Pharmacology Biochemistry & Behavior, Vol 26, pp 521–526 Copyright © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Benzodiazepine Self-Administration in Rhesus Monkeys: Estazolam, Flurazepam and Lorazepam[1]

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Received 22 April 1986

JOHANSON, C E *Benzodiazepine self-administration in rhesus monkeys Estazolam, flurazepam and lorazepam* PHARMACOL BIOCHEM BEHAV 26(3) 521–526, 1987 —The ability of three benzodiazepines to maintain self-administration behavior was studied in rhesus monkeys using a substitution procedure Lever-press responding was maintained in six monkeys under a fixed-ratio schedule of IV pentobarbital delivery in daily sessions of 3 hr duration Each of several doses of flurazepam, lorazepam and estazolam as well as saline and vehicle was periodically substituted for 4–13 consecutive sessions Between dose or vehicle substitutions, responding was maintained by pentobarbital All six monkeys self-administered flurazepam above vehicle or saline levels In addition four of five monkeys tested with lorazepam and four of six tested with estazolam self-administered at least one dose of drug above control levels These results indicate that self-administration performance can be reliably maintained in rhesus monkeys by certain benzodiazepines under appropriate experimental conditions

Benzodiazepines Flurazepam Lorazepam Estazolam Pentobarbital Drug self-administration Rhesus monkeys

EXPERIMENTAL studies of the dependence potential of benzodiazepines have been conducted using both humans and monkeys Studies in sedative abusers have shown that diazepam and other benzodiazepines have positive reinforcing properties as well as subjective effects characteristic of drugs of abuse such as the barbiturates [14, 15, 17, 19]. In contrast, studies conducted in normal volunteers have indicated that benzodiazepines do not have positive reinforcing properties in the large majority of experimental subjects [5–7, 22]

Self-administration studies in animals with benzodiazepines have also yielded variable results [13] For instance, when diazepam was substituted for codeine in rhesus monkeys it was reported to maintain responding in one study [20] but not in another [18] When substituted for cocaine in baboons, Griffiths *et al* [16] found that diazepam and several other benzodiazepines maintained modest but variable levels of self-administration. In that same study, barbiturates such as pentobarbital consistently maintained levels of responding similar to those maintained by cocaine Bergman and Johanson [3] evaluated the reinforcing properties of diazepam when substituted for either cocaine or pentobarbital. Although the results varied among animals, the probability of diazepam functioning as a positive reinforcer was higher when it was substituted for pentobarbital The present study is an extension of the Bergman and Johanson [3] study and was designed to assess the reinforcing properties of estazolam, flurazepam and lorazepam when substituted for pentobarbital in rhesus monkeys. Estazolam and lorazepam while differing in chemical structure are both used therapeutically as anxiolytics, have short to intermediate half-lives, and have no active metabolites [1, 10, 12]. In contrast, flurazepam is marketed as an hypnotic and has two active metabolites one of which has a very long elimination half-life [8, 10, 11]

METHOD

Animals

Four female (9027, 9058, 9079, 9083) and two male (0025, 2036) rhesus monkeys (*Macaca mulatta)* were used in this experiment. Three monkeys were experimentally naive and the other three monkeys (0025, 9027 and 9058) had participated in previous studies that were similar to the present one, with responding maintained by a variety of drugs.

Each monkey was equipped with a single-lumen silicone venous catheter (Rodhelm Reiss Co., Belle Mead, NJ), implanted under sodium pentobarbital anesthesia (up to 30 mg/kg IV, as needed). The catheter was inserted into a major

[1]Portions of this research were supported by USPHS research grant DA 00250 (C R Schuster, Principal Investigator), and a research grant from Abbott Laboratories (North Chicago, IL) Portions of these data were published in Johanson [21]

vein (internal jugular, external jugular or femoral) for a distance calculated to terminate in or near the right atrium, the distal end was passed under the skin, exiting the body through an incision in the back of the monkey It was not always possible to maintain a single catheter for the duration of the experiment. When a catheter became dislodged, the monkey was removed from the experiment for several days. Another catheter was surgically implanted in one of the remaining veins and the monkey was returned to the experiment 1 or 2 days later

All monkeys had continuous access to water and were given supplemental vitamins several days each week Monkeys were given sufficient food (Purina Monkey Chow) following experimental sessions to maintain free-feeding weight. In addition, the diet was occasionally supplemented with fresh fruit. When necessary, antibiotics were administered to treat a catheter tract infection.

Apparatus

Each monkey was housed in a sound-attenuating wooden cubicle (inside dimensions: 70×80×70 cm) that served as the experimental space Each cubicle was equipped with a ventilation fan that also masked extraneous sounds. The front door of each cubicle had a Plexiglas window which allowed the monkey visual access to the room. This window was covered during experimental sessions. Mounted on the inside of the cubicle door were two metal boxes located 23 cm apart Each box contained a response lever (PRL-001, BRS/LVE, Beltsville, MD) and four Dialco stimulus lights, two covered with white lens caps and two covered with red lens caps The Plexiglas ceiling of the cubicle could be transilluminated by either a white or red light

Each monkey wore a stainless steel harness connected to a spring arm (E & H Engineering Co., Chicago, IL) which was 46 cm long and bolted through the rear wall of the cubicle This arrangement allowed the monkey relatively unrestricted movement within the cubicle and protected the catheter which was threaded through the spring arm. Outside the cubicle, the catheter was connected to a peristaltic infusion pump (7540X, Cole-Parmer Instrument Co., Chicago, IL), which delivered solutions at the rate of 6 ml/min Solid state equipment located in an adjacent room controlled stimulus light presentation, drug delivery and recorded lever responses

Procedure

The previously trained monkeys required no additional training and were exposed immediately to the terminal conditions as described below, with responding maintained by 0.25 or 0 5 mg/kg/infusion pentobarbital during 3-hr sessions The three experimentally naive monkeys were trained to respond on the right lever to receive an infusion of 0 1 mg/kg cocaine During the initial session, the operative lever was baited with a preferred food and all other food was removed from the cubicle. When responding occurred reliably, the number of responses required for drug delivery was increased to 10 (fixed-ratio 10, FR 10) over the course of several sessions Saline was then substituted for cocaine during daily sessions until responding declined to low levels Next, 0.1 mg/kg/infusion cocaine was available during the next 1 or 2 sessions until responding increased. Pentobarbital at a dose of 0 25 mg/kg/infusion was then substituted for cocaine under a FR 1 schedule of delivery The response requirement for drug delivery was gradually increased to 10 over the next 6–8 sessions

The terminal conditions of the experiment were as follows Each pentobarbital infusion was delivered over a 10-sec period upon the completion of a FR 10 on the right lever in the presence of an illuminated white ceiling light and white stimulus lights above both levers During each infusion, all white lights were extinguished and the red ceiling light and red stimulus lights above both levers were illuminated. Responding during the pentobarbital infusion as well as all responding on the left lever had no programmed consequences and were not recorded Experimental sessions were 3 hr in duration and were conducted 7 days a week

After responding maintained by pentobarbital delivery was stable, saline was substituted under the same schedule requirement until responding declined to low levels Monkeys were then returned to the baseline condition, i.e , pentobarbital-maintained responding, until responding returned to the previous baseline level after which an Emulphor-based vehicle (see below) was substituted in a similar manner Next, each of a range of doses of flurazepam, lorazepam and estazolam was substituted, in most cases for at least the same number of sessions as saline or Emulphor vehicle Occasionally the substitution period was extended if responding was erratic or was shortened if responding rapidly declined to low levels comparable to saline or vehicle. Between each substitution period, monkeys were returned to the baseline condition until responding returned to the previous baseline level or stabilized at a new level The order of testing the three drugs varied among monkeys Generally all doses of one drug were tested prior to testing another drug Occasionally, vehicle or saline substitutions were repeated between the testing of two drugs

Data Analysis

During each session, the number of infusions delivered and total responses emitted were recorded Pentobarbital control values were computed by averaging the means of baseline sessions prior to all substitutions. Pentobarbital baseline sessions prior to each substitution were continued until responding was at previous baseline levels. This required between 1 and 3 sessions. The mean number of infusions during the last 2 sessions was calculated for each of the substitutions of drug dose, Emulphor vehicle and saline A dose of a drug was considered to maintain responding, i.e , to function as a positive reinforcer, if the range of infusions during the final 2 sessions of the substitution exceeded the range of infusions of saline and Emulphor vehicle in the corresponding period under the same schedule requirement

Drugs

Pentobarbital sodium and flurazepam hydrochloride were prepared using sterile saline (0 9% NaCl) for solutions All doses are expressed as the salt. Lorazepam and estazolam were prepared using a previously reported suspension system suitable for water insoluble compounds [4]. Specifically, they were dissolved in a small quantity of 95% ethyl alcohol to which polyethoxylated vegetable oil (Emulphor, EL-620,GAF) was added in a 1:1 ratio. The concentration was 20 mg/ml for lorazepam and 40 mg/ml for estazolam Solutions for administration were prepared fresh daily by adding this solution to saline to achieve the final concentration The Emulphor vehicle consisted of the alcohol-Emulphor mixture diluted in saline

TABLE 1

AVERAGES OF THE MEAN NUMBER OF PENTOBARBITAL INFUSIONS PRIOR TO ALL SUBSTITUTIONS AND MEAN NUMBER OF SALINE AND VEHICLE INFUSIONS PER 3-HR SESSION

	Pentobarbital*		Saline‡		Vehicle‡	
	Mean	(SE)	Mean (Range)	Sessions	Mean (Range)	Sessions
0025	63 7	(3 0)	*11 0 (10–12)*§	5	1 5 (1–2)	11
2036	95 9	(2 7)	14 5 (9–20)	5	*27 5 (26–29)*	6
9027†	44 9	(1 2)	0 5 (0–1)	5	*21 5 (21–22)*	9
					18 5 (17–20)	7
9058†	56 4	(2 8)	*35 0 (32–38)*	13	17 5 (16–19)	10
9079	53 9	(2 4)	16 0 (9–23)	4	14 5 (5–24)	7
			15 0 (6–24)	4		
9083	47 6	(2 1)	16 0 (16–16)	6	19 5 (16–23)	8
					21 0 (19–23)	8

*The means are based on 7 (0025, 9079), 10 (9027), 11 (2036), 12 (9058) or 14 (9083) observations

†Pentobarbital dose was 0 5 mg/kg/infusion The dose was 0 25 mg/kg/infusion for the other monkeys For monkey 9058, responding was maintained under a FR 5

‡Saline or vehicle was substituted until responding declined to low levels for 2 sessions The mean and range were calculated from these final sessions only Total sessions required for decreased responding are indicated

§The averages in italics are the control values shown in Figs 1–3

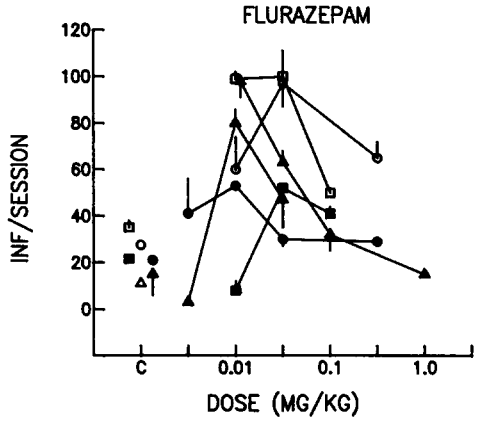

FIG 1 The mean number of infusions of flurazepam per 3-hr session when this drug was substituted for 0 25 or 0 5 (9027, 9058) mg/kg/infusion pentobarbital Responding was maintained under a fixed-ratio 5 (9058) or 10 schedule of drug delivery The doses shown on the abscissa are on a log scale Each point represents the mean over the last 2 sessions of each substitution Range is indicated by the vertical line The values above C are the highest vehicle or saline substitution (see Table 1) △ 0025, ○ 2036, ■ 9027, □ 9058, ▲ 9079, ● 9083

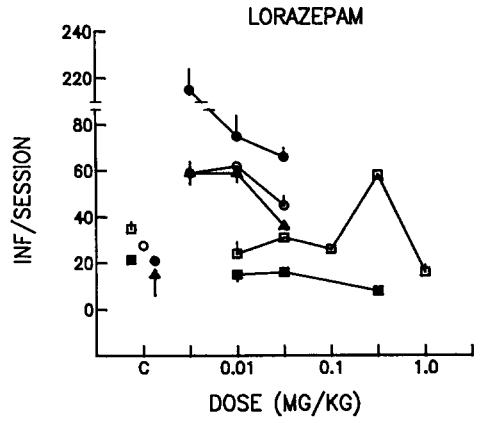

FIG 2 The mean number of infusions of lorazepam per 3-hr session when this drug was substituted for pentobarbital Other details as in Fig 1

RESULTS

Table 1 shows the mean number of pentobarbital infusions self-administered by each of the monkeys averaged across all periods prior to each substitution. This mean varied between 45 and 64 infusions for five of the monkeys but was higher for monkey 2036 Responding for pentobarbital fluctuated somewhat across the entire experiment but there was no evidence of a consistent trend When saline or vehicle was substituted responding declined to low levels after 4 to 13 sessions (Table 1).

All three benzodiazepines maintained responding above vehicle and saline levels in the majority of the monkeys tested. Flurazepam at doses between 0.003 and 0.3 mg/kg/infusion maintained responding in all six monkeys evaluated (Fig 1). The highest level of self-administration varied between 53 and 100 infusions/session and this peak responding occurred at a dose of 0.01 or 0.03 mg/kg/infusion. Lorazepam maintained responding above vehicle or saline levels in three of the five monkeys tested at doses between 0.003 and 0.03 mg/kg/infusion (Fig. 2) In these three mon-

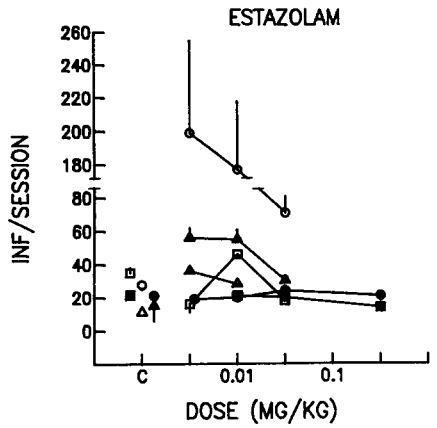

FIG 3 The mean number of infusions of estazolam per 3-hr session when this drug was substituted for pentobarbital Other details as in Fig 1

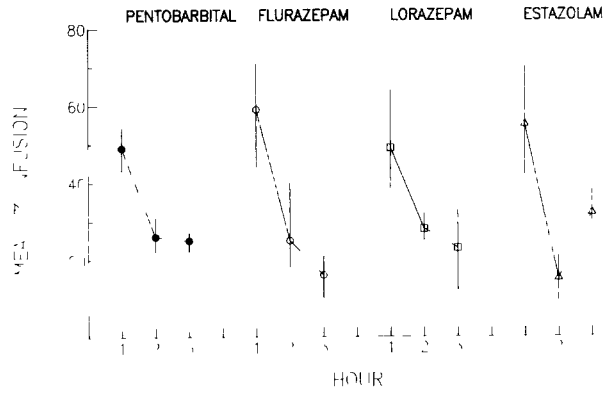

FIG 4 The percent of total infusions of pentobarbital (0 25 or 0 5 mg/kg) or 0 01 mg/kg/infusion flurazepam, lorazepam and estazolam self-administered during each hour of the 3-hr session averaged across monkeys For flurazepam, lorazepam and estazolam only the results from monkeys that self-administered the dose above both vehicle and saline levels are included Vertical line is range across monkeys

keys, peak levels of responding varied between 59 and 215 infusions/session at a dose of 0 003 or 0.01 mg/kg/infusion In an additional monkey (9058), responding was maintained by lorazepam above saline or vehicle only at the dose of 0.3 mg/kg Estazolam was similar to lorazepam in that 4 of the 6 monkeys self-administered it above saline or vehicle levels at doses between 0 003 and 0 03 mg/kg/infusion (Fig. 3). Furthermore, there was considerable variability in peak levels ranging from 36 to 198 at doses of 0 003 or 0.01 mg/kg/infusion While complete dose-response functions were not always obtained, the doses of the three benzodiazepines that maintained the highest rates of responding were similar across drugs.

The pattern of pentobarbital responding across the 3-hr period for all control periods is shown in Fig 4 averaged across all 6 monkeys. For all six monkeys the pattern was similar, i e., a greater proportion of the infusions were taken during the first hour with intake consistent across the remaining 2 hr. For the purposes of comparison the pattern of intake of a single dose (0.01 mg/kg/infusion) of each drug was evaluated and averaged only across monkeys that self-administered the dose above vehicle levels As shown in Fig 4, the pattern for all three drugs was similar to pentobarbital, i.e , the number of infusions during the first hour was greatest

DISCUSSION

All three benzodiazepines tested maintained responding above control levels in the majority of monkeys tested The results with flurazepam were consistent with findings by Griffiths *et al* [16] showing that this drug maintained responding when substituted for cocaine in baboons and extend these findings to another species. Although it can be misleading to compare across studies that differ in many parameters, flurazepam was self-administered by all monkeys in the present study at rates of responding comparable to those maintained by barbiturates tested under similar conditions [27] In contrast, in the Griffiths *et al* [16] study, the maximum responding engendered by pentobarbital exceeded flurazepam levels Although there were individual differences among monkeys in the maximum number of flurazepam infusions taken and the dose at which this maximum occurred, individual differences were more pronounced with lorazepam and estazolam as indicated by the finding that a few of the monkeys tested did not self-administer these drugs above control levels at any dose The monkey that did not self-administer lorazepam at any dose (9027) also failed to respond for estazolam and a second monkey (9058) only self-administered one dose of each of these 2 drugs This difference between flurazepam and the other drugs could have been due to the use of a suspension with lorazepam and estazolam

The benzodiazepines tested have been shown in previous studies to be similar in their behavioral profiles of action For instance, all have antipunishment properties in experimental studies [25] and flurazepam and lorazepam have similar discriminative stimulus properties in animals and subjective effects in humans [5, 7, 26] As a discriminative stimulus in rats, lorazepam was approximately 14 times more potent than flurazepam [26]. In humans, the recommended therapeutic dose of flurazepam is 10 to 15 times greater than for lorazepam and 2 mg lorazepam and 30 mg flurazepam produce comparable changes in scores on subjective effects questionnaires [5,7]. However in the present study these two drugs were similar in potency

Despite similarities in behavioral action, the three drugs tested differ in terms of pharmacokinetics Flurazepam, marketed as an hypnotic, has two active metabolites, desalkylflurazepam and hydroxyethylflurazepam which are rapidly formed [8, 10, 11] While flurazepam itself has a relatively short half-life, desalkylflurazepam has a very long elimination half-life so that multiple infusions during self-administration sessions very likely resulted in significant accumulation even across sessions [10] In contrast, lorazepam, a 3-hydroxybenzodiazepine derivative, is metabolized by conjugation producing no active metabolites

[10,12]. Although the half-life of this drug is short to intermediate, several studies have shown that its behavioral actions cannot be predicted from plasma or even brain levels [5, 9, 23] Estazolam is a triazolobenzodiazepine derivative with a half-life comparable to that of lorazepam [1]. Despite these pharmacokinetic differences the results were similar for all three drugs and where there were differences, it is difficult to relate them to differences in pharmacokinetics. However, it is also important to note that the pharmacokinetics of these drugs have not been established in monkeys. In this regard, it is interesting to note that the distribution of responding over the 3-hr sessions, which is presumed related to differences in duration of action and drug accumulation, was similar for all three drugs at the dose selected for comparison

In summary, the present study indicates that flurazepam, lorazepam and estazolam can all function as positive reinforcers in rhesus monkeys This study combined with others [3,16] suggests that the likelihood of maintaining responding with benzodiazepines is increased when they are substituted for pentobarbital For instance, in the Griffiths *et al* [16] study where benzodiazepines were substituted for cocaine, rates of flurazepam self-administration were relatively low. In the Bergman and Johanson [3] study diazepam was a reinforcer in all monkeys when substituted for pentobarbital but in only 3 of 11 monkeys when substituted for cocaine In sedative abusers many benzodiazepines were self-administered [14, 15, 17] whereas in subjects without a history of sedative abuse, diazepam [6,22], flurazepam [7] and lorazepam [5] were not self-administered. Taken together, these studies provide evidence of the influence of drug history on self-administration. The mechanism underlying the differences in the reinforcing properties of benzodiazepines in organisms with a history of sedative use is not clear. On one hand, it could be related to the similarity in the stimulus properties of the test drugs and pentobarbital or cross-tolerance to effects that might interfere with self-administration On the other hand, failure to obtain self-administration of drugs following exposure to cocaine may be due to a contrast in these properties. Further studies are clearly needed to resolve this issue since it may have importance for finding factors which place some humans at risk for the abuse of benzodiazepines.

ACKNOWLEDGEMENTS

The author would like to thank Rene de la Garza, Mike Monaghan and Suzette Evans for their technical assistance Flurazepam, lorazepam and estazolam were gifts of Abbott Laboratories

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