Pharmacology Biochemistry & Behavior, Vol. 5, pp. 35–37.

Alterations in the Behavioral Effects of LSD by Motivational and Neurohumoral Variables[1]

J. A. JOSEPH AND J. B. APPEL

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

(Received 6 February 1976)

JOSEPH, J. A. AND J. B. APPEL. *Alterations in the behavioral effects of LSD by motivational and neurohumoral variables.* PHARMAC. BIOCHEM. BEHAV. 5(1) 35–37, 1976. – Forty naive male albino rats were trained to press a bar on a fixed-ratio (FR 32) schedule of water reinforcement. They were then divided into two groups, one of which (N = 20) received 5 min of extra water 12 hr before each experimental session; the other group (N = 20) received no extra water. Half of the animals in each group was then given three daily doses (100 mg/kg) of the tryptophan hydroxylase inhibitor p-chlorophenylalanine methyl ester (PCPA) while the remaining animals were given control injections of the PCPA vehicle. Ten days following the last administration of PCPA (or vehicle) all animals were given a low dose of LSD (20 μg/kg). Bar-pressing behavior was significantly disrupted only in those animals receiving both PCPA and extra water. Central (whole brain) concentrations of serotonin (5-HT) were significantly lower in all animals which had been treated with PCPA. These results, along with those previously reported, suggest that amount of deprivation can be an important determinant of both the ability of drugs to alter behavior and the dependence of such alterations upon underlying neuronal activity.

LSD PCPA 5-HT Motivation Fixed-ratio

RECENT results have supported the hypothesis that the behavioral effects of LSD and related indoleamine and phenylethylamine hallucinogens are mediated by central serotonin-containing neurons [1, 4, 5, 7, 10, 11, 12]. For example, in one series of experiments, it was shown that the ability of relatively low doses of LSD (20–40 μg/kg) to disrupt the bar-pressing behavior of rats maintained by fixed-ratio (FR) schedules of food reinforcement was enhanced by manipulations which significantly lowered the concentration of serotonin (5-HT) in brain. These included (1) administration of three daily doses (100 mg/kg) of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA) [4], and (2) lesions of the medial raphe [5]. Comparable doses of PCPA were also found to potentiate (1) the discriminative stimulus properties of mescaline [8] and LSD [9], and (2) a characteristic hyperactivity syndrome induced by much larger doses of LSD, e.g., 260–520 μg/kg [12].

Attempts to analyze further these relationships between changes in the functional activity of amines such as 5-HT and drug-induced alterations in behavior have been complicated by the observation that the extent of these alterations is highly dependent on numerous behavioral as well as pharmacological variables [3] one of the most important of which appears to be the amount of deprivation of the reinforcer used to maintain on-going behavior (motivation). Thus, in animals trained to press a bar on an FR schedule of food reinforcement, increasing deprivation (by decreasing body weight) was found to attenuate the effect of LSD over a wide range of doses, such that animals maintained at 80% of their ad lib body weight showed very little LSD-induced disruption at doses of 80 μg/kg while animals maintained at 90% of their free-feeding weights were disrupted by doses as low as 40 μg/kg [6].

In the present experiment we show that similar increases in deprivation will attenuate PCPA-induced hypersensitivity to LSD. That is, a low dose of LSD (20 μg/kg) disrupts bar pressing for water reinforcement only in animals which have been depleted of central 5-HT (by PCPA) and which have been given a small amount (5 min) of extra water (i.e., are not too severely deprived).

METHOD

Animals

Forty, naive male albino Sprague-Dawley rats obtained from Charles River Laboratories, Wilmington, MA were used. They were housed in individual cages in a room of constant temperature (75°F) and humidity (40–50%) and maintained on a 12 hr day–night cycle. Each animal was approximately 90 days old and weighed 200–250 g at the beginning of the experiment.

[1] This research was supported by USPHS Research Grants MH-24,333 and MH-24,593 from the National Institute of Mental Health. We thank Earl Utsey for technical assistance and Cheryl Funderburk for typing the manuscript. Reprint requests should be sent to J. B. Appel.

Apparatus

The behavioral studies were carried out in four commercially available experimental chambers (BRS/LVE Model 143-24). Each box contained a single lever or bar located to the right of one of the sides, a dim 28 V house light, and a dipper which delivered 0.05 ml of tap water. A force of 10–15 g was required to activate the lever. Each chamber was housed in a shell that provided sound- and light-attenuation (BRS/LVE Model 132-02). All experimental events were programmed by solid state circuitry located in an adjoining room. Bar-pressing responses were recorded on electromagnetic counters and cumulative recorders.

Procedures

Upon arrival, each rat was weighed, coded and placed into its home cage. For at least one week, it was allowed free access to food and water. During the second week, daily weights were recorded and the animals were deprived of water until they reached 80% of their ad lib weight. Preliminary training was then begun.

The animal was placed in a chamber and the reinforcement dipper was activated manually until the animal drank as soon as the dipper was presented; next, a shaping procedure was instituted to condition the bar-press response. In subsequent 40 min sessions, the number of responses required to obtain reinforcement was raised from 1 to 32 (FR 32). The animals were given no water outside the chamber during training.

When response rates were relatively stable on the FR 32 schedule, (1.79 responses/sec) the animals were divided into two groups in such a manner that the mean response rate between these groups did not differ by more than 50 responses. Each of the animals in one of the groups (N = 20) received 5 min of water 12 hr before each experimental session during which they typically drink about 14 ml; the animals in the other group (N = 20) received no extra water. In order to insure that the animals in the extra water groups were, in fact, less motivated to work for water reinforcement, testing continued until the mean number of responses of the group had declined by 1000 (to 1.43 responses/sec). The two groups were then divided further such that 1/2 of the animals in each group (N = 10) received intraperitoneal (IP) injections of 100 mg/kg of PCPA methyl ester (Obtained from Regis Biochemicals, Chicago, IL.) for 3 successive days (3 × 100 mg/kg) immediately before behavioral testing; the other 1/2 of each group (N = 10) received the PCPA vehicle. Animals within each subgroup (extra water and no extra water) were chosen so that the average predrug and vehicle response rates were approximately equal.

The animals were given IP injections of saline for 9 days after the three days of PCPA or vehicle. On the 10th day, the 12th day after the first exposure to PCPA (or vehicle), all animals were given 20 μg/kg of LSD (obtained from the National Institute of Drug Abuse) IP immediately prior to FR testing. These temporal and dosage parameters were selected on the basis of previous research [4].

To control for possible differences in response rates prior to drug administration, rates during the LSD session were converted to a per cent control score by dividing each animal's response rate following LSD by its mean rate during the 4 days prior to PCPA or vehicle administration (base line rate) and multiplying by 100 (LSD Response Rate/Base Line Response Rate × 100).

Following testing, 1/2 the animals in each group were sacrificed by decapitation; central 5-HT levels were determined by the column extraction procedure described by Anden and Magnusson [2].

RESULTS

Figure 1 shows the per cent control FR response rates for the four sessions prior to PCPA or vehicle administration (Sessions 1–4), the sessions of PCPA or vehicle administration (Sessions 5–7), and the session of LSD (20 μg/kg) (Session 17). As can be seen, there are no differences among groups prior to LSD administration. Following LSD, however, a lower average response rate occurs in all groups; the lowest rate occurs when PCPA and extra water are given.

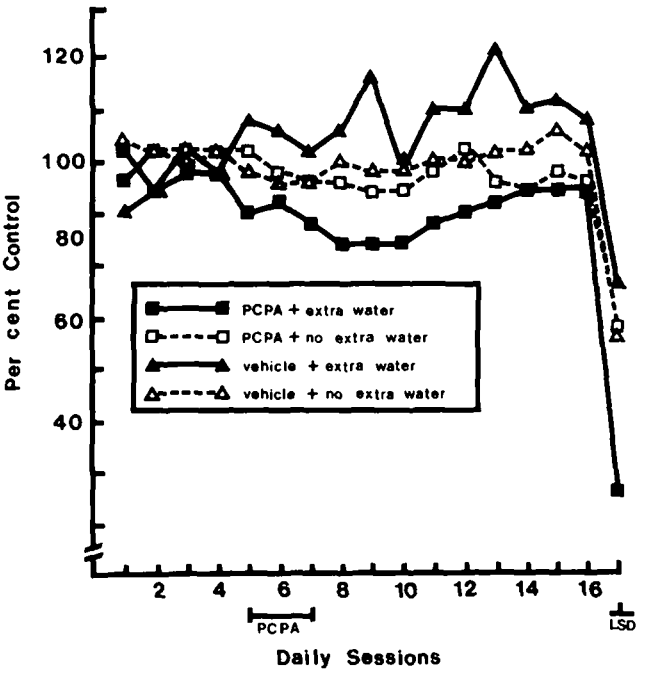

FIG. 1. Mean per cent of control (FR) response rate following treatment with PCPA methyl ester (100 mg/kg) of PCPA methyl ester vehicle on Days 5, 6, and 7. LSD (20 μg/kg) was given to all animals on Day 17 and NaCl (isotonic saline) was given on all other days.

An analysis of variance conducted on the data obtained on the LSD-day showed that the animals which had received PCPA tended to differ significantly from those which had received the vehicle $F(1,36) = 5.10$, $p<0.03$. However, this analysis also revealed that there was a significant interaction between the PCPA and water variables $F(1,36) = 5.22$, $p<0.03$. In addition, Duncan's post-hoc tests showed that the group which had received both treatments (PCPA and extra water) showed the lowest response rate and differed significantly from all other groups ($df = 36$, $p<0.05$); the other groups did not differ significantly from each other ($df = 36$, $p>0.05$). Thus, the significant interaction was due primarily to the disruption in the extra water-PCPA group.

It is interesting to note here that the group which had received extra water but no PCPA (Fig. 1) actually showed the least amount of disruption following LSD (response

rate was 80% of normal); this indicates that (1) extra water alone did not just simply lower response rate and (2) the rank order of effects did not parallel the amount of deprivation.

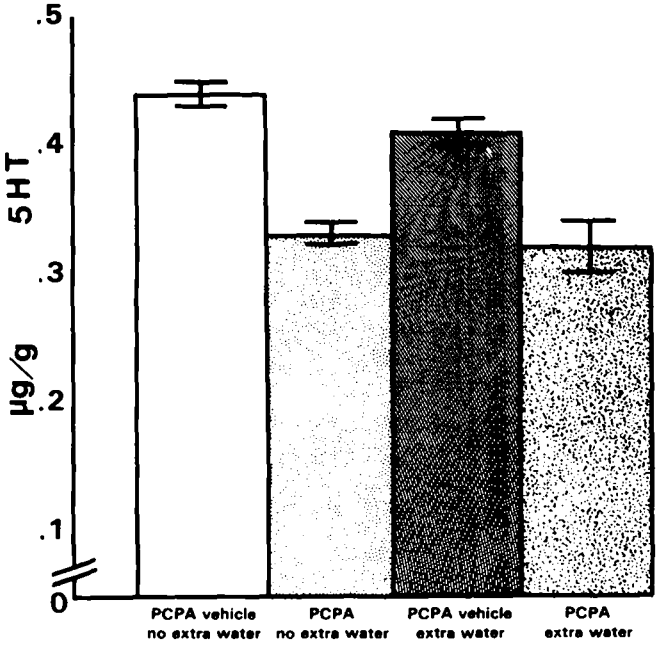

FIG. 2. Whole brain serotonin (5-HT) concentrations on the 17th day of the experiment (the 10th day after the final PCPA (or vehicle) treatment.

The results of the biochemical analysis are shown in Fig. 2. It can be seen that while PCPA reduced central 5-HT concentrations, there were no differences in depletion between animals receiving extra water and PCPA and those receiving PCPA alone. An analysis of variance showed that these effects of PCPA were significant $F(1,6) = 17.95$, $p<0.0009$, and that there was no differential depletion of 5-HT by extra water, i.e., the interaction between PCPA and water variables was not significant $F(1,16) = <1$, $p>0.9$.

DISCUSSION

While the behavioral effects of only one dose of LSD were examined in this experiment, the results clearly indicate that increasing water deprivation can attenuate, if not overcome, increased sensitivity to this compound following central 5-HT depletion [4]. Moreover, preliminary observations in our laboratory suggest that these relationships persist even when PCPA-induced depletion of 5-HT is as great as 50–60%. These results, along with those of Appel *et al.*, [6] reemphasize the point that the ability of relatively potent compounds to disrupt the frequency of on-going behavior and the dependency of these drug-behavior interactions on underlying neuronal activity are critically determined by the degree of motivation of the animal (i.e., the amount of deprivation of whatever reinforcer is maintaining its behavior); the methodological as well as the clinical implications of this observation should be obvious.

REFERENCES

1. Aghajanian, G. K. LSD and CNS transmission. *Ann. Rev. Pharmac.* 12: 157–168, 1972.
2. Anden, N. E. and T. Magnusson. An improved method for the fluorimetric determination of 5-hydroxytryptamine in tissues. *Acta Physiol. scand.* 69: 87–94, 1967.
3. Appel, J. B. The effects of "psychotomimetic" drugs on animal behavior. In: *Psychopharmacology: A Review of Progress 1957–1967*, edited by D. Efron. U.S. Public Health Service Publication No. 1836, 1968, pp. 1211–1222.
4. Appel, J. B., R. A. Lovell and D. X. Freedman. Alterations in the behavioral effects of lysergic acid diethylamide by pretreatment with p-chlorophenylalanine and alpha-methyl-p-tyrosine. *Psychopharmacology* 18: 387–406, 1970.
5. Appel, J. B., M. H. Sheard and D. X. Freedman. Alterations in the behavioral effects of LSD by midbrain raphe lesions. *Communs behav. Biol.* 5: 237–241, 1970.
6. Appel, J. B., W. E. Whitehead and D. X. Freedman. Motivation and the behavioral effects of LSD. *Psychon. Sci.* 12: 305–306, 1968.
7. Brawley, P. and J. C. Duffield. The pharmacology of hallucinogens. *Pharmac. Rev.* 24: 31–66, 1972.
8. Browne, R. G. and B. T. Ho. The role of serotonin in the discriminative stimulus properties of mescaline. *Pharmac. Biochem. Behav.* 3: 429–435, 1975.
9. Cameron, O. G. and J. B. Appel. A behavioral and pharmacological analysis of some discriminable properties of d-LSD in rats. *Psychopharmacology* 33: 117–134, 1973.
10. Greenberg, I., D. M. Kuhn and J. B. Appel. Behaviorally-induced sensitivity to the discriminable properties of LSD. *Psychopharmacology* 43: 229–232, 1975.
11. Haigler, H. J. and G. K. Aghajanian. Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. *J. neuron. Trans.* 35: 257–273, 1974.
12. Kuhn, D. M. and J. B. Appel. Effect of serotonin agonists and antagonists on motor activity in rats. Paper read at Society for Neuroscience. New York, 1975.