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STUDIES ON THE CONCURRENT BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF PSYCHOACTIVE DRUGS USING THE PUSH-PULL CANNULA[1,2]

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ABSTRACT

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Intraventricular infusions of ^{3}H-norepinephrine or ^{14}C-serotonin were given 1 hour or 30 minutes, respectively, before rats had their lateral ventricles perfused with artificial cerebrospinal fluid through chronic-indwelling push-pull cannulas while responding for food on a fixed-ratio 30 schedule of reinforcement. Twelve to 15 minutes into the session, they were injected i.p. with *d*-amphetamine (1.0, 2.5 or 5.0 mg/kg), mescaline (10, 15 or 20 mg/kg), *d*-lysergic acid diethylamide (LSD) (0.4 mg/kg) or 0.5 ml of isotonic NaCl. Only *d*-amphetamine produced a dose-dependent increase of tritium in the perfusate after injection, in addition to dose-related shortening of latencies to behavioral disruption. Thin-layer chromatographic analysis of the perfusate, taken during the 5.0 mg of *d*-amphetamine per kg session, revealed increased concentrations of radioactivity in segments having R_f values of authentic norepinephrine and normetanephrine. Mescaline produced similar effects on behavior, but produced dose-dependent increases only in ^{14}C-radioactivity soon after drug administration. Thin-layer chromatographic separation of the perfusate after administration of 20 mg of mescaline per kg revealed apparent increased proportions of ^{14}C-5-hydroxyindoleacetic acid. LSD (0.4 mg/kg) disrupted fixed-ratio responding in a manner similar to the high doses of mescaline and *d*-amphetamine but produced a significant decrease in ^{14}C released without altering the metabolic disposition of 5-hydroxytryptamine or its acid metabolite. These data suggest that mescaline and *d*-amphetamine may act presynaptically to produce the release and/or block the reuptake of serotonin and norepinephrine, respectively. LSD, on the other hand, produces an apparent inhibition of release of 5-hydroxytryptamine, perhaps *via* a presynaptic action or through postsynaptic feedback inhibition.

The alteration in the disposition of biogenic amines has been implicated in the mechanism

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[2] A preliminary report of some of this work has been presented elsewhere (79th Proceedings of the American Psychological Association, pp. 739–740, 1971).

[3] This work represents a portion of a thesis submitted to the Faculty of the University of Minnesota by H.A.T. in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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action of several psychoactive substances. High doses of *d*-amphetamine, for example, have been shown to deplete brain catecholamines (CA) (McLean and McCartney, 1961; Sanan and Vogt, 1962) and in more moderate doses are believed to act by producing the release and/or by blocking the reuptake of norepinephrine (NE) (Sulser and Sanders-Bush, 1971). *d*-Amphetamine does not consistently lower brain serotonin (5-hydroxytryptamine, 5-HT) (Paasonen and Vogt, 1956; McLean and McCartney, 1961), although high doses have been shown to increase 5-HT turnover (Reid, 1970). Psychotomimetics have been reported to produce small decreases in brain CA levels (Freedman, 1963; Sugrue, 1969), but the mechanism appears not to be one of release from the synapse (Leonard and Tonge, 1969). In addition, small increases in brain 5-HT levels have been reported (Giarman and Freedman, 1965) and it has been suggested that *d*-lysergic acid diethylamide (LSD) may block the release and/or decrease the turnover of 5-HT (Freedman *et al.*, 1970). Because of the similarity in behavioral effects between LSD and mescaline, it has been suggested that these two hallucinogens act through common site(s) (Giarman and Freedman, 1965).

In vivo evidence for *d*-amphetamine-induced release of CA in solid brain tissue has been reported using the push-pull cannula (McKenzie and Szerb, 1968; Stein and Wise, 1969). The use of this technic, however, has been criticized since tissue damage at the tip of the cannula might possibly alter the integrity of the perfused area (Chase and Kopin, 1968; Portig and Vogt, 1969). Concentrations of biogenic amines and their metabolites in the cerebrospinal fluid (CSF) appear to be a sensitive measure of brain metabolism of the transmitter candidates (Ashcroft *et al.*, 1969; Moir *et al.*, 1970; Cserr, 1971) and we have recently demonstrated the feasibility of perfusion of the lateral ventricles of rats with push-pull cannulas while they were responding on an operant schedule for food reinforcement (Tilson and Sparber, 1970). Subsequently, we have reported what appears to be a schedule-induced (*i.e.*, extinction) and specific *d*-amphetamine-elicited release of ^{3}H-NE (and metabolites) in the ventricular perfusate (Sparber and Tilson, 1972). We have expanded upon this work and report dose-related behavioral disruption and release of ^{3}H-NE and ^{14}C-5-HT after i.p. injections of *d*-amphetamine and mescaline, respectively. Lysergic acid diethylamine-25 (LSD), at the dose used, produced behavioral disruption similar to 15 to 20 mg of mescaline per kg but contrary to mescaline produced a decrease in the total amount of 5-HT in the perfusate after injection.

Methods

Subjects and ventricular implantation. Seven male rats (Long-Evans; Simonsen Laboratories, Gilroy, Calif.) were housed individually in a room with a 12-hour day-night cycle (6:45 A.M. to 6:45 P.M.) and relatively constant environment (27°C and 50% humidity). The animals were anesthetized with sodium pentobarbital (35 mg/kg) and had their right lateral ventricles implanted with push-pull cannulas (Gaddum, 1961). The cannulas were constructed of stainless-steel tubing, the inside tube being 29 gauge (0.014 inch outside diameter) and the outside, 20 gauge (0.023 inch inside diameter). Optimal protrusion of the inner tube was 0.5 mm (Szerb, 1967). Coordinates for the implant were determined by measuring 1 mm laterally and 1 mm posteriorly from the anterior bregma and lowering the cannula 3.45 to 3.55 mm into the brain through a hole in the skull (Noble *et al.*, 1967). After permanent attachment of the cannula with acrylic dental cement and screws anchored to the skull, the wound was closed and the animals allowed to recover for at least two weeks.

Conditioning and Drug Sessions. The animals (350–400 g) were food-deprived and maintained at 75 to 80% of their free-feeding body weight. They then were trained to lever-press for food reinforcement (45-mg pellets, P. J. Noyes Co., Lancaster, N.H.) and eventually stabilized on a fixed-ratio (FR) 30 schedule of reinforcement (*i.e.*, every 30th response provided access to a food pellet). The operant chamber (Lehigh Valley Electronics, Inc., Fogelsville, Pa.; model 143-22) was contained within a sound-attenuated chamber (Lehigh Valley Electronics, Inc. model 132-02) equipped with a masking noise. All responses and schedule events were recorded and controlled automatically with standard electromechanical equipment.

After stabilization of FR 30 responding, the rats were habituated to having their lateral ventricles perfused with artificial CSF (Robinson *et al.*, 1968) at a rate of 20 μl-min during behavioral sessions which were terminated after the collection of 15 two-minute samples. The method of perfusion and automatic collection of samples of ventricular perfusate have been described in detail elsewhere (Sparber and Tilson, 1972).

Drug sessions followed habituation to the perfusion procedure and were separated by at least

72 hours to reduce possible formation of tolerance to the behavioral effects of the drugs (Freedman *et al.*, 1958; Tilson and Sparber, 1971). One hour before the rats were placed into the operant chamber, they were slowly injected intraventricularly (I.v.) with 5 μl of an acetic acid solution (0.1 N) containing 6.4 to 85 m μg of 3H-*dl*-NE (0.25–5.0 μc) *via* the inner cannula tube. When release of 5-HT was studied, similar infusions of ^{14}C-5-HT (9.0–9.96 μg; 0.11–0.50 μc) were given 30 minutes before the session began. The animals then were placed into the operant chamber, connected to the perfusion device and immediately allowed to lever-press for food. Approximately 12 to 15 minutes after the establishment of the proper flow rate (20 μl/min), they were injected (i.p.) with 0.5 ml of isotonic saline (NaCl), mescaline hydrochloride (10, 15 or 20 mg/kg), *d*-amphetamine sulfate (1.0, 2.5 or 5.0 mg/kg) or *d*-LSD (0.4 mg/kg). This dose of LSD was used because it has been shown to produce an increase in brain 5-HT (Freedman *et al.*, 1970) and to produce an onset and duration of disruption of FR responding similar to 15 to 20 mg of mescaline per kg (Sparber and Tilson, 1971).

Assay of perfusate. Immediately after the perfusion, 10-μl aliquots from each of the 15 samples of perfusate were placed in counting vials containing 10 ml of scintillator liquid (6 g of 2,5-diphenyloxazole per l of toluene) and 50 μl BBS-3 (Bio-Solv, Beckman Instruments, Inc., Fullerton, Calif.). They then were counted in a Beckman DPM-100 liquid scintillation spectrometer at ambient temperature. Perfusate obtained during sessions using NaCl, 5.0 mg of *d*-amphetamine per kg, 20 mg of mescaline per kg or 0.4 mg of LSD per kg was analyzed further for ^{3}H-NE, ^{14}C-5-HT and their metabolites. Ten microliters of the remaining perfusate from a sample two to four minutes prior to (sample no. 4), two to four minutes after (sample no. 8) and 14 to 16 minutes after (sample no. 15) drug injection were spotted on plastic thin-layer chromatography (TLC) plates precoated with cellulose MN 300 (Brinkman Instruments, Inc., Waterbury, N.Y.). To each spot were added 5 μl of 0.01 N HCl solution containing a mixture of 0.5 μg each of NE, normetanephrine (NM) and 3,4-dihydroxymandelic acid or 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) (Calbiochem, Los Angeles, Calif.). Replicate TLC plates containing the standard NE or 5-HT and metabolites were allowed to develop concurrently in an acidified butanol solvent system (butanol, 3 N HCl, 100:35) in an ascending chromatography tank at 27°C. Visualization of the standards was by *p*-nitroanaline (De Potter *et al.*, 1965). The TLC plates containing the perfusate and cold carrier were cut into 1.0 cm^2 segments around R_f values of the standards and continued back to the origin and up to the solvent front. The serial segments were then placed into counting vials containing 10 ml of scintillation liquid (without BBS-3), shaken and counted. Since counting efficiencies for all segments containing the isotope in use during that experiment were approximately the same, no attempt was made to convert from counts per minute to disintegrations per minute. The results are, therefore, expressed as a percentage of radioactivity in the individual segments using total counts per minute on the TLC plate as 100%. In the case of NE and its metabolites, radioactivity (in counts per minute) other than that residing in the segments thought to contain NE and NM is referred to as deaminated-O-methylated metabolites (and/or conjugated and unidentified substances, DOM).

Drugs and radioactive materials. The drugs used were mescaline hydrochloride (Aldrich Chemical Co., Milwaukee, Wisc.), *d*-amphetamine sulfate (K & K Laboratories, Inc., Plainview, N.Y.) and *d*-lysergic acid diethylamide-25 (LSD) tartrate in saline (FDA-PHS Psychotomimetics Agents Advisory Committee). *d*-Amphetamine and mescaline were injected i.p., as the salt, whereas the dose of LSD is expressed as actual LSD substance. The *dl*-norepinephrine-7-^{3}H (6.6–9.95 c/mmol) and serotonin-^{14}C (12.8–15.3 mc/mmol) were obtained from New England Nuclear Corporation (Boston, Mass.).

Results

Drug-induced appearance of ^{3}H-NE or ^{14}C-5-HT in the ventricular perfusate. Previous studies in this laboratory (Sparber and Tilson, 1972) have indicated that there is a high level of radioactivity in initial samples. A "washout" follows which is characterized by a rapid drop of counts in successive samples reaching an asymptotic level at about the fourth sample. The profile of tritium (^{3}H) in each sample after the injection of *d*-amphetamine and NaCl, therefore, are reported relative to sample no. 4 (fig. 1). There were dose-related increases in radioactivity which were significantly different from NaCl control values two to six minutes after the injection of 2.5 and 5.0 mg of *d*-amphetamine per kg. The lower dose of *d*-amphetamine (1.0 mg/kg) produced increases in the total radioactivity in samples after the injection, but the peak effect (sample no. 8) did not significantly differ from the controls because of variability. Since all three drugs produced behavioral effects which lasted throughout the remainder of the

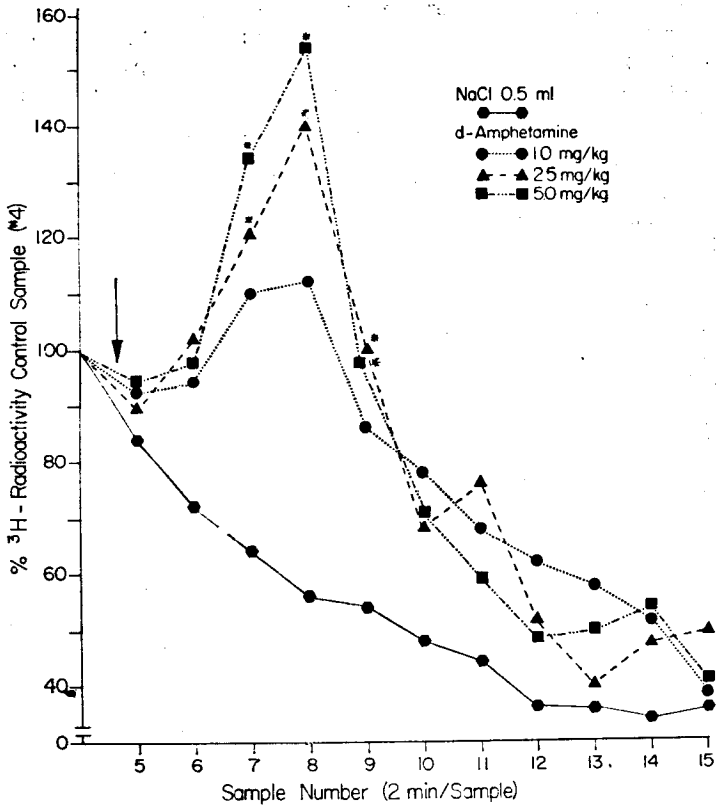

FIG. 1. Release of radioactivity from ^{3}H-NE into the perfusate from the ventricles after the i.p. injection of various doses of *d*-amphetamine or NaCl. Results are expressed as a percentage of radioactivity compared to sample no. 4 (100%). Each point is the mean of three experiments for the two lower doses and six for the 5.0 mg/kg dose and the NaCl experiments. * indicates a significant difference from NaCl levels using a matched pair *t* test, $P < .05$. ↓ indicates the approximate point of injection.

session, we also compared the radioactivity in sample no. 4 (preinjection) with the average radioactivity in samples after drug injection (samples 5–15) (table 1). As can be seen, this measure shows that all three doses of *d*-amphetamine produced significant increases in radioactivity in samples after injection, whereas mescaline or LSD did not produce a peak or trough (*i.e.*, did not significantly alter the total ^{3}H in subsequent samples).

Mescaline (15 and 20 mg/kg) produced significant peaks in ^{14}C-radioactivity two to six minutes after the injection, but the lower dose of mescaline (10 mg/kg) did not appear to alter the disposition of ^{14}C-5-HT (and metabolites) as analyzed by this method (fig. 2). Table 1 also shows that the two higher doses of mescaline produced significant increases in average total radioactivity in samples following the injection, whereas the lower dose likewise did not differ from NaCl controls using this method of analysis. In contrast to mescaline, LSD produced a decrease in the amount of ^{14}C-radioactivity recovered in the perfusate starting almost immediately after i.p. administration and attained a level of statistical significance (at least $P < .05$) in samples 10 to 15 (about 10–20 minutes postinjection). As can be seen in table 1, which analyzes the effect in terms of an average decrease in samples after, relative to a sample before injection, LSD (0.4 mg/kg) produced a significantly lower ($P < .05$) total amount of radioactivity in the perfusate than was obtained following NaCl. Table 1 also shows that *d*-amphetamine, at the doses used, had no effect upon radioactivity derived from ^{14}C-5-HT.

TLC analysis of perfusate. There were marked changes in the content of unchanged NE and its metabolites in the perfusate after administration of 5.0 mg of *d*-amphetamine per kg and the

results are summarized in table 2. The percentages of NE, NM and DOM in the perfusate of the sample before the injection (sample no. 4) are almost identical to similar samples taken during control sessions. In addition, the ratio of DOM to NE and NM [(*i.e.*, DOM/(NE + NM)] was also comparable. Two to four minutes after 5.0 mg of *d*-amphetamine per kg, there were increases in the percentage of NE and NM as well as a decrease in the percentage of DOM with a corresponding decrease in the ratio from 2.07 to 0.30. This was not the result of merely a shift in relative distribution of NE, NM and DOM, but more a reflection of increased radioactivity in perfusate (release) which appeared as unchanged NE. There was little change in the appearance of ^{3}H-NE or metabolites two to four minutes (sample no. 8) after injections of NaCl, mescaline (20 mg/kg) or LSD (0.4 mg/kg). An analysis of the perfusate taken approximately 15 minutes after the injection of *d*-amphetamine (sample no. 5) indicates continued changes in CA metabolism. The percentage of unchanged NE fell to within predrug levels, but NM was still elevated. In addition, there was also a corresponding decrease in relative values of DOM and the ratio remained diminished (0.45). Neither mescaline nor LSD produced significant alterations in the pattern of radioactivity in these samples.

Table 3 summarizes the data obtained from the chromatographic separation of ^{14}C (5-HT and 5-HIAA). The preinjection distribution of radioactivity in sample no. 4 revealed no significant differences in the pattern of radioactivity for any of the treatment conditions. Mescaline (20 mg/kg) almost doubled the percentage of 5-HIAA in sample no. 8 compared with sample no. 4 (preinjection). In addition, there was a significant decrease in the percentage of 5-HT in that sample (no. 8) as well as an increase in the percentage of radioactivity in the other segments of the TLC plate. Sample no. 15 also contained less 5-HT with a concurrent tripling in radioactivity at the 5-HIAA segment. LSD or *d*-amphetamine, on the other hand, did not alter the pattern of the distribution of radioactiviy compared to preinjection levels.

As can be seen in tables 2 and 3, approximately 60 and 40% of the radioactivity on the TLC plates could not be attributed to NE and 5-HT (or their major metabolites), respectively. It is interesting to note that under NaCl control conditions or when release did not follow drug administration, approximately 25% of the total radioactivity remained at the origin. This radioactivity also varied according to the neurotransmitter candidate and the drug investigated. For example, after the administration of 5 mg of *d*-amphetamine per kg (table 2), increases of NE and NM were associated with decreases in both the absolute and relative levels of radioactivity remaining at the origin. On the other hand, there were increases in both relative and absolute amounts of ^{14}C-radioactivity from ^{14}C-5-HT remaining at the origin two to four (sample no. 8) and 13 to 15 (sample no. 15) minutes after the injection of 20 mg of mescaline per kg (table 3).

The patterns of radioactivity obtained during those sessions when NaCl was injected after prelabeling with ^{14}C-5-HT or ^{3}H-NE were similar in their shape (see figs. 1 and 2). This may be important in view of earlier observations that when reported as a semilog plot, ^{3}H-NE produces what appears to be a declining, multi-

TABLE 1

Effect of i.p. drug administration on appearance of radioactivity in the ventricular perfusate

^{3}H-NE or ^{14}C-5-HT were infused l.v. one hour or 30 minutes before perfusion sessions, respectively, and drugs or NaCl were injected i.p. 12 to 15 minutes into the session. Results are expressed as mean ± S.E.M. percentage of radioactivity in samples subsequent to the injection relative to sample no. 4. Number of subjects is in parentheses.

Treatment	Mean % Radioactivity of Sample No. 4	
	^{14}C	^{3}H
NaCl	38.0 ± 2.8 (7)	51.1 ± 5.3 (6)
d-Amphetamine		
1.0 mg/kg	42.6 ± 1.5 (4)	72.6 ± 0.7[a] (3)
2.5 mg/kg	47.1 ± 5.4 (4)	78.5 ± 3.2[a] (3)
5.0 mg/kg	41.9 ± 1.8 (7)	89.3 ± 8.6[a] (6)
Mescaline		
10 mg/kg	46.2 ± 3.4 (4)	56.0 ± 4.5 (3)
15 mg/kg	65.7 ± 6.5[a] (4)	51.4 ± 3.9 (3)
20 mg/kg	68.9 ± 5.7[a] (7)	56.8 ± 4.7 (6)
d-LSD		
0.4 mg/kg	28.7 ± 3.6[a] (3)	42.6 ± 6.3 (3)

[a] Significantly different from NaCl value, matched pair *t* test, $P < .05$.

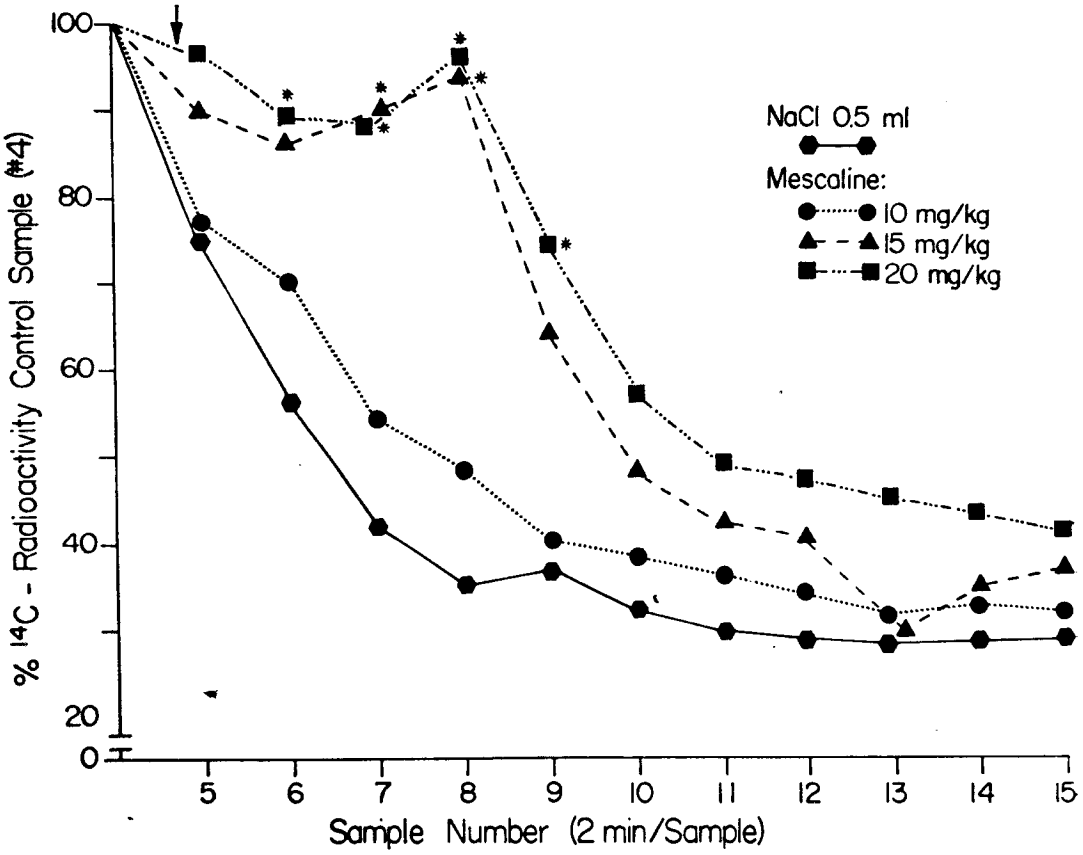

FIG. 2. Release of radioactivity from ^{14}C-5-HT into the perfusate from the ventricles after the i.p. administration of various doses of mescaline or NaCl. Results are mean percentage of radioactivity compared with sample no. 4 taken as 100%. Four animals were used for the experiments involving lower doses of mescaline and seven for the 20 mg of mescaline per kg and NaCl experiments. * indicates a significant difference from NaCl levels using a matched pair t test, $P < .05$. ↓ indicates the approximate point of injection.

exponential curve, which is in contrast to the single, rapidly declining curves obtained with ^{14}C-urea (Sparber and Tilson, 1972). Figure 3 shows a semilog plot of the perfusion curve taken 30 minutes after l.v. prelabeling with ^{14}C-5-HT and subsequent i.p. injection of NaCl and, as can be seen, there is a decremental curve which likewise appears to have more than one function. This would imply multicompartmental distribution rather than being extracellular and dependent mostly upon CSF flow and diffusion.

Drug effects upon FR responding during perfusion. There was dose-dependent disruption of responding after i.p. administration of both *d*-amphetamine and mescaline. The latency to onset of behavioral action (*i.e.*, no reinforcers for at least two minutes) followed a dose-related pattern with a lower dose requiring longer to disrupt behavior than higher doses. Although in most cases responding did not resume before the end of the session (about 30 minutes after injection), it has been shown previously that latency is a good indicator of drug effect and well correlated with duration of behavioral disruption of FR responding (Sparber and Tilson, 1971). The equieffectiveness of LSD (0.4 mg/kg), mescaline (20 mg/kg) and *d*-amphetamine (5 mg/kg) is exhibited by their similar latencies to onset of action (table 4). Figure 4 contains sample cumulative records obtained from rat A-26 during the perfusions when ^{14}C-5-HT was injected 30 minutes before perfusion. There was no observable effect of the perfusion process on FR responding and pauses occurred only after reinforcement. Note that the responding before the

TABLE 2

Effect of i.p. administration of drugs on percentage of ^{3}H-NE and ^{3}H-metabolites of total radioactivity in the perfusate

Samples of perfusate before and after injection of psychoactive substances were analyzed by TLC for percentage of ^{3}H-NE and ^{3}H-NM of total counts per minute on the TLC plate. All other counts on the plate are ^{3}H-DOM. Results are mean ± S.E.M. of percentage of total radioactivity and each value is the mean of the same three subjects.

Treatment	Sample No.	Mean Percent—Total Radioactivity			R = DOM/(NE + NM)
		% NE	% NM	% DOM	
NaCl, 0.5 ml	4	28.2 ± 2.4	9.7 ± 2.8	63.9 ± 1.6	1.78 ± .18
	8	32.8 ± 5.2	8.8 ± 0.6	58.4 ± 5.8	1.52 ± .40
	15	29.4 ± 2.2	8.8 ± 1.0	62.0 ± 2.0	1.65 ± .15
d-Amphetamine, 5.0 mg/kg	4	26.9 ± 0.7	8.2 ± 2.0	67.4 ± 2.9	2.07 ± .08
	8	55.4 ± 3.9[a]	21.9 ± 2.4[a]	22.8 ± 2.7[a]	0.30 ± .05[a]
	15	33.5 ± 5.1	38.3 ± 10.9[a]	28.2 ± 9.4[a]	0.45 ± .21[a]
Mescaline, 20 mg/kg	4	28.8 ± 0.9	5.5 ± 1.5	65.8 ± 1.0	1.93 ± .09
	8	29.6 ± 2.3	5.8 ± 1.2	64.6 ± 2.6	1.86 ± .22
	15	30.1 ± 0.8	5.6 ± 0.7	64.3 ± 1.8	1.81 ± .10
d-LSD, 0.4 mg/kg	4	33.6 ± 1.8	9.0 ± 0.6	60.1 ± 2.6	1.53 ± .17
	8	32.5 ± 1.1	4.0 ± 1.2	63.6 ± 2.3	1.76 ± .16
	15	30.1 ± 0.5	5.7 ± 1.5	63.4 ± 1.3	1.74 ± .09

[a] Significantly different from predrug value of same treatment, matched pair *t* test, $P < .05$.

TABLE 3

Effect of drugs administered i.p. on percent ^{14}C-5-HT and ^{14}C-5-HIAA of total radioactivity in the perfusate

Samples of perfusate before and after i.p. injection of psychoactive substances were analyzed by TLC for percentage of ^{14}C-5-HT or ^{14}C-5-HIAA. Results are mean ± S.E.M. of percentage of total radioactivity and each value is the mean of the same three subjects.

Treatment	Sample No.	Mean Percent—Total Radioactivity		
		% 5-HT	% 5-HIAA	% Other
NaCl, 0.5 ml	4	62.3 ± 3.3	3.9 ± 0.4	33.8 ± 3.6
	8	59.9 ± 6.7	1.6 ± 0.1	48.7 ± 6.5
	15	61.5 ± 1.3	1.2 ± 0.1	37.3 ± 1.2
d-Amphetamine, 5.0 mg/kg	4	57.2 ± 3.2	3.9 ± 0.1	38.9 ± 3.2
	8	63.0 ± 3.6	2.3 ± 0.5	34.7 ± 6.3
	15	60.9 ± 5.3	2.8 ± 0.7	36.3 ± 4.6
Mescaline, 20 mg/kg	4	62.6 ± 1.5	5.4 ± 1.8	31.9 ± 0.3
	8	35.5 ± 0.4[a]	8.5 ± 0.9[a]	55.4 ± 3.5[a]
	15	30.5 ± 3.7[a]	16.4 ± 2.6[a]	53.0 ± 5.7[a]
d-LSD, 0.4 mg/kg	4	54.8 ± 7.2	3.1 ± 0.5	43.9 ± 6.9
	8	55.9 ± 3.0	2.8 ± 0.3	41.4 ± 2.7
	15	55.0 ± 8.9	2.9 ± 0.8	42.3 ± 7.8

[a] Significantly different from preinjection level of same treatment, matched pair *t* test, $P < .05$.

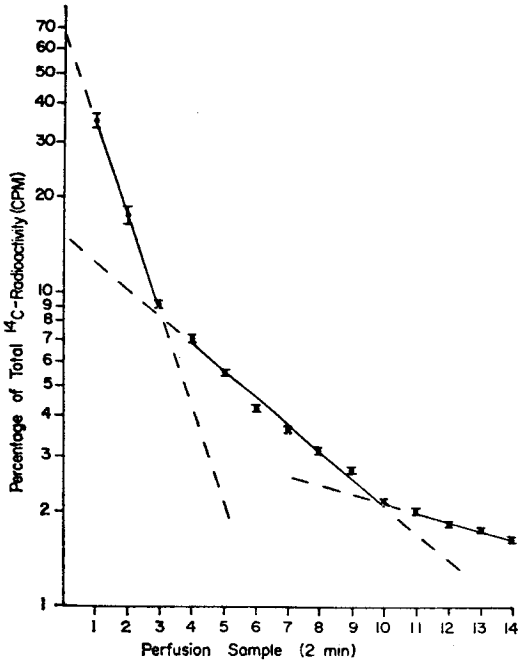

FIG. 3. Semi-logarithmic plot of radioactivity in 10-μl aliquots of two-minute serial samples of ventricular perfusate. The total radioactivity (in counts per minute) for each perfusion session was taken as 100%. Each point is the mean ± S.E.M. percentage of total counts per minute and is based upon observations from seven different subjects. The points of extrapolation were arbitrary and are merely to show that the declining curve is comprised of at least two functions.

drug injections (indicated by arrow) is stable and comparable to that seen during the control session when NaCl was injected.

Discussion

The data presented in this investigation support earlier observations that perfusion of the lateral ventricles of rats with push-pull cannulas provides useful information concerning the relase and/or uptake processes of suspected neurotransmitters in areas surrounding the cerebroventricular system (Sparber and Tilson, 1972). The advantage of this technique is that it allows for the concurrent measurement of dose-related neurochemical and behavioral effects of drugs in the same animals, without having to rely upon correlative data obtained from separate groups of subjects. Furthermore, this combined procedure has allowed for the demonstration of what appears to be specific release of 5-HT or NE without the use of extracellular "markers" such as ^{14}C-urea. Mescaline and *d*-amphetamine, for example, both produced a dose-dependent latency to onset of behavioral disruption which was accompanied by a specific dose-related increase in radioactivity from either ^{14}C-5-HT or ^{3}H-NE, respectively, in the perfusate after i.p. drug injection. LSD (0.4 mg/kg) produced a behavioral alteration similar to the higher doses of mescaline and *d*-amphetamine but instead decreased the total amount of ^{14}C-radioactivity from ^{14}C-5-HT, having no discernible action on catecholaminergic pools. Additional evidence for specificity of drug action lies in the distribution of metabolites separated from perfusion samples two to four and 14 to 16 minutes after the administration of *d*-amphetamine and mescaline. Therefore, these results indicate that *d*-amphetamine's operant behavioral disruptive action is temporally related to the release and/or blockade of the reuptake of NE, especially at the higher doses, since they produced a significant increase of ^{3}H-radioactivity in the perfusate almost immediately after the injection. In addition, *d*-amphetamine produced a significant increase in unchanged ^{3}H-NE as well as ^{3}H-NM, indicating that NE was being released from functional stores and acted upon by catechol-O-methyltransferase. These data also

TABLE 4

Latency to onset of behavioral disruption after drug administration

Rats were injected i.p. with drugs 10 to 15 minutes into the perfusion session. Latency to drug action is defined as the period between injection of the drug and the point in time when no reinforcers were delivered for two minutes. Each point is the mean ± S.E.M. Numbers in parentheses represent the number of experiments performed at that dose.

Treatment	Latency
	min ± S.E.M.
Mescaline	
10 mg/kg (7)	4.5 ± 0.3
15 mg/kg (7)	3.6 ± 0.2
20 mg/kg (13)	2.9 ± 0.2
d-Amphetamine	
1.0 mg/kg (7)	6.5 ± 0.8
2.5 mg/kg (7)	3.9 ± 0.2
5.0 mg/kg (13)	2.7 ± 0.2
d-LSD	
0.4 mg/kg (6)	2.8 ± 0.2

support other investigations showing this drug acts to produce alterations in catecholamines in structures near the cerebroventricular system (Javoy *et al.*, 1970; Besson *et al.*, 1971). Fuxe and Ungerstedt (1968), using a fluorescence histochemical technique, have also reported what appears to be release of extragranular catecholamines from central noradrenergic and dopaminergic neurons near the cerebroventricular space after *d*-amphetamine. Finally, the perfusion data showing increased concentrations of NE (and/or metabolites) are consistent with those who have reported *in vivo* release of catecholamines in the brain after peripheral administration of *d*-amphetamine (Stein and Wise, 1969; Sparber and Tilson, 1972) and central perfusion of the caudate nucleus (McKenzie and Szerb, 1968; Riddell and Szerb, 1971) or the lateral ventricles (Carr and Moore, 1970) with an amphetamine solution.

LSD and mescaline did not significantly alter the disposition of ^{3}H-NE or its metabolites after i.p. injection, suggesting they did not facilitate release and/or block the reuptake of NE from functional stores in structures bordering the ventricles. These data appear to be inconsistent with those of Leonard and Tonge (1969) who have reported that LSD and mescaline significantly increased the NE depletion after inhibition of catecholamine synthesis by α-methyl-*p*-tyrosine. On the other hand, these investigators found that 30 minutes after the injection of 0.4 mg of LSD per kg or 20 mg of mescaline per kg there was no apparent increase in the level of NM in rat brain, suggesting that these drugs may act to release NE intraneuronally. LSD and mescaline might have produced a release of NE, as measured by our method, if higher doses had been used. Goldstein *et al.* (1970), for example, have reported that approximately 20 minutes after the injection of 1.3 mg of LSD per kg, there were increases in ^{3}H-NM and ^{3}H-deaminated metabolites associated with decreases in endogenous NE levels. The increase in NE metabolites reported by Goldstein *et al.* (1970) might have been more a reflection of increased activity of afferents to the central nervous system resulting from LSD's autonomic stimulatory action (Freedman *et al.*, 1958) and not its so-called psychotomimetic action. The dose Goldstein *et al.* (1970) employed was 3 times that which, in our hands, produced 45 to 60 minutes of complete operant behavioral disruption.

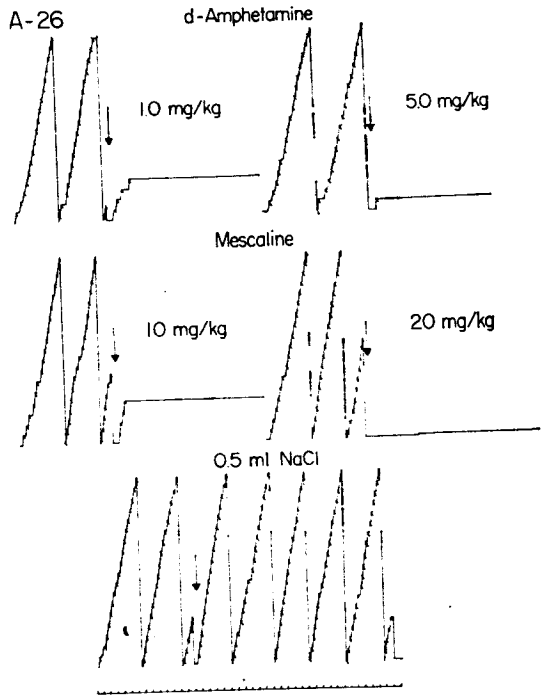

FIG. 4. Sample cumulative records from rat A26 showing dose-related disruption of FR 30 responding. Mescaline, *d*-amphetamine or NaCl were injected i.p. 10–15 minutes into the session which is indicated by ↓ and resetting of the recorder pen. Each excursion of the event pen at the bottom of the record indicates one minute and the recorder pen automatically resets after 550 responses. Note that the higher doses of *d*-amphetamine and mescaline produced a more rapid onset of behavioral disruption than the lower doses. The rate of responding before drug injection is similar to that obtained before and after the injection of NaCl, indicating that the perfusion procedure and injection *per se* did not affect food-reinforced responding.

The decrease in total ^{14}C-radioactivity (from 5-HT) in samples after injection of LSD is in accord with other data indicating that this substance inhibits the release of 5-HT from electrically stimulated brain slices (Chase *et al.*, 1967) and decreases the turnover rate of brain 5-HT (Freedman, 1961; Rosecrans *et al.*, 1967; Andén *et al.*, 1968). Other studies also have shown that LSD decreases the conversion of ^{14}C-tryptophan to ^{14}C-5-HT in rats (Lin *et al.*, 1969) and mice (Schubert *et al.*, 1970), as well as decreasing the brain levels of radioactive deaminated metabolites of ^{14}C-5-HT after preinjection into the ventricular system of rats (Schildkraut *et al.*, 1969). LSD has also been reported to decrease the amount of 5-HT and 5-HIAA in the

forebrain after electrical stimulation of the midbrain raphé (Randic and Padjen, 1971), to decrease the firing rate of these neurons after i.v. administration (Aghajanian *et al.*, 1968) and to antagonize 5-HT-excitation of single neurons when LSD is applied iontophoretically or administered i.v. (Boakes *et al.*, 1970). Mescaline, on the other hand, has been shown to increase the firing rate of some subgroups of neurons in the midbrain raphé (Aghajanian *et al.*, 1970). Since electrical stimulation of the area produces increases in 5-HT and 5-HIAA in the forebrain of rats (Aghajanian *et al.*, 1967) as well as in the CSF of cats (Sheard and Zolovick, 1971), mescaline, at 15 and 20 mg/kg, may be acting to produce the release, enhance the synthesis and/or block the reuptake of 5-HT. This suggestion is also supported by the investigations of others showing that 5 mg of mescaline per kg i.p. in rats significantly decreased brain levels of tryptophan, the amino acid precursor of 5-HT (Tonge and Leonard, 1970) and that mescaline (5–15 mg/kg) produces slight but significant increases in both whole brain 5-HT and 5-HIAA (Freedman *et al.*, 1970). In the study of Tonge and Leonard (1970), it was also reported that LSD (0.1 mg/kg) produced an increase in tryptophan levels, suggesting an inhibition of 5-HT synthesis and/or block of 5-HT release. The report by Kopin (1970) indicating a similar action of mescaline and LSD on 5-HT release form stimulated brain slices (both decreased release) initially appears contrary to the above hypothesis. However, the lowest extrapolated concentration of mescaline necessary to produce an inhibition of 5-HT release is 10^{-4} M, about 10 times greater than the concentration of mescaline found in whole brain of rats 20 to 60 minutes after i.p. administration of 10 to 18 mg/kg (Denber and Teller, 1969).

Using a solvent system similar to ours, Giese *et al.* (1967) reported that approximately 25% of total radioactivity on the TLC plate remained at the origin after separation of ^{3}H-catecholamines from nonhydrolyzed samples of brain tissue. In attempting to separate radioactive biogenic amines from metabolites in ventricular perfusate, a similar effect was obtained in these investigations. This suggested the presence of a nonmigratory substance occurring in the CSF which may have functional significance. For example, the release of transmitter candidates such as acetylcholine and NE from intraneuronal stores has been reported to occur with the simultaneous release of soluble proteins which may be bound to the transmitter (Kirshner *et al.*, 1966; Matsuda *et al.*, 1971). Axon-like processes with possible neurosecretory function have been observed in the lateral ventricles of cats (Noack and Wolff, 1971) and offer an anatomical basis for released substances of vesicular origin in the CSF. The contention for the presence of a protein-like substance in the CSF, which is dependent upon tonic release of stored NE, is supported by the observation that 5.0 mg of *d*-amphetamine per kg [which is believed to act by releasing a nonstored, functional pool of NE (Sulser and Sanders-Bush, 1971)], significantly reduced the proportion of counts remaining at the origin in samples after the injection. A different mechanism of release of 5-HT by 20 mg of mescaline per kg is suggested by the observation that there were significant increases in the proportion of radioactivity in this segment for samples taken two to four minutes after the injection.

Conclusions

This investigation shows that the push-pull cannula can be used to perfuse the lateral ventricles of rats while they are engaged in an operant response so that simultaneous dose-related drug alterations of neurochemistry and behavior can be obtained and studied. Our data suggest that *d*-amphetamine and mescaline act to produce a dose-related release and/or to block the reuptake of NE and 5-HT, respectively. Furthermore, the action appears to occur in a temporally related fashion to the onset of behavioral disruption. LSD, on the other hand, appears to produce a net inhibition of the release of 5-HT while producing its operant behavioral disruptive effects.

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