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The Inhibitory Effect of Diazepam on Conditioned Defensive Burying is Reversed by Picrotoxin

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TREIT, D., J. P. J. PINEL AND H. C. FIBIGER. *The inhibitory effect of diazepam on conditioned defensive burying is reversed by picrotoxin.* PHARMAC. BIOCHEM. BEHAV. 17(2) 359–361, 1982.—The ability of picrotoxin to reverse the effect of diazepam was studied using the conditioned defensive burying paradigm. Although picrotoxin alone had no detectable effect on the conditioned defensive burying response of rats, picrotoxin was able to reverse the usual inhibitory effect of diazepam on defensive burying. These results suggest that the anxiolytic effect of diazepam may depend upon the integrity of GABAergic neural systems.

Anxiolytics Conditioned defensive burying Animal models Diazepam Picrotoxin GABA

RECENTLY Treit, Pinel, and Fibiger [8] introduced the conditioned defensive burying paradigm [3] as an animal model for the study of anxiolytic agents. It was demonstrated that anxiolytic drugs reliably suppressed the species-typical propensity of rats to bury objects associated with aversive stimulation. In addition, the relative potency of these drugs in the burying paradigm was similar to their relative potency in clinical settings. In contrast, nonanxiolytic compounds either had no effect on conditioned defensive burying or had effects that could be dissociated from those of known anxiolytics.

In addition to its apparent ability to meet the criteria of sensitivity, relative potency, and selectivity [8], the conditioned defensive burying paradigm possesses other attributes that facilitate its use as a model for studying anxiolytic agents. Unlike the most common behavioral test of anxiolytic action, the Geller conflict test, the burying test does not require lengthy periods of pretraining, elaborate instrumentation, or repeated exposures to the test compound. The burying response occurs reliably after only a single exposure to an aversive stimulus, and it is suppressed within minutes of a single injection of an anxiolytic agent [8]. Furthermore, because the burying response can be produced without food reinforcement, antianxiety effects in this paradigm are not confounded with effects on appetitively motivated behaviors, as they are in the conflict test.

A number of pharmacological observations suggest that anxiolytics such as diazepam may exert their effects by interacting with gamma aminobutyric acid [GABA] neuronal mechanisms [2,4]. However, evidence relating GABAergic function to the behavioral actions of diazepam and other benzodiazepines has been less persuasive. For example, attempts to facilitate the effect of benzodiazepines in the conflict test by administering the GABA transaminase inhibitor, aminooxyacetic acid have been largely unsuccessful [1], and attempts to inhibit the behavioral effects of benzodiazepines with GABA antagonists such as picrotoxin have produced contradictory results [4,6]. Thus, a relationship between GABA and the behavioral actions of benzodiazepines has not been firmly established using conventional animal models of anxiolytic drug action.

In view of the potential advantages of the conditioned defensive burying paradigm as a model for studying the mechanisms of anxiolytic drug action, it seemed worthwhile to use this model to investigate the putative relationship between GABAergic neural mechanisms and the anxiolytic action of benzodiazepines. Accordingly, the present experiment was designed to determine whether picrotoxin would reverse the inhibitory effect of diazepam on conditioned defensive burying.

METHOD

Subjects

The subjects were 40 naive, 250–450 g, male hooded rats (Canadian Breeding Farm Laboratories, La Prairie, Quebec).

Apparatus

The test apparatus was a 44×30×44 cm Plexiglas chamber, the floor of which was evenly covered with bedding material (San-i-cel, Paxton Processing Co., Paxton, IL). A small hole was centered on one wall 2 cm above the level

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of the San-i-cel, through which a 6.5×0.5×0.5 cm wire-wrapped dowel (i.e., the shock prod) could be inserted [8].

Procedure

Habituation. Before the test day the rats were placed in the test chamber in groups of five for 30-min periods on each of 4 consecutive days.

Drug administration. On the fifth day, the rats were randomly assigned to one of four conditions (n=10). Rats in the three drug conditions received an intraperitoneal injection of either picrotoxin (1 mg/kg), diazepam (1 mg/kg), or picrotoxin plus diazepam (1 mg/kg; 1 mg/kg). Injections of diazepam were administered 30 min before the conditioning session, whereas injections of picrotoxin were administered 15 min before the session. Rats in the vehicle control group received an intraperitoneal injection of the diazepam vehicle (1 ml/kg of 40% propylene glycol and 10% ethanol) 30 min before the session and an injection of the picrotoxin vehicle (1 ml/kg of 5% acacia gum) 15 min before the session.

Shock administration. Before the conditioning session, the shock prod was inserted 6 cm into the test chamber through the hole in the chamber wall and fixed there. Each animal was then placed individually in the center of the chamber to begin the session. When the animal first touched the prod with its forepaw, it received a brief, 1 mA shock from an 800 V power source [3, 8, 9].

Behavioral observation. Immediately after shock administration, the behavior of each rat was viewed for 15 min from a separate room via closed circuit television, and the duration of each burying sequence [3,8] was recorded on a chart recorder.

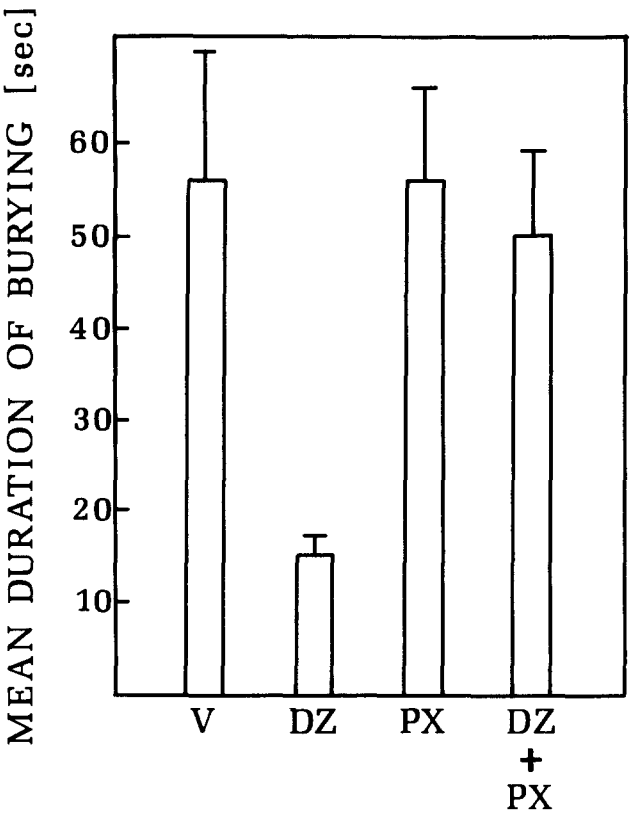

FIG. 1. Mean duration (±S.E.M.) of burying by rats in the vehicle (V), diazepam (DZ), picrotoxin (PX), and diazepam plus picrotoxin (DZ + PX) conditions.

RESULTS AND DISCUSSION

As can be seen from Fig. 1, diazepam produced a substantial suppression of conditioned defensive burying, whereas picrotoxin by itself had no detectable effect. Both of these results confirm those of a previous investigation [8]. However, the most important finding of the present study was that picrotoxin reversed the suppression of conditioned defensive burying normally produced by diazepam.

A one-way analysis of variance of these data revealed a significant main effect of groups, $F(3,36)=3.93$, $p<0.02$. Subsequent pair-wise comparisons (Duncan's, $p=0.05$) confirmed that the rats injected with diazepam alone spent significantly less time burying the shock prod than did rats in the vehicle control group, the picrotoxin alone group, or the picrotoxin plus diazepam group. There were no significant differences in the duration of burying displayed by rats in these latter three groups. Thus, the present data show that the suppression of conditioned defensive burying normally produced by diazepam can be reversed by picrotoxin, thus implicating GABAergic neural mechanisms in the antiburying action of benzodiazepines.

A large body of pharmacological data suggests that benzodiazepines exert their effects through a synergistic interaction with GABA [2,4]. For example, recent pharmacological investigations have revealed that benzodiazepine binding in neural tissue is facilitated by GABA and inhibited by bicuculline, a GABA antagonist [7,10]. The present results are noteworthy because they provide clear behavioral evidence of a relationship between the anxiolytic effects of benzodiazepines and their interaction with GABAergic mechanisms. The finding that picrotoxin, a GABA antagonist, reversed the suppressive effect of diazepam on conditioned burying suggests that the action of GABA receptors is necessary for benzodiazepines to exert at least some of their behavioral effects.

Although the present results are consistent with the view that benzodiazepines exert their anxiolytic effects through central GABAergic neuronal mechanisms, other interpretations of the results should also be considered. It is possible, for example, that picrotoxin did not reverse the effect of diazepam through a direct antagonism of GABA receptors, but instead through an indirect action via an ancilliary chloride ionophore, (cf. [5]). Another possibility is that picrotoxin reduced the efficacy of diazepam through some peripheral effect on its absorption, uptake or metabolism. Thus, further research on the role of GABAergic neural mechanisms in the behavioral effects of benzodiazepines is clearly required. The present data suggest that the conditioned defensive burying paradigm may prove to be a particularly valuable model for studying these putative GABAergic mechanisms of anxiolytic drug action.

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