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Convulsant Benzodiazepine Ro 5-3663 Has Anxiolytic Properties

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FELDON, J. AND M. MYSLOBODSKY. *Convulsant benzodiazepine Ro 5-3663 has anxiolytic properties.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 689–691, 1982.—A hypothesis was tested that convulsant benzodiazepine would have a pronounced pro-anxiety action. Contrary to expectation, convulsant benzodiazepine with GABA antagonistic properties, Ro 5-3663, administered alone (1 mg/kg, IP) or together with chlordiazepoxide (5 mg/kg, IP) antagonized response suppression in the one-trial inhibitory learning task.

Anxiety GABA Convulsant benzodiazepine

ALTHOUGH benzodiazepines are reputed to be the most potent currently available anti-anxiety drugs [1] the nature of their anxiolytic action remains obscure. Their "anti-catecholaminergic" properties [17] and ability to interfere with the serotonergic transmission [17–20] are believed to contribute to the release of punishment-induced behavioral suppression. An alternative hypothesis postulates that benzodiazepines facilitate GABAergic transmission by removing the endogenous protein regulator from the high affinity GABA receptors [4,8]. The fact that most of the benzodiazepines have a pronounced anticonvulsant activity in experimental animals and man [9] clearly supports this hypothesis as epilepsy is believed to be associated with the "weakness" of the GABA system [15]. This hypothesis also implies that the anticonvulsant and anti-anxiety actions of benzodiazepines are related.

Given this background one can anticipate that GABA antagonists and especially convulsant benzodiazepines should have a pronounced pro-anxiety action [13]. Schlosser and Franco [16] have recently described the effects of a powerful convulsant benzodiazepine, Ro 5-3663 (1,3-dihydro-5-methyl-2H-1,4-benzodiazepine-2-one) which had *in vivo* potency near that of picrotoxin. In a preliminary experiment with this drug we noticed that it reduced postictal analgesia tested by the tail-pressure agitation test [12]. However, the nature of this effect and its relevance to anxiety is not clear. We therefore tested Ro 5-3663 in a more conventional behavioral paradigm.

In an experiment employing electric shock as an aversive stimulus, benzodiazepines are known to antagonize the response suppression produced by punishment [7]. In view of this evidence we opted for the one-trial inhibitory learning task ("passive avoidance").

METHOD

Subjects and Materials

Experimentally naive male 200 g Charles-River rats were housed in a standard laboratory environment with food and water ad lib. Night-day cycle (12 hr darkness/12 hr light) was maintained by artificial lighting.

The following compounds were used: Ro 5-3663 (Hoffmann, La Roche, Inc.) and chlordiazepoxide HCl (CDP, Hoffmann, La Roche, Inc.). The doses given in the text refer to their salts.

Procedure and Apparatus

Rats were randomly allocated to four equal groups (n=5) and trained and tested in a testing chamber (15×20×17 cm) containing a drinking tube. During training, the rats were allowed to drink tap water in the testing chamber for four consecutive sessions (10 min session/day). The latency to the first lick was about 4 sec and the amount of time to complete 300 licks varied from 38 to 82 seconds. There were no significant differences between the four groups on either measure. On the fifth day the Grason-Stadler shocker was connected to the drinking tube (the other pole was on the grid floor) and the rat received an electric current (0.5 mA) every time it attempted to drink. Twenty-four hours later rats of each group were administered IP with chlordiazepoxide HCl (CDP, 5 mg/kg); Ro 5-3663 (1 mg/kg); CDP (5 mg/kg) + Ro 5-3663 (1 mg/kg); and Saline (1 ml/kg). CDP and Ro 5-3663 were given 10 and 5 min prior to the test, respectively. Thereafter, each rat was placed into the testing chamber with the shocker disconnected from the tube and the latency to the first lick, time to complete 300 licks, and total number of licks per session were noted.

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TABLE 1

MEAN SELECTED PARAMETERS OF DRINKING BEHAVIOR (LATENCY TO THE FIRST LICK, TIME IN SEC TO COMPLETE 300 LICKS, TOTAL NUMBER IN A 10 MIN SESSION) AFTER PRETREATMENT WITH CHLORDIAZEPOXIDE HCl (CDP), Ro 5-3663, CDP + Ro 5-3663 AND SALINE (n=5 PER GROUP)

Group (dose)	Latency to the 1st lick (sec)	Time (sec) to complete 300 licks	Number licks/ session
Saline (1 ml/kg)	85.2 ± 16.8	134.0 ± 19.2	2478 ± 218
CDP (5 mg/kg)	15.8 ± 5.4†	88.0 ± 19.2	2808 ± 309
Ro 5-3663 (1 mg/kg)	37.8 ± 8.5†	111.0 ± 12.0	2544 ± 227
CDP (5 mg/kg) + Ro 5-3663 (1 mg/kg)	14.0 ± 8.7†	72.0 ± 6.3*	2894 ± 76

None of these parameters proved significantly different before the test. The values represent means ± S.E.M. One-way ANOVA yielded a significant effect of latency, $F(3,16)=9.75$, $p<0.01$. Post hoc Duncan's multiple range test showed that it was due to a latency decrement under CDP, Ro 5-3663 and CDP + Ro 5-3663.

*$p<0.05$, †$p<0.01$ refer to the significance as compared to the saline group.

The time and dose of CDP injection were selected on the basis of previous findings [14]. Ro 5-3663 dose was based on a pilot electrophysiological study conducted with a separate group of rats as described elsewhere [11,12].

This study showed that a dose of 1 mg/kg produced a clear facilitation of the secondary components of visual evoked potentials, the slow negative wave and photically-induced afterdischarge. ECoG showed hypersynchronous 6–7 c/s spindles unaccompanied by behavioral epileptiform manifestations (data not shown). Higher doses of Ro 5-3663 (about 2 mg/kg) were noticed to induce myoclonic jerks and hence cannot be used for behavioral studies.

RESULTS

The results of the study are summarized in Table 1. It demonstrates that CDP and Ro 5-3663 affect rats' drinking behavior in the same direction. While changes under CDP were more pronounced than after Ro 5-3663 this difference was not supported by statistical analysis. Both drugs, however, affected behavior differently from saline.

Comparison of Ro 5-3663 with CDP shows that the former has anxiolytic properties of its own and acts agonistically to CDP.

DISCUSSION

Given that Ro 5-3663 inhibits GABA-stimulated enhancement of diazepam binding [13] and in electrophysiological experiments acts in a manner opposite to the depressant benzodiazepines [16], the present findings are consistent with recent findings [10] that the anticonflict activity of benzodiazepines and their anticonvulsant properties may be dissociated.

An alternative possibility is suggested by recent findings [3] demonstrating that a convulsant, metrazol, may antagonize response-suppression induced by punishment. While this phenomenon has been interpreted as result of retention impairment [3] caused by EEG abnormality, it is known that EEG synchronization (and probably EEG hypersynchronization caused by a drug), may also interfere with motivational dimensions of pain [11]. As mentioned above, Ro 5-3663, although used in subconvulsive doses caused facilitation of the secondary components of VEP, slow secondary negativity and sensory afterdischarge. The VEP facilitation of this magnitude may lead to recall impairment [10]. Also, secondary VEP components are known to undergo facilitation in a state of reduced fear, when habituation to the environment is attained [2], under conditions of withdrawal of noxious stimulation [11]; or when the animal's behavior is rewarded with a palatable food or liquid [5,11]. These states correlate with the low frequency theta (below 7 Hz in a rat) of Gray [6] which has been designated by one of us as a "sensory theta," related to the mechanism of reward [11]. Given this evidence, these changes of VEP wave-form suggest (however tentatively) that Ro 5-3663 may have a peculiar effect (perhaps through a primary action on GABA mechanisms) within the circuits modulating thalamo-cortical activity which causes electrocortical synchrony, inducing thereby a state of mild tranquility.

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