In contrast, CIMI at a low dose (5 mg/kg) did not reduce 5-HT efflux, despite an inhibition in impulse flow. At a high dose (20 mg/kg), CIMI produced an increase in $^3$H-5-HT efflux. We conclude that 1) LSD decreases $^3$H-5-HT efflux by directly inhibiting impulse flow in 5-HT neurons and/or by a local effect on 5-HT terminals and 2) a low dose of CIMI produces no net change in $^3$H-5-HT efflux because a reduction in impulse flow-dependent 5-HT release compensates for blockade by CIMI of 5-HT reuptake.

The hallucinogenic agent, *d*-lysergic acid diethylamide (LSD), and the tricyclic antidepressant, chlorimipramine (CIMI), have been shown to have similar effects on the firing rate of serotonergic neurons located in the midbrain raphe area (Aghajanian *et al.*, 1968; Sheard *et al.*, 1972). The systemic administration of both LSD and CIMI uniformly inhibits raphe neuronal firing. Since release of serotonin (5-hydroxytryptamine; 5-HT) is based at least in part on impulse flow (Andén *et al.*, 1966; Sheard and Aghajanian, 1968; Holman and Vogt, 1970, 1972), both drugs might be expected to decrease the release of 5-HT from serotonergic nerve terminals. *In vivo* studies suggest that LSD decreases 5-HT release (Randic and Padjen, 1971; Tilson and Sparber, 1972). Parallel *in vivo* studies using CIMI have not been done. However, contrary to expecta-

Received for publication September 24, 1974.
[1] This work was supported in part by Grants MH-17871 and PHS-NS-05706 and by the State of Connecticut.

**Send reprint requests to:** Dorothy W. Gallager, Department of Psychiatry, Yale University School of Medicine, CMHC, 34 Park St., New Haven, Conn. 06508.

tions, in *in vitro* experiments CIMI have been shown to cause an increased 5-HT "efflux" or release (Farnebo and Hamberger, 1971). Despite these differences in their effects on release, both CIMI and LSD have similar effects on 5-HT metabolism. *In vivo* biochemical studies have shown that both drugs increase endogenous levels of 5-HT and decrease endogenous levels of 5-hydroxyindoleacetic acid (5-HIAA) (Rosecrans *et al.*, 1967; Freedman *et al.*, 1970; Halaris *et al.*, 1973). CIMI and LSD have also been reported to cause a reduction in 5-HT synthesis (Lin *et al.*, 1969; Schubert *et al.*, 1970a,b; Andén *et al.*, 1968; Corrodi and Fuxe, 1969; Shields and Eccleston, 1973; Hamon *et al.*, 1974).

In the present studies, we have investigated in parallel the effects of LSD and CIMI on the firing rate of 5-HT neurons and on the *in vivo* efflux of 5-HT. While it is not yet technically possible to measure directly the synaptic *release* of a transmitter *in vivo*, it is possible to measure the *efflux* of a transmitter, which is the net result of the complex of events: release, reuptake and/or metabolism. Previous *in vivo* studies on release and metabolism have generally used doses of LSD and CIMI (Rosecrans *et al.*, 1967; Andén *et al.*, 1968; Lin *et al.*, 1969; Corrodi and Fuxe, 1969; Schubert *et al.*, 1970a,b; Randić and Padjen, 1971; Tilson and Sparber, 1972; Halaris *et al.*, 1973) which are much higher than those needed to inhibit firing (Aghajanian *et al.*, 1968; Sheard *et al.*, 1972) making the relationship between firing rate and efflux unclear. We have given low enough doses of both drugs to permit at least partial recovery from total inhibition of cell firing during the period of perfusion. In addition, by employing doses of LSD and CIMI which cause equivalent inhibition of raphe cell firing, we can begin to separate impulse-flow dependent release from other possible effects of these drugs.

Because endogenous 5-HT efflux from serotonergic nerve terminals is low (Feldberg and Myers, 1966) and often masked by the presence of non-neuronal 5-HT (Portig and Vogt, 1969), we have used precursor-formed $^3$H-5-HT as a marker for endogenous 5-HT. Chiueh and Moore (1973) have reported on the use of catechols synthesized from $^3$H-tyrosine in their *in vivo* release studies. However, the use of precursor-formed indoles has not yet been applied to *in vivo* 5-HT release studies. This labeling technique provides major advantages: 5-HT formed from tryptophan has been shown to be specific for serotonergic neurons (Moir and Eccleston, 1968; Kuhar *et al.*, 1972; Aghajanian and Asher, 1971; Aghajanian *et al.*, 1973). In addition, *in vitro* studies by Snodgrass and Iverson (1974) have shown that release of $^3$H-tryptophan ($^3$H-TRP) along with $^3$H-5-HT is associated with nonspecific tissue damage or to unphysiological effects. Thus measuring $^3$H-TRP efflux as well as precursor-formed $^3$H-5-HT provides a check on the specificity of the effects observed.

## Methods

***Experimental subjects and recording studies.*** Male albino rats (Charles River, 220–280 g) were used in these experiments. For purposes of recording, animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic apparatus. Animals were kept anesthetized with additional injections of chloral hydrate as needed throughout the experiment. In previous studies, it has been shown that the dose of LSD required to inhibit raphe neurons in chloral hydrate-anesthetized rats (Aghajanian *et al.*, 1970) was virtually identical to the dose in unanesthetized rats (Haigler and Aghajanian, 1974). Micropipettes filled with 2 M NaCl saturated with fast green dye (tip diameters 1-2 $\mu$m) were lowered into the midbrain raphe region with a hydraulic microdrive system. Electrode potentials were passed through a high-impedence amplifier and monitored on an oscilloscope. Integrated firing rate was recorded by means of a rate meter as described previously (Aghajanian *et al.*, 1970). Rat temperature was monitored by Tele-Thermometer and maintained between 36–37°C. Under these conditions, raphe units display a regular rhythm and a rate of 0.5 to 2.5 spikes/second as described previously (Aghajanian *et al.*, 1968). After observing and recording a base-line firing rate for at least 5 minutes, drugs were administered intraperitoneally. At the end of each experiment, the site of the electrode tip was marked by iontophoretic ejection of fast green (Thomas and Wilson, 1965). Precise location of electrode tip was later determined by histological examination.

***Ventricular implantation and perfusion.*** Rats were anesthetized as above and placed in a stereotaxic apparatus. Two cannulae were implanted

in each rat: a polyethylene cannula (constructed and implanted according to deBalbian Verster *et al.*, 1971) for the intraventricular administration of $^3$H-TRP and a Gaddum type push-pull cannula for perfusion (Gaddum, 1961). The lateral ventricle was chosen as the perfusion site for two reasons. First, the possibility of direct tissue damage by the perfusion cannula (Chase and Kopin, 1968; Portig and Vogt, 1969) is decreased by ventricular placement. Secondly, studies of Richards *et al.* (1973) have demonstrated the presence of serotonergic nerve terminals lining the rat cerebral ventricles. By both fluorescence and electron microscopy, we have found that these nerve terminals are abolished by midbrain raphe lesions establishing this area as the origin of these 5-HT terminals (Aghajanian and Gallager, 1975).

The push-pull cannula was constructed from stainless steel tubing the inside tube being 27 gauge and the outside 20 gauge. Optimal protrusion of the inner tube was 0.5 mm (Szerb, 1967). Two adjacent burr holes were drilled at 1.5 and 2 mm lateral to the intersection of the coronal and saggital sutures (bregma zero) and the push-pull and ventricular cannulae were inserted below the skull surface, 4.2 and 3.5 mm respectively. After permanent attachment to the skull, an inner (protection) stylet was inserted into the outer cannula. After the wound was closed, the animals were allowed to recover from the anesthesia. Sixteen to 20 hours after the implantation of these cannulae, $^3$H-TRP (0.2 mc specific activity: 4.6 c/mmol, Schwarz-Mann, Orangeburg, N.Y.) was injected *via* the polyethelene cannula into the lateral ventricle in a volume of 20 $\mu$l. Perfusion with artificial cerebrospinal fluid (Merlis, 1940) was begun through the adjacent push-pull cannula 1 hour after the administration of labeled tryptophan in the nonanesthetized, unrestrained rats. A constant perfusion rate of 50 $\mu$l/min was maintained by a Manostat peristaltic pump (Manostat, New York, N.Y.). Consecutive 10-minute perfusate samples were collected in test tubes containing 0.1 ml of 1% cysteine, 200 $\mu$g of cold TRP and 100 $\mu$g of cold 5-HT. A variation of more than 10% between the volume infused and that recovered was taken as evidence of a faulty perfusion and the data were discarded. At the end of each ventricular perfusion, rats were anethetized and perfused through the heart with 10% buffered formalin. Serial frozen sections (50 $\mu$m in thickness) were cut, mounted and stained to verify cannula placement. The area of cannulation corresponded to the frontal planes A7470 through A6450 of König and Klippel (1970). Although four animals were run simultaneously, the absolute amounts of labeled tryptophan and 5-HT collected in perfusate samples were quite variable between animals. Therefore, four 10-minute perfusate samples were collected prior to the administration of drugs so that each animal could serve as his own control. The data were then expressed as a percentage of the fourth (control) period. Statistical significance was calculated by Student's *t* test (two-tailed).

***Assay of perfusate.*** The perfusate samples (0.5 ml/sample) were lyophilized to dryness and redissolved in 100 $\mu$l of 80% ethanol. After centrifugation, 50 $\mu$l of each sample were applied to silica gel (LQ6D Linear-Q™, Quantum Industries, Fairfield, N.J.) thin-layer plates. Since the radioactivity associated with tryptophan was in great excess, a solvent system in which $^3$H-TRP remained near the origin was chosen to prevent streaking and masking of the smaller $^3$H-5-HT peak. However, no single chromatographic system was available in which both $^3$H-5-HT and $^3$H-5-HIAA were separated cleanly from $^3$H-TRP. Although attempted, the ion exchange method of Schubert (1974) was not satisfactory for separating $^3$H-TRP from $^3$H-5-HIAA and $^3$H-5-HT in perfusate samples. $^3$H-TRP was in such excess that its 0.1 to 0.2% overlap with $^3$H-5-HIAA using multiple exchange resins masked the small amounts of the labeled acid metabolite. Thus, attempts to quantitate this indole metabolite were abandoned. The thin-layer plates were developed in ethyl acetate-*n*-propanol–10% ammonia (4:3:1) for 2.0 hours and the Rf values for 5-HIAA, TRP and 5-HT were 0.24, 0.27 and 0.65, respectively (Aures *et al.*, 1968). The remaining 50 $\mu$l from each sample were counted in Aquasol (New England Nuclear Corporation, Boston, Mass.) to determine total radioactivity. Since radioactivity was limited to positions coinciding with those of added authentic 5-HT and TRP (Kuhar *et al.*, 1971), the fluorescent areas associated with cold carriers of the respective indoles were visualized under ultraviolet light, scraped into counting vials containing Aquasol and quantitated by scintillation counting. Recoveries from known standards run simultaneously were 60% for TRP and 80% for 5-HT. Rats were pretreated for 30 minutes with Ro 4-4602, in a dose sufficient to totally block 5-HT synthesis (800 mg/kg i.p.; Bédard *et al.*, 1971). After the injection of $^3$H-TRP, no radioactivity in perfusate samples from these animals was found to be associated with the authentic 5-HT peak. This further indicated that the radioactivity coinciding with authentic 5-HT was $^3$H-5-HT.

***Determination of endogenous substances.*** Endogenous TRP, 5-HT and 5-HIAA were analyzed in the same tissue samples by a combina-

tion of the column method of Schubert *et al.* (1970a) and the fluorimetric *o*-phthalaldehyde condensation method of Maickel and Miller (1966). Recoveries for tryptophan, 5-HT and 5-HIAA were 100, 71 and 90%, respectively.

***Drugs and radioactive materials.*** Chlorimipramine hydrochloride (Anafranil, Ciba Pharmaceutical Company, Summit, N.J.), Ro 4-4602 ([N-(DL-seryl)-N-(2,3,4-trihydroxybenzyl) hydrazine], Hoffmann-La Roche, Inc., Nutley, N.J.) and *d*-lysergic acid diethylamide bitartrate were administered i.p., doses of drugs were expressed in terms of their free base. $^3$H-tryptophan (4.6 c/mmol, generally labeled) was obtained from Schwarz-Mann. Prior to use, 1.0 ml of the ethanol-water solution was evaporated to dryness and redissolved in 100 $\mu$l of artificial cerebrospinal fluid. Twenty microliters of this solution were injected intraventricularly in an approximate concentration of 10 $\mu$c/$\mu$l.

## Results

**Recording studies.** The spontaneous rates of neuronal units tested in the dorsal raphe ranged from 0.5 to 2.5 spikes/sec. Administration of an intraperitoneal dose of 5 mg/kg of CIMI produced a total inhibition of raphe cell firing for approximately 1 hour (fig. 1, top trace). An i.p. injection of LSD (75 $\mu$g/kg) also produced a total inhibition of raphe cell firing for approximately 1 hour (fig. 1, bottom trace). As previously reported (Haigler and Aghajanian, 1973), the inhibition of raphe firing by LSD was not found to be associated with a decrease in action potential size. This was also observed with CIMI indicating that neither drug produces its inhibition by a local anesthetic effect.

**Perfusion studies.** We attempted to determine how these drugs, at doses which have similar effects on raphe firing, would affect 5-HT efflux. LSD (75 $\mu$g/kg i.p.) administered at the end of the control perfusion period produced a significant decrease ($P < .025$) in the efflux of $^3$H-5-HT (fig. 2). Higher doses of LSD (150 and even 300 $\mu$g/kg, not shown) were not able to reduce further the efflux of $^3$H-5-HT (mean of six rats at each dose ± S.E.). $^3$H-5-HT efflux did not return to base line but remained depressed and parallel to the control curve throughout the perfusion period. In a separate group of animals, 75 $\mu$g/kg (i.p.) of LSD produced a total inhibition of raphe firing for approximately 63 minutes (fig. 2, shaded bar). LSD did not alter the efflux of $^3$H-TRP at any time during the perfusion period (table 1).

In contrast, CIMI at a dose of 5 mg/kg i.p. did not, except for one point, significantly alter

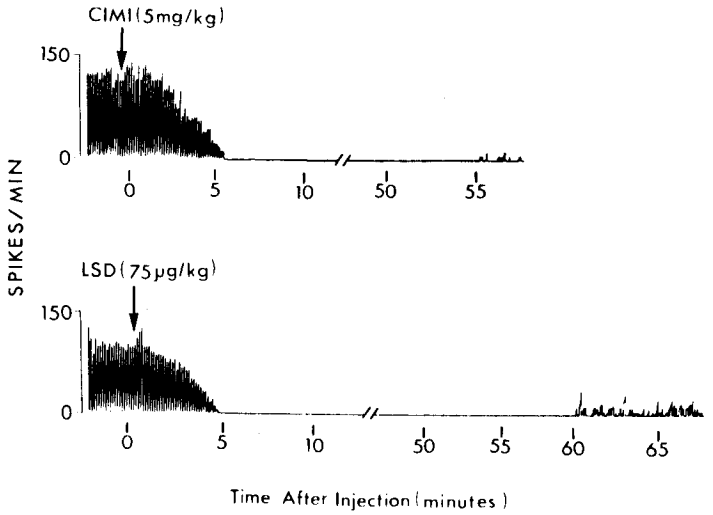


FIG. 1. A comparison of the response of cells in the dorsal raphe nucleus to the systemic administration of CIMI and LSD. The arrow in both cases marks the point at which drugs were administered i.p. In the top trace, a raphe cell is totally inhibited for 55 minutes by the i.p. injection of 5 mg/kg of CIMI. In the lower trace another raphe cell is inhibited for 60 minutes by the i.p. injection of 75 $\mu$g/kg of LSD. Both traces shown here are integrated rate records, with the ordinate indicating the firing rate in spikes per minute of neurons in the dorsal raphe nucleus.

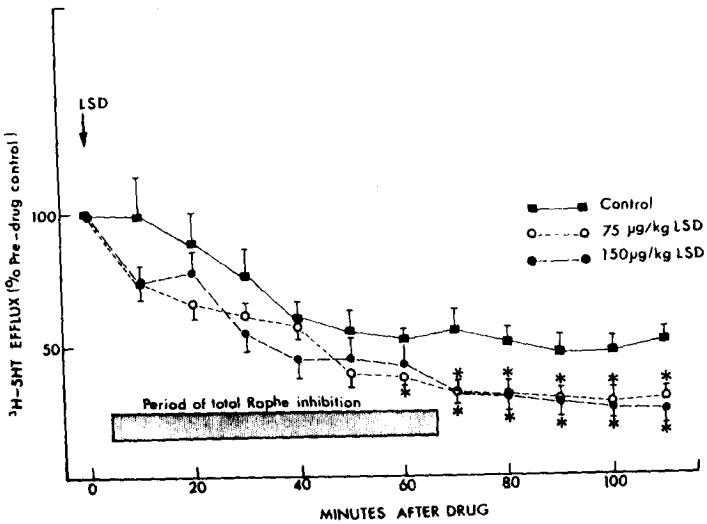


FIG. 2. The effects of LSD (75 and 150 µg/kg, i.p.) on the spontaneous efflux of $^3$H-5-HT into the ventricular perfusate and on the firing rate of raphe neurons. $^3$H-TRP (200 µc) was injected intraventricularly 1 hour prior to the start of perfusion. LSD was administered after a predrug perfusion period. The mean absolute amount of $^3$H-5-HT recovered in the predrug perfusion period was 0.66 ± 0.31 µc/10 min (75 µg/kg of LSD; $N$ = 6) or 0.49 ± 0.09 nc/10 min (150 µg/kg of LSD; $N$ = 6). The mean absolute amount of $^3$H-5-HT recovered from the perfusate of the same period for control animals was 0.25 ± 0.07 nc/10 min, $N$ = 11. $^3$H-5-HT efflux in subsequent samples was expressed as a percentage of the respective predrug amounts of $^3$H-5-HT. In a separate group of rats, represented by the shaded bar, the response of raphe cell firing was followed after an i.p. dose of 75 µg/kg of LSD. Dorsal raphe cells were totally inhibited for 63.3 ± 2.9 minutes ($N$ = 10). *Indicates a significant difference from control levels ($P < .05$).

the efflux of $^3$H-5-HT from that of control rats (fig. 3), although this dose of CIMI totally inhibited raphe neuronal firing for approximately the same duration as the 75 µg/kg dose of LSD (fig. 3, shaded bar). However, CIMI at a dose of 20 mg/kg produced a significant increase ($P < .05$) in $^3$H-5-HT efflux (mean of six rats at each dose ± S.E.). This increased efflux of $^3$H-5-HT returned to control levels approximately 90 minutes after the administration of CIMI. CIMI did not alter the efflux of $^3$H-TRP from control values at any time during the perfusion period (table 1).

Both LSD and CIMI have been reported to inhibit 5-HT synthesis (Lin *et al.*, 1969; Schubert *et al.*, 1970a,b; Andén *et al.*, 1968; Corrodi and Fuxe, 1969). Since efflux of 5-HT was measured by the presence in the perfusate of $^3$H-5-HT formed from $^3$H-TRP, it was necessary to eliminate the possibility that $^3$H-5-HT synthesis was occurring during the perfusion interval. Schubert and Sedvall (1972) have reported that 1 hour after precursor administration, synthesis of $^3$H-5-HT from $^3$H-TRP was negligible. To confirm this in our system, we administered the aromatic L-amino acid decarboxylase inhibitor, Ro 4-4602, in a dose sufficient to block totally 5-HT synthesis (800 mg/kg i.p.; Bédard *et al.*, 1971). This synthesis inhibitor did not significantly alter the rate of $^3$H-5-HT efflux as compared to saline-treated animals (mean of six rats ± S.E., fig. 4). This indicates that $^3$H-5-HT synthesis was negligible during the perfusion period suggesting that the efflux of preformed $^3$H-5-HT was the only phenomenon being measured in these experiments.

Other control experiments were also necessitated by the use of precursor-formed $^3$H-5-HT. While the intraventricular administration of labeled TRP permitted high specific activities of the precursor and product to be measured in the limited area of brain sampled by perfusion, it was assumed that the fate of labeled 5-HT reflected that of the endogenous amine. It has been shown that when TRP is employed as the precursor, the product, 5-HT, is specific for serotonergic neurons (Moir and Eccleston, 1968; Kuhar *et al.*, 1972; Aghajanian and

TABLE 1

*Spontaneous $^3$H-tryptophan efflux in control and drug-treated rats*[a]

| Time after injection | Treatment[b] | | | |
|---|---|---|---|---|
| | Saline | LSD | CIMI | Ro 4-1602 |
| *min* | | | | |
| 0 | 100 | 100 | 100 | 100 |
| 10 | 97 ± 10 | 78 ± 9 | 82 ± 9 | 90 ± 4 |
| 20 | 81 ± 9 | 87 ± 6 | 77 ± 8 | 76 ± 5 |
| 30 | 64 ± 6 | 66 ± 8 | 79 ± 15 | 67 ± 4 |
| 40 | 55 ± 4 | 66 ± 11 | 61 ± 8 | 62 ± 4 |
| 50 | 46 ± 5 | 52 ± 9 | 57 ± 8 | 53 ± 3 |
| 60 | 43 ± 4 | 50 ± 9 | 52 ± 7 | 49 ± 6 |
| 70 | 39 ± 6 | 40 ± 8 | 49 ± 9 | 42 ± 6 |
| 80 | 38 ± 3 | 48 ± 11 | 49 ± 6 | 47 ± 4 |
| 90 | 29 ± 3 | 40 ± 10 | 48 ± 12 | 38 ± 4 |
| 100 | 30 ± 4 | 36 ± 10 | 41 ± 7 | 35 ± 4 |
| 110 | 32 ± 2 | 33 ± 7 | 39 ± 8 | 38 ± 5 |

[a] A pulse dose of $^3$H-TRP (200 μc) was injected intraventricularly 60 minutes prior to the start of perfusion. One hundred minutes after $^3$H-TRP or 40 minutes after the start of perfusion, saline, LSD (75 μg/kg), CIMI (5 mg/kg) or Ro 4-4602 (800 mg/kg) was administered i.p.

[b] The mean absolute amounts of $^3$H-TRP recovered in predrug perfusion periods were: saline 14.7 ± 3.8 nc/10 min, $N$ = 11; LSD 10.4 ± 2.3 nc/10 min, $N$ = 6; CIMI 10.7 ± 1.5 nc/10 min, $N$ = 6; Ro 4-4602 13.5 ± 3.0 nc/10 min, $N$ = 6. $^3$H-TRP efflux in subsequent samples was expressed as the mean percentage of the respective predrug amounts of $^3$H-TRP ± S.E.

Asher, 1971; Aghajanian *et al.*, 1973). However, several investigators (Asheroft *et al.*, 1965; Moir and Eccleston, 1968; Fernstrom and Wurtman, 1971) have demonstrated that TRP availability can alter brain 5-HT levels and turnover. To be sure that steady-state conditions were not affected by our labeling procedure, we evaluated the effects of the 8.9-μg dose of labeled TRP on endogenous levels of indoles in brain (table 2). One hour after intraventricular administration of this pulse dose of $^3$H-TRP, the endogenous levels of the precursor (TRP) product (5-HT) and metabolite (5-HIAA) were not altered. Nonlabeled TRP in approximately the same dose (10 μg) also had no effect on endogenous indole levels, suggesting that labeled and authentic TRP behave similarly. TRP (100 μg) given intraventricularly 1 hour prior to sacrifice significantly increased endogenous tryptophan and 5-HIAA levels in rat brain. These data suggest that the label did not represent a "loading dose" of TRP at the time of perfusion.

It has also been postulated that certain drugs could alter TRP availability (Tagliamonte *et al.*, 1971; Bruinvels, 1972). Since this could result in changes of brain 5-HT as well, $^3$H-TRP efflux was measured after each drug. In addition, Snodgrass and Iverson (1974) have shown that release of the precursor, $^3$H-TRP, in addition to $^3$H-5-HT, is associated with nonspecific, non-neuronal effects. Since $^3$H-TRP efflux was not affected by any drug treatment (table 1), the data suggest that $^3$H-TRP accumulation in tissues was not significantly altered by these drugs and that changes in $^3$H-5-HT efflux were specific drug effects.

## Discussion

Our results show that LSD and CIMI, at doses which cause a similar degree of inhibition of raphe cell firing, affected 5-HT efflux differently: a decrease occurred after LSD but there was no change in efflux with the low dose of CIMI.

In studies in which LSD was administered to perfused rats, at both the 75 and 150 μg/kg doses, a similar decrease in $^3$H-5-HT efflux was observed. While other investigators have suggested that LSD blocks release of 5-HT (Chase *et al.*, 1967; Farnebo and Hamberger, 1971; Tilson and Sparber, 1972), there is little information about how the hallucinogenic agent accomplishes this. Haigler and Aghajanian (1974) have shown that LSD causes a direct inhibition of raphe cell firing. This inhibition of firing could lead to a reduction in impulse flow-dependent 5-HT release from serotonergic terminals as suggested by our data. However, LSD has also been shown to decrease the formation of 5-HT formed from labeled tryptophan (Lin *et al.*, 1969; Schubert *et al.*, 1970a). It is therefore significant that although efflux of $^3$H-5-HT was decreased by LSD, $^3$H-5-HT synthesis was negligible during the perfusion period. Thus inhibition of synthesis is not sufficient to explain the reduction of $^3$H-5-HT efflux by LSD.

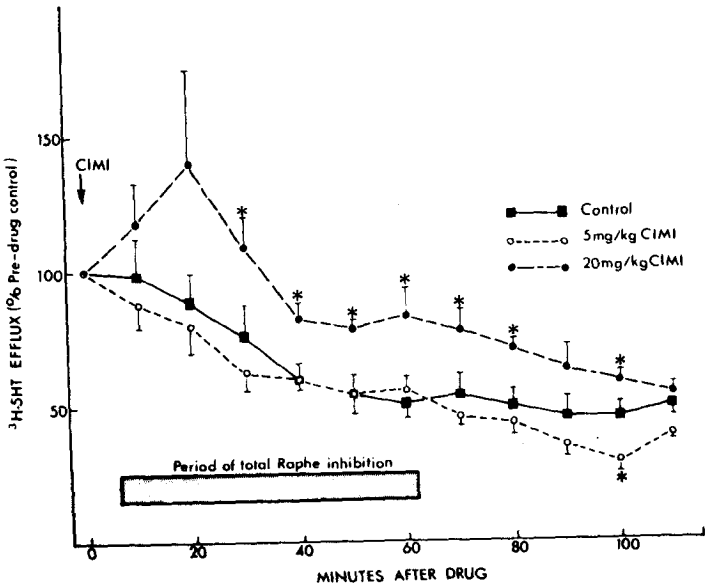


FIG. 3. Effects of chlorimipramine (CIMI, 5 and 20 mg/kg i.p.) on the spontaneous efflux of $^3$H-5-HT measured in ventricular perfusate and on the firing rate of raphe neurons. CIMI was administered i.p. (5 or 20 mg/kg) after a predrug perfusion period ($N$ = 6). The mean absolute amount of $^3$H-5-HT recovered in the predrug perfusion period is 0.18 ± 0.05 nc/10 min (5 mg/kg of CIMI) or 0.14 ± 0.03 nc/10 min (20 mg/kg of CIMI). The mean absolute amount of $^3$H-5-HT recovered from the perfusate of the same period for control animals was 0.25 ± 0.07 nc/10 min, $N$ = 11. $^3$H-5-HT efflux in subsequent samples is expressed as a percentage of the respective predrug amounts of $^3$H-5-HT. In a separate group of rats, represented by the shaded bar, the response of raphe cell firing was measured after an i.p. dose of 5 mg/kg of CIMI. Dorsal raphe cells were totally inhibited for 56.2 ± 3.6 minutes ($N$ = 8).

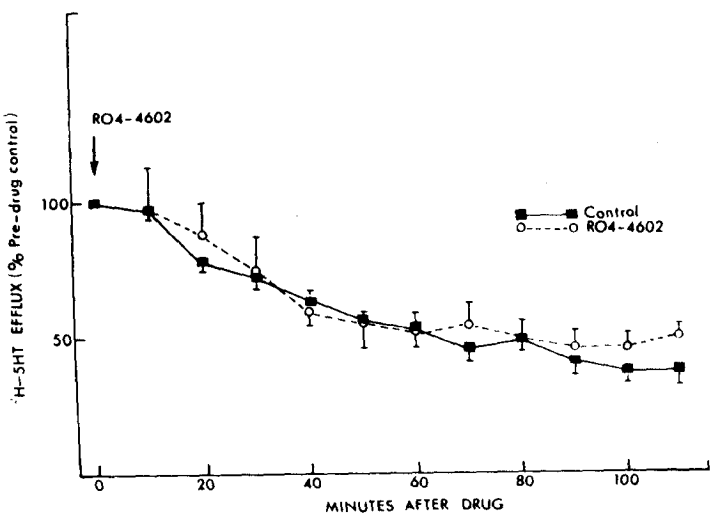


FIG. 4. A comparison of the spontaneous efflux of $^3$H-5-HT in control rats to the efflux of $^3$H-5-HT after Ro 4-4602. $^3$H-TRP (200 μc) was injected intraventricularly 1 hour prior to the start of perfusion. At the end of a predrug perfusion period, Ro 4-4602 (800 mg/kg i.p.) was administered to rats and ventricular perfusion was continued for 11 additional collection periods of 10 minutes. The mean absolute amount of $^3$H-5-HT recovered in the predrug perfusion period was 0.28 ± 0.11 nc/10 min; $N$ = 6. The mean absolute amount of $^3$H-5-HT recovered from the perfusate of same period for control animals was 0.25± 0.07 nc/10 min; $N$ = 11. $^3$H-5-HT efflux in subsequent samples is expressed as a percentage of this predrug $^3$H-5-HT.

TABLE 2

*Concentrations of tryptophan, 5-HT and 5-HIAA in whole rat brain tissue following the intraventricular administration of tryptophan*

| Tryptophan[a] | Tryptophan | 5HT | 5HIAA |
|---|---|---|---|
| μg | μg/g ± S.D. | μg/g ± S.D. | μg/g ± S.D. |
| 0 | 4.59 ± 0.47 | 0.62 ± 0.14 | 0.27 ± 0.04 |
| 8.9[b] | 5.06 ± 0.46 | 0.66 ± 0.08 | 0.28 ± 0.04 |
| 10 | 5.00 ± 0.18 | 0.68 ± 0.04 | 0.28 ± 0.03 |
| 100 | 7.40 ± 0.63[c] | 0.66 ± 0.07 | 0.44 ± 0.23[d] |

[a] Tryptophan was administered in a constant volume of 20 μl *via* polyethelyne cannula implanted according to the method of deBalbian Verster *et al.* (1971). Rats were implanted 36 hours prior to treatment and sacrificed 1 hour after the intraventricular injection. Values are the mean of 5 rats ± S.D.

[b] This dose of $^3$H-TRP (200 μc) was computed to represent 8.9 μc of the amino acid.

[c] $P < .01$.

[d] $P < .05$.

Another possible explanation for the ability of LSD to inhibit 5-HT efflux is based on the potent presynaptic effect of the hallucinogen (Haigler and Aghajanian, 1974). Presynaptic receptor theory suggests that, in some systems, there are receptors on the presynaptic membrane which are capable of modulating transmitter release (Kirpekar and Puig, 1971; Starke, 1971; Enero *et al.*, 1972). Thus LSD could inhibit transmitter release by activating presynaptic receptors located on serotonergic terminals. *In vitro* studies by Chase *et al.* (1967), Katz and Kopin (1969) and Farnebo and Hamberger (1971) have shown that LSD causes a reduction in stimulation-induced release of 5-HT in brain tissue slices. Since it is unlikely that intact serotonergic pathways exist in brain slices, such presynaptic receptors could explain the effects produced by LSD in these experiments where nerve terminal areas are separated from their soma. Because the inhibition of impulse flow by LSD and its possible presynaptic effects were not separated in our *in vivo* experiments, we cannot distinguish between these possibilities. It is interesting to note that although raphe cell firing was inhibited for approximately 1 hour and complete recovery of neuronal firing to control rates required an additional 20 to 30 minutes (Aghajanian *et al.*, 1968), efflux of $^3$H-5-HT did not return to control levels at any time during this interval. These data suggest that inhibition of firing may not be a complete explanation for the diminished efflux of $^3$H-5-HT during the period of recovery from LSD.

CIMI at a dose (5 mg/kg) that totally inhibited raphe neuronal firing for approximately the same duration as LSD did not decrease the efflux of $^3$H-5-HT. Furthermore, CIMI at a dose of 20 mg/kg produced a significant increase in $^3$H-5-HT efflux. Thus, it is clear that changes in firing rate cannot alone explain the dose-dependent effects of CIMI on efflux.

It has been postulated that CIMI inhibits raphe neuronal firing through an indirect mechanism by first blocking 5-HT uptake; the resulting excess of 5-HT in the synaptic cleft could then lead to a negative feedback inhibition of raphe neurons (Corrodi and Fuxe, 1969). The indirect action of CIMI on raphe cell firing is supported by the observation that the depression of raphe firing by CIMI can be blocked by pretreatment with the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (Sheard *et al.*, 1972). In terms of the negative feedback model, the reduction in impulse flow-dependent 5-HT release may entirely compensate for the block in 5-HT uptake by low doses of CIMI.

Presynaptic receptor theory has been used to explain why potent inhibitors of norepinephrine (NE) neuronal uptake are not very effective in increasing "transmitter overflow" or efflux (Enero *et al.*, 1972). When neuronal uptake is impaired, presynaptic inhibition of transmitter release is enhanced because a higher synaptic gap concentration of NE is available for activation of presynaptic receptors (Langer, 1974). A parallel serotonergic presynaptic receptor could also account for the lack of effect of CIMI on 5-HT efflux as seen in our system. Since evidence is accumulating that 5-HT is inhibitory on the raphe (presynaptic) system (Aghajanian *et al.*, 1972; Haigler and Aghajanian, 1974) perhaps the CIMI effects are suggestive of a presynaptic 5-HT receptor. However, efflux of $^3$H-5-HT is increased, not decreased, by a high dose (20 mg/kg) of CIMI. Thus presynaptic inhibition of 5-HT release cannot explain this effect of CIMI. The increase in $^3$H-5-HT efflux at a high dose of CIMI (20 mg/kg) may be due to a more complete blockade of 5-HT reuptake. Carlsson (1970) has reported that a dose of 7.5 mg/kg of CIMI (intraperitoneal

administration in mice) is the ED50 for the chlorimipramine-induced blockade of the membrane pump in 5-HT neurons. Thus, uptake blockade of 5-HT is a dose-dependent effect of CIMI which may be correlated with the effects of this drug on 5-HT efflux. Increased efflux of $^3$H-5-HT returns to control levels 90 minutes after the administration of 20 mg/kg of CIMI. While the metabolic fate of CIMI has not been determined, a closely related compound, imipramine, has a half-life of 0.6 hour in brain tissue (Bickel and Weder, 1968). In addition, liver microsomal studies indicate that CIMI and imipramine are metabolized at the same rate (J. Schenkman and H. Nybäck, personal communication). Thus, the time required for $^3$H-5-HT efflux to return to control levels could be related to the rate of degradation of CIMI.

The increase in 5-HT efflux caused by the high dose of CIMI could conceivably result from a direct release of 5-HT. However, data of Halaris *et al.* (1973) show that the endogenous levels of 5-HT are increased and levels of 5-HIAA are decreased by this treatment. Since release of 5-HT is associated with a lowering of 5-HT as well as an increase in 5-HIAA (Sheard and Aghajanian, 1968; Gumulka *et al.*, 1969; Kostowski *et al.*, 1969), these data are not consistent with a direct release of 5-HT by CIMI.

In conclusion, these data suggest that LSD and CIMI, at doses which cause a similar degree of inhibition of raphe cell firing, affect 5-HT efflux differently and by different mechanisms. It appears that LSD, by directly inhibiting impulse flow produces a reduction in $^3$H-5-HT efflux. However, a possible local presynaptic inhibition of 5-HT release by LSD must also be considered. In contrast, a low dose of CIMI does not cause a decrease in $^3$H-5-HT efflux despite an inhibition of raphe firing. Although a block in 5-HT reuptake by low doses of CIMI might be expected to increase efflux, this is apparently counterbalanced by the reduction in impulse flow-related 5-HT release, resulting in no net change in $^3$H-5-HT efflux. This is consistent with the view that the primary action of CIMI is to block 5-HT reuptake and that the inhibition of firing is a compensatory response to this diminished neuronal reuptake.

**Acknowledgments.** We would like to thank Dr. W. Scott of Hoffmann-La Roche, Inc., Nutley, N.J. for supplying the Ro 4-4602; Ciba Pharmaceutical Co., Summit, N.J. for supplying the chlorimipramine HCl (Anafranil); and the National Insitute of Mental Health Center for Studies of Narcotics and Drug Abuse for providing the LSD used in these experiments.

## References

Aghajanian, G. K. and Asher, I.: Histochemical fluorescence of raphe neurons: Selective enhancement by tryptophan. Science (Washington) **172:** 1159–1161, 1971.

Aghajanian, G. K., Foote, W. and Sheard, M.: Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. Science (Washington) **161:** 706–708, 1968.

Aghajanian, G. K., Foote, W. E. and Sheard, M. H.: Action of psychotogenic drugs on single midbrain raphe neurons. J. Pharmacol. Exp. Ther. **171:** 178–187, 1970.

Aghajanian, G. K. and Gallager, D. W.: Raphe origin of serotonergic nerves terminating in the cerebral ventricles. Brain Res., in press, 1975.

Aghajanian, G. K., Haigler, H. and Bloom, F.: Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons. Life Sci. Part I Physiol. Pharmacol. **11:** 615–622, 1972.

Aghajanian, G. K., Kuhar, M. J. and Roth, R. H.: Serotonin-containing neuronal perikarya and terminals: Differential effects of *p*-chlorophenylalanine. Brain Res. **53:** 85–101, 1973.

Andén, N.-E., Corrodi, H., Fuxe, K. and Hökfelt, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Brit. J. Pharmacol. **34:** 1–7, 1968.

Andén, N.-E., Fuxe, K. and Hökfelt, T.: The importance of nervous impulse flow for the depletion of the monoamines from central neurons by some drugs. J. Pharm. Pharmacol. **18:** 630–632, 1966.

Ashcroft, G. W., Eccleston, D. and Crawford, T. B.: 5-Hydroxyindole metabolism in rat brain. A study of intermediate metabolism using the technique of tryptophan loading—I Methods. J. Neurochem. **12:** 483–492, 1965.

Aures, D., Fleming, R. and Håkanson, R.: Separation and detection of biogenic amines by thin-layer chromatography. J. Chromatogr. **33:** 480–493, 1968.

Bédard, P., Carlsson, A., Fuxe, K. and Lindqvist, M.: Origin of 5-hydroxytryptophan and L-dopa accumulating in brain following decarboxylase inhibition. Naunyn-Schmiedebergs Arch. Pharmakol. **269:** 1–6, 1971.

Bickel, M. H. and Weder, H. J.: The total fate of a drug: Kinetics of distribution, excretion and formation of 14 metabolites in rats treated with imipramine. Arch. Int. Pharmacodyn. Ther. **173:** 433–463, 1968.

Bruinvels, J.: Inhibition of the biosynthesis of 5-hydroxytryptamine in rat brain by imipramine. Eur. J. Pharmacol. **20:** 231–237, 1972.

CARLSSON, A.: Effect of drugs on amine uptake mechanisms in the brain. *In* Bayer-Symposium II, pp. 223–233, Springer-Verlag, Heidelberg, 1970.

CHASE, T., BREESE, G. AND KOPIN, I.: Serotonin release from brain slices by electrical stimulation: Regional differences and effect of LSD. Science (Washington) **157:** 1461–1463, 1967.

CHASE, T. AND KOPIN, I.: Stimulus-induced release of substances from olfactory bulb using the push-pull cannula. Nature (London) **217:** 466–467, 1968.

CHIUEH, C. C. AND MOORE, K. E.: Release of endogenously synthesized catechols from the caudate nucleus by stimulation of the nigrostriatal pathway and by administration of *d*-amphetamine. Brain Res. **50:** 221–225, 1973.

CORRODI, H. AND FUXE, K.: Decreased turnover in central 5HT nerve terminals induced by antidepressant drugs of the imipramine type. Eur. J. Pharmacol. **7:** 56–59, 1969.

DEBALBIAN VERSTER, F., ROBINSON, C. A., HENGEVELD, C. A. AND SANDERS-BUSH, E.: Freehand cerebroventricular injection technique for unanesthetized rats. Life Sci. Part I Physiol. Pharmacol. **10:** 1395–1402, 1971.

ENERO, M. A., LANGER, S. Z., ROTHLIN, R. P. AND STEFANO, F. J. E.: Role of the alpha-adrenoceptor in regulating nonadrenaline overflow by nerve stimulation. Brit. J. Pharmacol. **44:** 672–688, 1972.

FARNEBO, L.-O. and HAMBERGER, B.: Drug-induced changes in the release of $^3$H-monoamines from field stimulated rat brain slices. Acta Physiol. Scand. **371:** 35–44, 1971.

FELDBERG, W. AND MYERS, R. D.: Appearance of 5-hydroxytryptamine and an unidentified pharmacologically active lipid acid in effluent from perfused ventricles. J. Physiol. (London) **184:** 837–855, 1966.

FERNSTROM, J. AND WURTMAN, R.: Brain serotonin content: Physiological dependence on plasma tryptophan levels. Science (Washington) **173:** 149–152, 1971.

FREEDMAN, D., GOTTLIEB, R. AND LOVELL, R.: Psychotomimetic drugs and brain 5-hydroxytryptamine metabolism. Biochem. Pharmacol. **19:** 1181–1188, 1970.

GADDUM, J. H.: Push-pull cannulae. J. Physiol. (London) **155:** 1–20, 1961.

GUMULKA, W., SAMANIN, R., GARATTINI, S. AND VALZELLI, L.: Effect of stimulation of midbrain raphe on serotonin (5HT) level and turnover in different areas of rat brain. Eur. J. Pharmacol. **8:** 380–384, 1969.

HAIGLER, H. J. AND AGHAJANIAN, G. K.: Mescaline and LSD: Direct and indirect effects on serotonin-containing neurons in brain. Eur. J. Pharmacol. **21:** 53–60, 1973.

HAIGLER, H. AND AGHAJANIAN, G. K.: Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. J. Pharmacol. Exp. Ther. **188:** 688–699, 1974.

HALARIS, A., LOVELL, R. AND FREEDMAN, D.: Effect of chlorimipramine on the metabolism of 5-hydroxytryptamine in the rat brain. Biochem. Pharmacol. **22:** 2200–2202, 1973.

HAMON, M., BOURGOIN, S., JAGGER, J. AND GLOWINSKI, J.: Effects of LSD on synthesis and release of 5HT in rat brain slices. Brain Res. **69:** 265–280, 1974.

HOLMAN, R. AND VOGT, M.: Release of 5-hydroxytryptamine (5HT) from septum and caudate nucleus. J. Physiol. (London) **210:** 163P, 1970.

HOLMAN, R. AND VOGT, M.: Release of 5-hydroxytryptamine from caudate nucleus and septum. J. Physiol. (London) **223:** 243–254, 1972.

KATZ, R. I. AND KOPIN, I. J.: Effect of *d*-LSD and related compounds on release of norepinephrine-H$^3$ and serotonin-H$^3$ evoked from brain slices by electrical stimulation. Pharmacol. Res. Commun. **1:** 54–62, 1969.

KIRPEKAR, S. M. AND PUIG, M.: Effect of flow-stop on noradrenaline release from normal spleens and spleens treated with cocaine, phentolamine or phenoxybenzamine. Brit. J. Pharmacol. **43:** 359–369, 1971.

KÖNIG, J. AND KLIPPEL, R.: The Rat Brain: A Stereotaxic Atlas, R. E. Krieger Publishing Co., Huntington, N.Y., 1970.

KOSTOWSKI, W., GIACALONE, E., GARATTINI, S. AND VALZELLI, L.: Electrical stimulation of midbrain raphe. Biochemical, behavioral and bioelectrical effects. Eur. J. Pharmacol. **7:** 170–175, 1969.

KUHAR, M., AGHAJANIAN, G. AND ROTH, R. H.: Tryptophan hydroxylase activity and synaptosomal uptake of serotonin in discrete brain regions after midbrain raphe lesions: Correlations with serotonin levels and histochemical fluorescence. Brain Res. **44:** 165–176, 1972.

KUHAR, M. J., ROTH, R. H. AND AGHAJANIAN, G. K.: Selective reduction of tryptophan hydroxylase activity in rat forebrain after midbrain raphe lesions. Brain Res. **35:** 167–176, 1971.

LANGER, S. Z.: Presynaptic regulation of catecholamine release. Biochem. Pharmacol. **23:** 1793–1800, 1974.

LIN, R., NGAI, S. AND COSTA, E.: Lysergic acid diethylamide: Role in conversion of plasma tryptophan to brain serotonin (5-hydroxytryptamine). Science (Washington) **166:** 237–239, 1969.

MAICKEL, R. P. AND MILLER, F. P.: Fluorescent products formed by reaction of indole derivatives and *o*-phthaldialdehyde. Anal. Chem. **38:** 1937–1938, 1966.

MERLIS, J. K.: The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. Amer. J. Physiol. **131:** 67–72, 1940.

MOIR, A. AND ECCLESTON, D.: The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J. Neurochem. **15:** 1093–1108, 1968.

PORTIG, P. AND VOGT, M.: Release into the cerebral ventricles of substances with possible transmitter function in the caudate nucleus. J. Physiol. (London) **204:** 687–715, 1969.

RANDIĆ, M. AND PADJEN, A.: Effect of N,N-dimethyltryptamine and *d*-lysergic acid diethylamide on the release of 5-hydroxyindoles in rat forebrain. Nature (London) **230:** 532–533, 1971.

RICHARDS, J., LOREZ, H. AND TRANZER, J.: Indolealkylamine nerve terminals in cerebral ventricles: Identification by electron microscopy and fluorescence histochemistry. Brain Res. **57:** 277–288, 1973.

ROSECRANS, J., LOVELL, R. AND FREEDMAN, D.:

Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine. Biochem. Pharmacol. **16:** 2011–2021, 1967.

SCHUBERT, J.: Labelled 5-hydroxytryptamine and 5-hydroxyindoleacetic acid formed *in vivo* from $^3$H-tryptophan in rat brain; effect of probenecid. Acta Physiol. Scand. **90:** 401–408, 1974.

SCHUBERT, J., NYBÄCK, H. AND SEDVALL, G.: Accumulation and disappearance of $^3$H-5-hydroxytryptamine formed from $^3$H-tryptophan in mouse brain: Effect of LSD-25. Eur. J. Pharmacol. **10:** 215–224, 1970a.

SCHUBERT, J., NYBÄCK, H. AND SEDVALL, G.: Effect of antidepressant drugs on accumulation and disappearance of monoamines formed *in vivo* from labeled precursors in mouse brain. J. Pharm. Pharmacol. **22:** 136–139, 1970b.

SCHUBERT, J. AND SEDVALL, G.: Accumulation and disappearance of $^3$H-5-hydroxytryptamine formed *in vivo* from $^3$H-tryptophan in various regions of the rat brain. Eur. J. Pharmacol. **17:** 75–80, 1972.

SHEARD, M. H. AND AGHAJANIAN, G. K.: Stimulation of the midbrain raphe: Effect on serotonin metabolism. J. Pharmacol. Exp. Ther. **163:** 425–430, 1968.

SHEARD, M., ZOLOVICK, A. AND AGHAJANIAN, G. K.: Raphe neurons: Effect of tricyclic antidepressant drugs. Brain Res. **43:** 690–694, 1972.

SHIELDS, P. J. AND ECCLESTON, D.: Evidence for the synthesis and storage of 5-hydroxytryptamine in two separate pools in the brain. J. Neurochem. **20:** 881–888, 1973.

SNODGRASS, S. R. AND IVERSON, L. L.: Formation and release of $^3$H-tryptamine from $^3$H-tryptophan in rat spinal cord slices. Advan. Biochem. Pharmacol. **10:** 141–150, 1974.

STARKE, K.: Influence of $\alpha$-receptor stimulants on noradrenaline release. Naturwissenschaften **58:** 420, 1971.

SZERB, J. C.: Model experiments with Gaddum's push-pull cannulae. Can. J. Physiol. Pharmacol. **45:** 613–620, 1967.

TAGLIAMONTE, A., TAGLIAMONTE, P., PEREZ-CRUET, J., STEIN, S. AND GESSA, G.: Effect of psychotropic drugs on tryptophan concentration in the rat brain. J. Pharmacol. Exp. Ther. **177:** 475–480, 1971.

THOMAS, R. C. AND WILSON, V. J.: Precise localization of Renshaw cells with a new marking technique. Nature (London) **206:** 211–213, 1965.

TILSON, H. A. AND SPARBER, S. S.: Studies on the concurrent behavioral and neurochemical effects of psychoactive drugs using the push-pull cannula. J. Pharmacol. Exp. Ther. **181:** 387–398, 1972.