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Effects of Diazepam and Ripazepam on Two Measures of Adjunctive Drinking in Rats[1]

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SANGER, D. J. AND D. E. BLACKMAN. *Effects of diazepam and ripazepam on two measures of adjunctive drinking in rats.* PHARMAC. BIOCHEM. BEHAV. 5(2) 139–142, 1976. Four rats were maintained at 85% of their pre-experimental body weights and were given daily 1 hr sessions during which they were each placed in a test chamber in which a 45 mg food pellet was delivered regularly every min independently of behavior. During these sessions water spouts were available to the rats and all 4 animals developed high levels of adjunctive drinking, a burst of licking typically following the consumption of each food pellet. This behavior was found to be sensitive to the effects of diazepam and ripazepam. Small doses of both drugs increased the volume of water consumed druing a session. The number of licks was not increased to the same extent, however. Larger doses of both drugs resulted in decreased numbers of licks and decreased water intake although licking appeared on several occasions to be more sensitive than water intake to this action of the drugs. A possible explanation of these effects is that the drugs affected the topography of the rats' licking at the water spouts. Whatever the mechanism involved, however, these results suggest that in such experiments measures of both water intake and number of licks should be obtained.

Diazepam Ripazepam Adjunctive drinking Licking

WHEN food deprived animals receive small portions of food delivered intermittently they have been observed to drink large quantities of water, a burst of drinking typically occurring after the consumption of each food portion [5]. This behavior has been described as schedule-induced polydipsia and it has been suggested that it is one of a class of behaviors which Falk [7] has named adjunctive.

Several reports have described the effects of amphetamines on adjunctive drinking, which is generally attenuated by the administration of these drugs [6, 11, 14, 15, 19]. Wayner et al. [18], however, observed increases in adjunctive drinking after the administration of small doses of d-amphetamine to a rat with a relatively low baseline rate of drinking. Decreases in adjunctive drinking have also been reported after administration of pentobarbital [6,15], atropine [3] and ethanol [8] and drinking has been found to be increased by certain doses of Δ^9 THC [17].

Recently a number of experiments have investigated the actions of chlordiazepoxide on adjunctive drinking. McKearney [11] studied the adjunctive licking of rats which were maintained on a fixed-interval schedule of reinforcement for which the operant response was also licking the water tube. Chlordiazepoxide was found to have little effect on or even to decrease the number of adjunctive licks, at doses which actually increased rates of operant licking. Barrett and Weinberg [2] also investigated adjunctive drinking on a fixed-interval schedule although in their experiment the animals were squirrel monkeys and the operant response was a lever press. These experimenters found that chlordiazepoxide increased adjunctive drinking, measured in this case as the amount of fluid (either water or an ethanol solution) consumed. A facilitation of adjunctive drinking after the administration of chlordiazepoxide has also been reported by Sanger and Blackman [13] who found that a dose of this drug enhanced the acquisition of adjunctive licking in one group of rats while reducing levels of established licking in a second group. More recently, Bacotti and Barrett [1] have also reported that chlordiazepoxide increases levels of adjunctive drinking in rats.

Although there are several differences between the results obtained in these experiments it seems clear that adjunctive drinking is sensitive to the actions of chlordiazepoxide. The present experiment was an attempt to obtain further information concerning the actions of similar drugs on this pattern of behavior. The drugs used were diazepam, which, like chlordiazepoxide, is a benzodiazepine, and ripazepam (formerly known as pyrazapon). The latter is a pyrazolodiazepinone, rather than a benzodiazepine, but has been shown to share several of the pharmacological and behavioral actions of chlordiazepoxide [12].

METHOD

Animals

Four female hooded rats, approximately 150 days old at the beginning of the experiment, served as test animals.

[1] We are grateful to Roche Products Limited who supplied the diazepam and to Parke Davis and Company who provided us with ripazepam.
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They were individually housed and maintained at 85% of their pre-experimental weights which were within the range 185–200 g. Water was continuously available in the home cages.

Apparatus

The apparatus consisted of 2 standard operant test chambers (L.V.E.) enclosed in sound and light proof outer cubicles. In each chamber the lever to the right of the food chute was removed as were the 3 stimulus lights situated above this lever. Pieces of steel were used to cover the resulting openings with the exception of that produced by the removal of the middle stimulus light (1.5 cm in dia.). A plastic water tube was securely fixed to the outside of each chamber so that the metal spout was positioned just behind (approximately 1–2 mm) this opening. When placed in the chambers the rats could reach through the openings to lick the water spouts. Each spout was approximately 5.5 cm to the right of and 8 cm higher than the food chute. Contacts with the spouts were sensed by contact relays which were connected to the spouts and the grid floors of the test chambers. Programming of the experiment and recording of licking were achieved by means of standard electro-mechanical equipment.

Procedure

The rats were given 2 one hr sessions to allow them to adapt to the test chambers. During these sessions a few food pellets (45 mg) were placed in the food chutes and the water tubes were in place. After this adaption to the chambers, the animals were given daily sessions during which 60 45 mg food pellets were delivered regularly at 1 min intervals to each animal independently of its behavior. The number of licks which occurred after the delivery of each pellet was recorded, as was the volume of water consumed during each session. At the end of each session the chambers were checked for possible spillage but under none of the conditions of the present experiment could spillage be detected.

When all four animals developed steady day to day drinking baselines, drug administration was begun. The drugs used were diazepam in doses of 0.1, 0.3, 1.0, 3.0 and 5.6 mg/kg and ripazepam in doses of 1, 3, 10, 30 and 56 mg/kg. Both drugs were injected IP as suspensions in 1% Tween 80 in physiological saline. Injections were given approximately 5 min before the start of a session and at least 3 nondrug days separated successive drug days, the vehicle being injected on all nondrug days. Administration of ripazepam preceded that of diazepam and the doses of both drugs were given in a mixed order, the order being different for each animal.

RESULTS

The four rats developed consistently high levels of adjunctive drinking. A burst of licking would typically follow immediately after consumption of each of 55 to 60 food pellets of the 60 delivered during each session. Each animal consumed approximately 15–25 ml of water during each session which represented approximately 5,000–10,000 licks per session. The effects of diazepam and ripazepam were assessed both in terms of the volume of water consumed and also in terms of the total number of licks.

Figure 1 presents dose-response curves showing the effects of both diazepam and ripazepam on levels of water intake in the 4 individual animals. Volumes of water consumed after each drug administration are shown in comparison with the mean and complete range of control values of this measure taken from the sessions immediatelv preceding drug sessions (i.e., 10 control sessions for each rat). The highest dose of both drugs reduced levels of water intake in 3 of the 4 rats while lower doses either had no effect on or increased these levels. At least one dose of diazepam produced a small increase in water intake in all but one (R 1) of the animals while ripazepam in doses of either 3.0 or 10 mg/kg produced larger increases in all 4 rats.

Dose-response curves showing the effects of the 2 drugs on amount of licking are presented in Fig. 2. Although these curves appear generally similar to those presented in Fig. 1 close comparison of these 2 figures shows a number of differences. The increases in levels of water intake produced by lower and intermediate doses of both diazepam and ripazepam are frequently not accompanied by increases in levels of licking. For instance, ripazepam at 3.0 mg/kg, which increased levels of water intake in three animals, did not increase numbers of licks. Licking was increased to values outside the range of control rates only by 0.1 mg/kg of diazepam in R 2 and R 4 and by 1.0 mg/kg of ripazepam in R 2. However, these doses did not increase levels of water intake in any animal. This apparent dissociation between the drug effects on water intake and

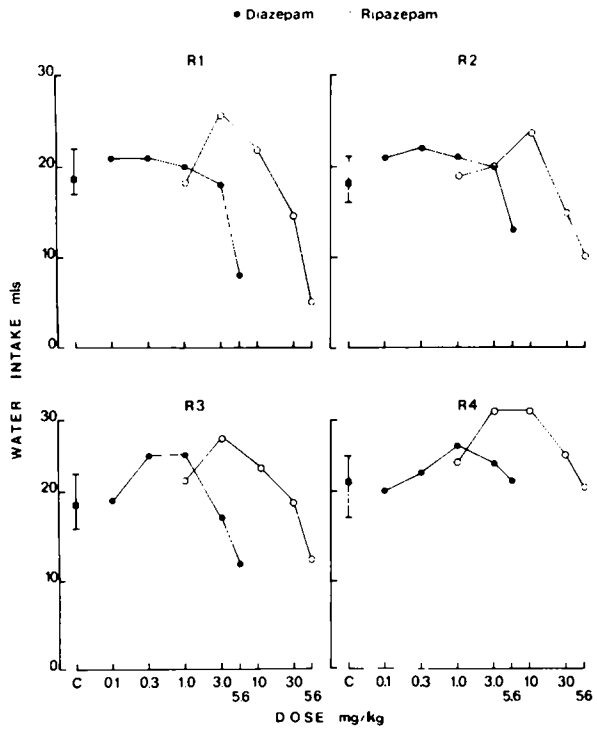

FIG. 1. Dose response curves showing the effects of diazepam and ripazepam on levels of water intake in the 4 individual rats. Each point shows the volume of water ingested after one administration of each dose except for the points at C which show the means and complete ranges of water intakes for sessions immediately preceding each drug session (i.e., 10 sessions for each animal).

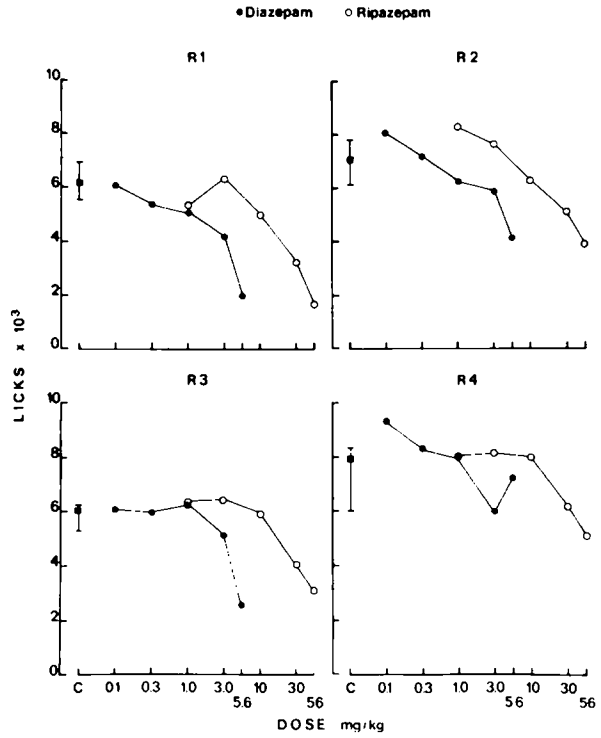

FIG. 2. Dose response curves showing the effects of diazepam and ripazepam on numbers of licks in the 4 individual rats. Each point shows the number of licks during a complete session after one administration of each dose exept for the points at C which show the means and complete ranges of numbers of licks for sessions immediately preceding each drug session (i.e., 10 sessions for each animal).

on licking was also shown at higher doses of both drugs when, on several occasions, lick rates were decreased to values outside the control range while levels of water intake were unaffected. Such an effect was seen with 3.0 mg/kg of diazepam in R 1, R2 and R 3, and with at least one dose of ripazepam with all 4 animals.

DISCUSSION

Both diazepam, a benzodiazepine, and ripazepam, which has been shown to have pharmacological actions similar to those of the benzodiazepines [12] were found to increase levels of adjunctive drinking in rats. This result is consistent with that obtained by Barrett and Weinberg [2] who found that levels of adjunctive drinking in squirrel monkeys were increased by the administration of chlordiazepoxide. The fact that there were several differences in procedure between the present experiment and that reported by Barrett and Weinberg, including the species of animal, the schedule on which food was delivered and the particular benzodiazepine used, suggests that anxiolytic drugs may enhance adjunctive drinking under a wide range of experimental conditions. An increase in levels of both water intake and licking has also been reported by Bacotti and Barrett [1] who administered chlordiazepoxide to rats whose food-reinforced lever pressing was maintained by a multiple fixed-interval fixed-ratio schedule. McKearney [11], however, did not find that chlordiazepoxide increased levels of adjunctive licking in rats. It is possible that this result may have been related to the fact that in McKearney's experiment licking was also the operant response. Another possibility, however, is that the failure to observe enhanced adjunctive drinking was due to drinking being measured only in terms of licks.

In the present experiment some dissociation was observed between the ways in which the two measures of adjunctive drinking were affected by the drugs. In general, it seemed that the measure of water intake was more sensitive to increases in drinking while licking was more sensitive to decreases. These results suggest that the drugs may have altered the topography of licking, that is the way in which the rats licked the water spouts. A further possibility, which might be mentioned, is that the drugs increased local rates of licking to such an extent that the electromechanical counters were unable to count every lick, although it appears from the available evidence that variations in rates of licking tend to be small [4].

Whatever the reason for these dissociations of water intake and numbers of licks the present results indicate that experiments in which the effects of drugs on adjunctive drinking, and indeed drinking in other situations, are studied should measure both the volume of fluid consumed and the number of licks. In most of the published experiments which have investigated the actions of drugs on adjunctive drinking only a single measure of drinking, usually licking, has been reported. The experiment described by Wayner et al. [18] in which the effects of d-amphetamine were studied provides an exception to this generalization. In this experiment, however, d-amphetamine generally had similar effects on both licking and on the volumes of water consumed, although at high doses these measures were depressed to different extents.

In this context it is interesting to note the results obtained by Knowler and Ukena [10]. These researchers studied the effects of several drugs, including chlordiazepoxide, on patterns of deprivation-induced drinking in rats. Certain doses of chlordiazepoxide were found to increase the volumes of water consumed but licking was also increased by these doses.

The results of the present experiment are consistent with those obtained in several previous studies [1, 2, 13] in showing that drugs with clinical anti-anxiety properties can increase levels of adjunctive drinking. This action is in contrast to those shown by a number of other classes of drugs, including stimulants and agents with anticholinergic properties, which have been found to depress levels of adjunctive drinking [3, 6, 11, 14, 15, 19]. It will be necessary for any theoretical view of adjunctive drinking to account for the sensitivity of such drinking to the effects of different drugs. At present, however, it would seem difficult for theories which view adjunctive drinking as the outcome of hypothesised emotional states such as anxiety [9] or frustation [16] to satisfactorily account for the actions of anxiolytic drugs since such drugs might be expected to reduce the intensity of any emotional state and thus not lead to increases in drinking.

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