Pharmacology Biochemistry & Behavior, Vol. 18, pp. 863–865, 1983. © Ankho International. Printed in the U.S.A.

Increased Consumption of Diazepam During Continuous Amphetamine Administration

LINDA R. NELSON,[1] ALLEN D. POTTHOFF, AND GAYLORD D. ELLISON

Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024

Received 27 May 1982

NELSON, L. R., A. D. POTTHOFF AND G. D. ELLISON. *Increased consumption of diazepam during chronic amphetamine administration.* PHARMACOL BIOCHEM BEHAV **18**(6) 863–865, 1983.—Previous investigations in our laboratory have demonstrated that after implantation of slow-release d-amphetamine pellets, rats with free access to water and a 10% ethanol solution selectively increase their consumption of the ethanol solution. We now report that this d-amphetamine treatment produces a similar increase in drinking of a benzodiazepine (diazepam) solution. Female albino rats were given free access to water and a 0.060 mg/ml diazepam solution and fluid intake was recorded every two days. The baseline consumption of diazepam averaged 25% of the total daily fluid intake. After d-amphetamine pellet implantation, rats increased their diazepam consumption to an average of 48% of total fluid intake, whereas rats implanted with control pellets containing vehicle only showed no change in diazepam drinking.

Chronic d-amphetamine Diazepam Valium Drug self-administration

CHRONIC amphetamine administration in rodents produces a variety of behavioral effects dependent on the dose, mode of administration, and length of time delivered (for review, see [8]). Our laboratory has been investigating several behavioral and biochemical effects of chronic amphetamine delivered continuously via slow release subcutaneous pellets [3,9]. Recently, we reported that rats increase their alcohol preference after implantation of a low-dose slow-release amphetamine pellet [11]. These subcutaneous pellets release an average of 1.1 mg/day of d-amphetamine base for 30 days and produce a small but significant increase in locomotor activity [10]. Rats with free access to water and a 10% v/v ethanol solution increased their intake of ethanol after d-amphetamine pellet implantation. This increased ethanol intake was not solely attributable to its caloric value nor its novel taste, for the intake of isocaloric or novel flavored solutions was not increased by d-amphetamine treatment. We suggested that rats increased their ethanol intake in order to counteract the chronic tension induced by the d-amphetamine pellet.

If the self-administration of ethanol was serving to reduce the tension generated by amphetamine treatment, we predicted that ingestion of other anxiolytic or sedative drugs would also increase after d-amphetamine pellet implantation. Although rats are generally reluctant to ingest drug solutions [2], Amit and Cohen [1] have reported diazepam drinking of approximately 20–30% of total daily fluid intake with low concentrations of diazepam (0.045–0.060 mg/ml), and Walton and Deutsch have reported that rats will ingest large amounts of diazepam, in solutions as concentrated as 1 mg/ml, on a water deprivation schedule [14]. Therefore, we chose the benzodiazepine, diazepam, to test the ability of continuous d-amphetamine to increase self-administration of an anxiolytic drug solution.

METHOD

Subjects

Sixteen female, albino rats (Simonsen Laboratories, Gilroy, CA) weighing approximately 220–320 g at the start of the experiment were housed individually in 17×24×20 wire mesh cages in a room with constant lighting. Food was available ad lib throughout the experiment.

Materials

Two drinking spouts from calibrated glass drinking tubes (Wahman Mfg. Co., Timonium, MD) were available to each rat; one contained water, the other a 0.060 mg/ml diazepam (Valium) solution. The diazepam drinking solution was prepared every second day from 5 mg/ml injectable diazepam (Valium, Roche Laboratories, Nutley, NJ) and water. Due to the solubilizers in the injectable diazepam, the diazepam drinking solution also contained 0.5% v/v propylene glycol and 0.12% v/v ethanol.

Silicone slow-release pellets containing either 235 mg/kg d-amphetamine base suspended in polyethylene glycol (PEG) or the vehicle alone were constructed according to the method of Nielsen and Ellison [10]. The d-amphetamine pellet releases 50% of the d-amphetamine content over 28 days.

Procedure

Subjects were acclimated to the diazepam drinking solutions for approximately one month, after which baseline in-

[1]Requests for reprints should be addressed to Linda R. Nelson, Department of Psychology, UCLA, 405 Hilgard Avenue, Los Angeles, CA 90024.

take was recorded for six days. The subjects were then randomly divided into two groups: group d-AMPHET received d-amphetamine pellets, while group PEG received vehicle only pellets. Lidocaine (2 mg/rat, SC) was injected in the rat's upper back region and the silicone pellet was implanted into the subcutaneous space.

After pellet implantation, the diazepam solution was removed for six days, during which time only water was available. This was done to minimize the possibility of the formation of a conditioned taste aversion to the diazepam solution through its association with the onset of the amphetamine effects (see [11]). During days 7–30 postimplantation, intake of both water and diazepam was recorded every second day, after which the bottles were refilled with fresh fluids and the positions (right or left) reversed. On day 30, the pellets were removed and fluid intake measurements continued for the next 6 days.

Data from each group were analyzed using a one-way ANOVA with repeated measures. Subsequent comparisons of baseline and postpellet values were made with Dunnett's test and a criterion of significance of $p<0.05$ was used [7].

RESULTS

The mean daily intake of diazepam and water for both groups is shown in Fig. 1. During the baseline period, both groups drank a similar amount of diazepam: 6.0±1.9 ml/day (mean±SEM) by the d-AMPHET animals and 5.3±1.6 ml/day by the PEG animals. This represents 25% and 27% of their respective total daily fluid intakes. Postpellet implantation, d-AMPHET rats significantly increased their diazepam intake above basline, $F(12,84)=3.74$, $p<0.01$. Dunnett's tests revealed that diazepam intake was elevated from day 7, when the diazepam was returned, through day 18 ($p<0.05$ for each comparison). During this time, d-AMPHET rats consumed 16.5±3.8 ml/day of diazepam which represents 48% of their total fluid intake. In contrast, the PEG rats showed no significant change in diazepam intake postpellet implantation.

Water consumption was elevated in both groups during days 1–6 postpellet implantation, when it was the only fluid available. There was no difference in water intake between the baseline period and days 7–30 postpellet implantation for d-AMPHET rats. In the same time interval, PEG rats showed a small but significant increase in water intake as compared to baseline, $F(12,84)=2.24$, $p<0.05$. However, Dunnett's tests indicated that there was only one significantly elevated two day period: 12–14 days postpellet.

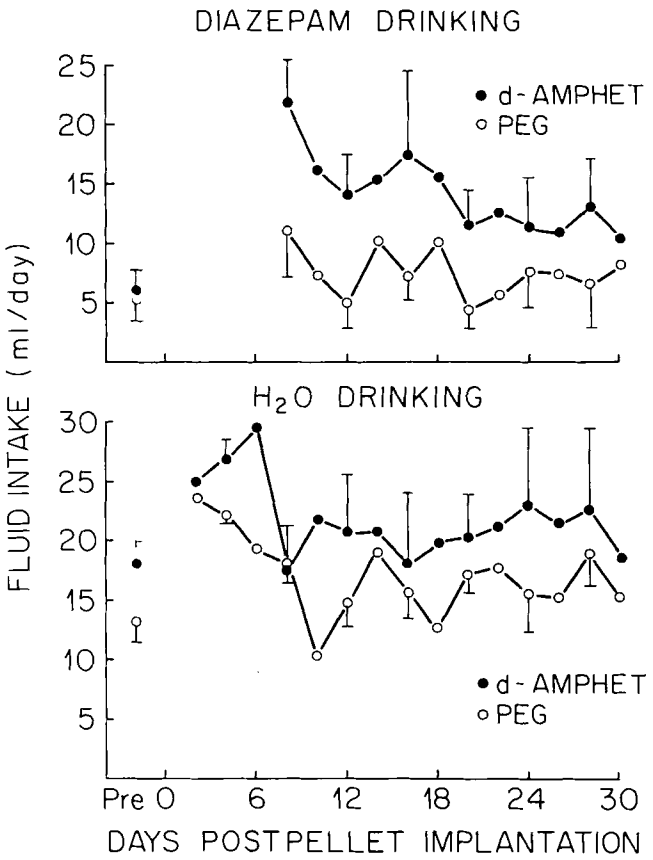

FIG. 1. Mean intake of diazepam and water by rats with slow-release d-amphetamine or polyethylene glycol pellets. Diazepam intake was significantly elevated in the d-amphetamine animals during days 7–18 post-implantation. Note that during days 0–6 post-implantation only water was available for drinking.

DISCUSSION

The mean baseline intake of diazepam in both groups, 25.6% of the total fluid intake, is in accordance with the results of Amit and Cohen [1]. They reported that Wistar rats drank between 20–30% of their total daily fluid as diazepam but two manipulations that reliably increase ethanol consumption: lateral hypothalamic stimulation and schedules of "periodic access" to the drinking solutions, failed to affect diazepam intake.

The present results, however, indicate that certain manipulations do similarly affect the consumption of ethanol and diazepam. As hypothesized, rats implanted with slow-release d-amphetamine pellets significantly increased their intake of the 0.060 mg/ml diazepam solution. There are, however, some differences between ethanol and diazepam ingestion postpellet implantation. Prior investigations demonstrated that ethanol intake remained elevated throughout the 30 day postpellet period [11], but the increased consumption of diazepam only persisted until day 18 postpellet. The time course of the elevation in diazepam ingestion is similar to the period of increased locomotor activity postpellet implantation, which has been reported to last through day 15 [10].

The chronic amphetamine regimen appears to produce a selective increase in the consumption of diazepam or ethanol solutions. This may be due to the sedative or anxiolytic properties common to these drugs, but there are alternative explanations which need to be examined. In the present study, the diazepam drinking solution contained 0.005 ml propylene glycol and 0.0012 ml ethanol per ml of solution. While it is possible that these ingredients affected the pattern of fluid intake, the daily volume drunk by a rat results in the ingestion of quantities of these agents almost certainly below minimally effective doses. Another possible explanation for the rise in diazepam consumption to almost 50% of daily total fluid intake is that the rat's ability to discriminate between the fluids offered was impaired. Potthoff and Ellison [11] addressed this problem by offering other caloric and/or flavored solutions (i.e., glucose and anise water), and found that their animals did not ingest more anise water or glucose during days 6 through 18 post amphetamine pellet implantation. Instead, they drank increased amounts of water during

this period, which indicates that, while the amphetamine pellet may make the animal hyperdipsic, discrimination of drinking fluids persists.

We suggest that continuous low level amphetamine is in some manner "stressful" or "tension producing" and that it induces rats to self-administer available solutions of anxiolytic or sedative agents, such as diazepam or ethanol. We do not yet know whether the self-administration of these solutions has any measurable effect on behavioral indicators of the pellet's action, such as locomotor stimulation. To address this, we intend to concomitantly assess the ingestion of diazepam or ethanol and the locomotor activity post d-amphetamine pellet implantation. In this situation, we predict that the anxiolytics will be able to antagonize some of the effects of the d-amphetamine pellet. In fact, we have previously shown that repeated injections of very low doses of ethanol (0.25 g/kg), roughly comparable to the amount drunk per hour, can antagonize the increased activity due to the d-amphetamine pellet [12]. However, several reviews of stimulant/anxiolytic and stimulant/depressant interactions have stressed that there does not appear to be a simple relationship between these classes of drugs [4–6, 13].

REFERENCES

1. Amit, Z. and J. Cohen. The effect of hypothalamic stimulation on oral ingestion of diazepam in rats. *Behav Biol* **10:** 223–229, 1974.
2. Cappell, H., A. E. LeBlanc and L. Endrenyi. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. *Psychopharmacologia* **29:** 239–246, 1973.
3. Ellison, G., M. Eison, H. Huberman and F. Daniel. Structural and biochemical alterations in dopaminergic innervation of the caudate nucleus following continuous amphetamine administration. *Science* **201:** 276–278, 1978.
4. Ford, R. D., R. H. Rech, R. L. Commissaris and L. Y. Meyer. Effects of acute and chronic interactions of diazepam and d-amphetamine on punished behavior of rats. *Psychopharmacology (Berlin)* **65:** 197–204, 1979.
5. Ford, R. D., R. H. Rech and D. Tobin. Interactions between stimulants and depressants on schedule-controlled behavior. *Pharmacol Biochem Behav* **12:** 657–661, 1979.
6. Holloway, J. A. and F. A. Holloway. Combined effects of ethanol and stimulants on behavior and physiology. *Neurosci Biobehav Rev* **3:** 137–148, 1979.
7. Keppel, G. *Design and Analysis: A Researcher's Handbook.* Engelwood Cliffs, NJ: Prentice-Hall, 1973.
8. Kokkinidis, L. and H. Anisman. Amphetamine models of paranoid schizophrenia: An overview and elaboration of animal experimentation. *Psychol Bull* **88:** 551–579, 1980.
9. Nelson, L. R. and G. D. Ellison. Enhanced stereotypies after repeated injections but not continuous amphetamine. *Neuropharmacology* **17:** 1081–1084, 1978.
10. Nielsen, E. B. and G. D. Ellison. A silicone pellet for longterm continuous administration of amphetamine. *Commun Psychopharmacol* **4:** 17–20, 1980.
11. Potthoff, A. D. and G. D. Ellison. Low-level continuous amphetamine administration selectively increases alcohol consumption. *Psychopharmacology (Berlin)* **77:** 242–245, 1982.
12. Potthoff, A. D. and G. D. Ellison. Continuous low level d-amphetamine administration: Effects on ethanol and on responses to low doses of ethanol. *Proc West Pharmacol Soc* **25:** 303–306, 1982.
13. Rech, R. H., M. K. Vomachka and D. E. Rickert. Interactions between depressants (alcohol-type) and stimulants (amphetamine-type). *Pharmacol Biochem Behav* **8:** 143–151, 1978.
14. Walton, N. Y. and J. A. Deutsch. Self-administration of diazepam by the rat. *Behav Biol* **24:** 533–538, 1978.