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The Effects of GABA and Benzodiazepine Receptor Antagonists on the Anti-Conflict Actions of Diazepam or Ethanol

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LILJEQUIST, S. AND J. A. ENGEL. *The effects of GABA and benzodiazepine receptor antagonists on the anti-conflict actions of diazepam or ethanol.* PHARMACOL BIOCHEM BEHAV **21**(4) 521–525 1984.—The effects of picrotoxin, bicuculline or RO 15-1788 on the anti-conflict action(s) of diazepam or ethanol were studied in rats using a modified Vogel's conflict test procedure. RO 15-1788 antagonized the anti-punishment effects of diazepam (2.5 mg/kg, IP), whereas various doses of bicuculline or picrotoxin did not interfere with diazepam's anti-conflict effect in this test situation. The anti-conflict action of ethanol (2 g/kg, IP) was antagonized by picrotoxin (1.0 mg/kg, IP), whereas both bicuculline and RO 15-1788 were without effect on the increased punishment response produced by ethanol. These data suggest that the anti-conflict properties of ethanol are at least partially mediated through an enhancement of central GABAergic activity.

Anti-conflict action Benzodiazepine Bicuculline Diazepam Ethanol GABA receptors Picrotoxin RO 15-1788

THE pharmacological profile of ethanol resembles that of most benzodiazepines (BDZ's). Thus, it has been demonstrated that ethanol—like the BDZ's—besides its sedative, anti-convulsant and muscle relaxant effects also exerts an anti-conflict action in certain experimental situations [5, 6, 15, 21, 25, 48]. The involvement of the inhibitory neurotransmitter, GABA, in the anti-convulsant and muscle relaxant properties of the BDZ's is today well-documented (for references, see [9] and [18]), whereas the role for GABA in the anxiolytic effects of these drugs appears to be more controversial. Thus, it has been reported by some investigators that drugs which interfere with GABAergic mechanisms, like picrotoxin [1,39] and bicuculline [49], are able to reverse the anti-conflict action of BDZ's, whereas others [27] only observed an antagonism of the ataxic and sedative actions of BDZ's following administration of picrotoxin. Intracerebral administration of GABAergic agonists like muscimol and 4,5,6,7-tetrahydroisoxazolo-(5,4-c)pyridin 3-ol (THIP) [4,8], or GABA itself [40], is reported to produce an anti-conflict action similar to that of the BDZ's. Systemic administration of muscimol or THIP does not, however, mimic the anti-conflict effects of BDZ's [17, 33, 35, 38].

Increasing evidence, from both behavioral [10, 15, 16, 23, 24, 26, 28], electrophysiological [11,30] and receptor binding studies [36, 41, 44], indicates that ethanol interferes with central GABAergic mechanisms. The possibility that GABA and/or BDZ-sensitive mechanisms may be involved in the recently demonstrated anti-conflict effect of ethanol has, to our knowledge, not been examined. In order to test this possibility and to compare the anxiolytic properties of BDZ's with those of ethanol, we have carried out a series of experiments in which we have compared the influence of various specific GABA or BDZ receptor antagonists on diazepam or an ethanol-induced increase of punished responding in a modified Vogel's conflict test situation.

METHOD

Animals

Male Sprague-Dawley rats (Anticimex; Sollentuna, Sweden) weighing 190–230 g were used. The animals were kept on a controlled light-dark cycle (light period between 5:30 a.m. and 5:30 p.m.) in a room with constant temperature (25°C) and humidity (65%). Upon arrival at the animal facilities of the department, there was a minimum of seven days of acclimation, during which the animals had free access to food and water until the start of the experiments. All animals were used only once in the experiments.

Apparatus and Experimental Procedure

A modified Vogel conflict test procedure [47] was employed using a Plexiglas box (inner dimension, 30×24×20 cm) enclosed in a sound-proof cage and equipped with a grid floor of stainless steel bars and a drinking bottle containing 5.5% (w/v) of glucose. An electric shock (with a current of 0.16 mA, and given for two seconds every three seconds) could be applied between the spout of the drinking bottle and the grid floor. On the first day of the experiment, the animals were adapted to the test chamber during a ten-minute session, after which the period of water deprivation began. After 24 hours of water deprivation, the animals were placed in the test chamber for a further five-minute adaptation, during which the animals had free access to the drinking bottle

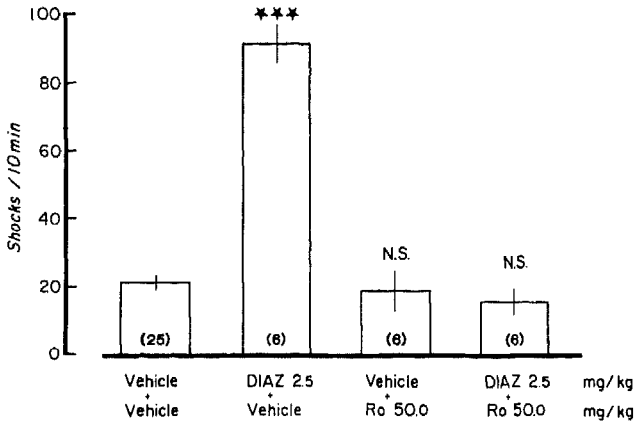

FIG. 1. Effect of RO 15-1788 on the anti-punishment effect of diazepam in rats. Diazepam (2.5 mg/kg, IP) and RO 15-1788 (50 mg/kg, PO) were administered 30 minutes prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. ***$p<0.001$.

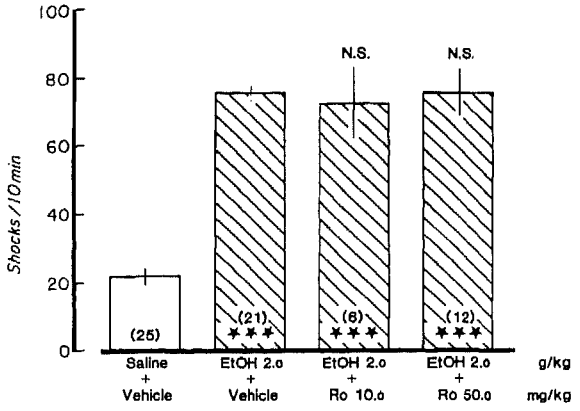

FIG. 2. Effect of various doses of RO 15-1788 on the anti-punishment effect of ethanol in rats. Ethanol (2 g/kg, IP) and RO 15-1788 (PO) were administered 50 and 30 minutes, respectively, prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. ***$p<0.001$ (p-values displayed at the bottom of the bars refer to the comparison with saline-pretreated controls, whereas p-values displayed at the top of the bars refer to the comparison with ethanol-pretreated animals.)

with the glucose solution. After another 24 hours of water deprivation, the rats were again placed in the test box. Upon finding the drinking spout (usually within 20 seconds), the animals were allowed to drink the glucose solution during 30 seconds, after which time the first electric shock was administered. Thereafter, every subsequent attempt of the animals to drink was punished with an electric shock during the ten-minute experimental session, and the number of shocks taken by the animals was recorded.

Drugs

All drugs (except ethanol) were administered in a volume of 2 ml/kg body weight. Diazepam was made from Stesolid (R) (Dumex A/S; Copenhagen, Denmark) ampullae by dilution with a solution of distilled water containing 4% ethyl alcohol and 4% propylenetylen-glycol and administered intraperitoneally (IP). The specific BDZ antagonist, ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-(1,5a)(1,4)-benzodiazepine-3-carboxylate (RO 15-1788; a generous gift from Dr. W. Haefely, Hoffman-LaRoche, Basel, Switzerland) [20] was suspended in distilled water and a few drops of Tween 80, and then administered orally. Bicuculline (Sigma Chemical; St. Louis, MO), a specific GABA receptor antagonist [32], was dissolved in 0.1 N HCl and neutralized by 0.1 N NaOH and administered IP. Picrotoxin HCl (Sigma Chemical; St. Louis, MO), a drug which probably suppresses GABAergic activity via a direct interaction on chloride ion channels [32,43], was dissolved in 0.9% NaCl and given IP. Ethanol was administered IP as a 20% (w/v) solution made from 0.9% NaCl and 95% ethyl alcohol (Svensk Spirit AB; Stockholm, Sweden).

Statistics

Statistical significance was calculated by a Student's t-test.

RESULTS

Preliminary dose-response studies showed that diazepam, in a dose of 2.5 mg/kg (given IP 30 minutes prior to the conflict test), and ethanol (2 g/kg given IP 50 minutes prior to the conflict test), were approximately equi-effective with regard to their anti-conflict properties in the present test situation, i.e., the diazepam- and ethanol group did not statistically significantly differ from each other with regard to number of shocks accepted in this conflict situation. However, it should be pointed out, that in accordance with the findings reported by Vogel *et al.* [48], we found that no consistent dose-relationship could be established between experimental sessions for the anticonflict action of ethanol with doses lower than 2 g/kg. Ethanol doses larger than 2 g/kg produced sedation in the animals, thus interfering with their performance in the operant test situation. Thus, when the drugs were given in these doses, diazepam did not cause any observable signs of sedation or motor disturbances as assessed by gross observation, whereas ethanol produced slight sedation and slight signs of ataxia.

Administration of the specific BDZ receptor antagonist, RO 15-1788 (50 mg/kg, given PO 30 minutes prior to the conflict test), completely antagonized the diazepam-induced increase of punished responding (Fig. 1). Lower doses of RO 15-1788 (10 and 30 mg/kg) have previously been shown to reverse the anti-conflict action of diazepam [2]. The anti-conflict effect of ethanol was unaffected by either a low (10 mg/kg) or a high (50 mg/kg) dose of RO 15-1788 (Fig. 2). These doses of RO 15-1788 did not exert any effect by themselves on the conflict behavior in this test situation (data not shown), which is in agreement with the results by Bonetti *et al.* [2], who found that RO 15-1788, in doses up to 100 mg/kg, had no effect *per se* in a Geller conflict situation. This by no means excludes the possibility that RO 15-1788 may have partial agonist properties in other behavioral test situations, as observed by Corda *et al.* [7] and Nutt *et al.* [31].

Administration of the specific GABA receptor antagonist, bicuculline (given IP 30 minutes prior to the conflict test), did not antagonize the anti-conflict actions of either diazepam or ethanol (Figs. 3 and 4). A higher dose (i.e., 4 mg/kg) of bicuculline could not be used since it produced initial signs of convulsions in both experimental groups, thus interfering

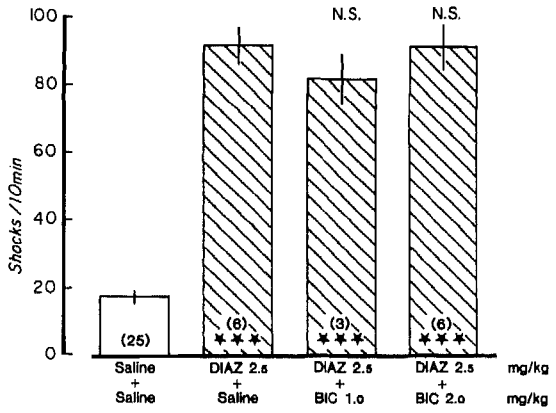

FIG. 3. Effect of various doses of bicuculline on the anti-punishment effect of diazepam in rats. Diazepam (2.5 mg/kg, IP) and bicuculline (IP) were administered 30 minutes prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. ***$p<0.001$ (p-values displayed at the bottom of the bars refer to the comparison with saline-pretreated controls, whereas p-values displayed at the top of the bars refer to the comparison with diazepam-pretreated animals.)

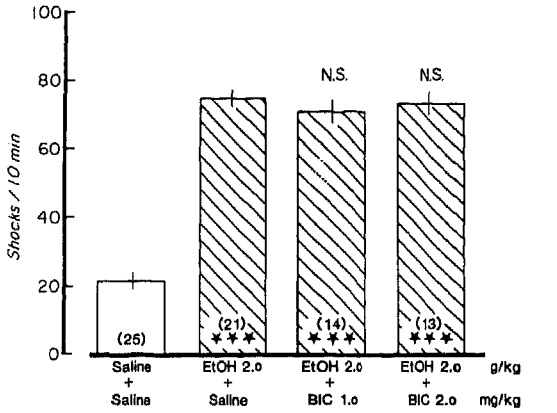

FIG. 4. Effect of various doses of bicuculline on the anti-punishment effect of ethanol in rats. Ethanol (2 g/kg, IP) and bicuculline (IP) were administered 50 and 30 minutes, respectively, prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. ***$p<0.001$ (p-values displayed at the bottom of the bars refer to the comparison with saline-pretreated controls, whereas p-values displayed at the top of the bars refer to the comparison with ethanol-pretreated animals.)

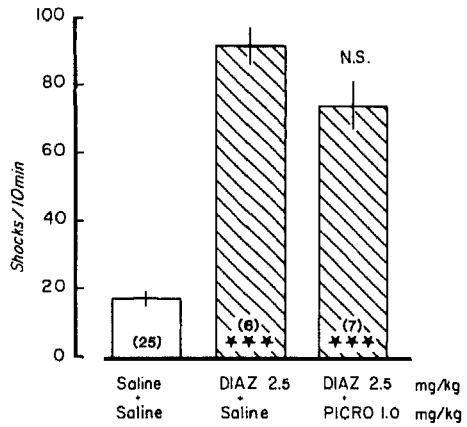

FIG. 5. Effect of picrotoxin (1 mg/kg, IP) on the anti-punishment effect of diazepam (2.5 mg/kg, IP). Diazepam and picrotoxin, respectively, were administered 30 minutes prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. ***$p<0.001$ (p-values displayed at the bottom of the bars refer to the comparison with saline-pretreated controls, whereas p-values displayed at the top of the bars refer to the comparison with diazepam-pretreated animals.)

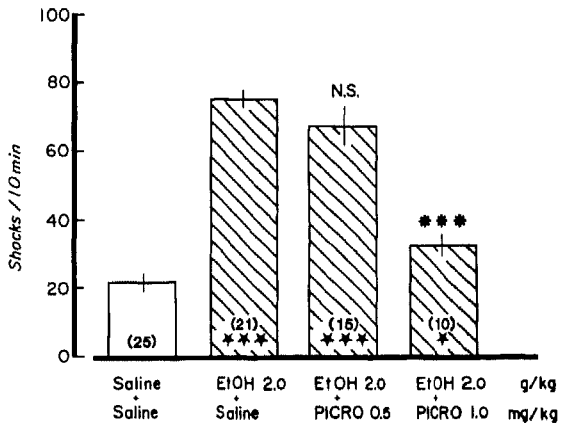

FIG. 6. Effect of various doses of picrotoxin on the anti-punishment effect of ethanol in rats. Ethanol (2 g/kg, IP) and picrotoxin (IP) were administered 50 minutes and 30 minutes, respectively, prior to the start of the recordings. The number of electric shocks accepted was recorded during ten minutes. Shown are the means±SEM. *$p<0.05$; ***$p<0.001$ (p-values displayed at the bottom of the bars refer to the comparison with saline-pretreated controls, whereas p-values displayed at the top of the bars refer to the comparison with ethanol-pretreated animals.)

with the anti-conflict behavior of the animals. In some experiments (data not shown), bicuculline (2 mg/kg) was administered ten minutes prior to the conflict test, a procedure which gave similar results as after administration of bicuculline 30 min prior to the experiments.

Administration of picrotoxin (1.0 mg/kg, given IP 30 minutes prior to the conflict test) had a slight, but statistically insignificant, effect on the anti-punishment action of diazepam (Fig. 5). The same dose of picrotoxin produced an almost complete antagonism of ethanol's anti-conflict activity (Fig. 6). As assessed by gross observation, a clear reversal of the slight ataxic effects of ethanol was observed following administration of picrotoxin and bicuculline. Higher doses of picrotoxin could not be used since they produced initial signs of convulsions (i.e., immobility and tremor) in both experimental groups, thus interfering with the anti-conflict behavior of the animals.

DISCUSSION

The findings in the present study, that picrotoxin almost completely reversed the ethanol-produced increase of punished responding, but did not affect the anti-conflict action of diazepam, indicate that different neurochemical mechanisms

may be responsible for the anti-conflict properties of these drugs. This view is further substantiated by the observation that the specific BDZ receptor antagonist was without any effect on the anti-punishment action of ethanol, whereas, in accordance with previous studies [2,34], it completely blocked the anti-conflict effects of diazepam. Although it appears less likely, it cannot be fully excluded that the inability of RO 15-1788 to block the anti-conflict action of ethanol could have been due to a decreased absorption of RO 15-1788, caused by the rather high dose of ethanol used in the present experiments.

Our study suggests that ethanol's anti-conflict effect may be due to an interference with central GABAergic activity through a picrotoxin-sensitive mechanism, since bicuculline was ineffective in reducing the ethanol-produced increase of punished responding. Previously, it has been found [26,28] that subconvulsive doses of picrotoxin effectively block the sedative effects of ethanol on locomotor activity, as well as ethanol-produced sleep. Further evidence for the contention that these pharmacological actions of ethanol might be mediated via a picrotoxin-sensitive site in the GABA-BDZ-receptor-ionophore complex [32] comes from recent receptor binding studies by Ticku and collaborators [12,42]. These authors found that ethanol produces an enhancement of ^{3}H-diazepam binding through an increase in receptor affinity, an effect which was blocked by picrotoxin.

Since it is known that ethanol may possess analgesic properties, the idea that an altered pain sensitivity might contribute to the anti-conflict actions of ethanol cannot be fully excluded. The role of analgesia in conflict experiments utilizing the delivery of electric shocks has been extensively discussed elsewhere [37], with the consensus being that changes in the pain threshold can hardly fully explain the anti-conflict actions of various drugs. For example, morphine, although a most potent analgesic, is devoid of anti-punishment effects in the type of conflict tests discussed here [37].

In agreement with numerous earlier observations (for references, see [19] and [37]), diazepam produced an increase of punished responding, an effect which was completely reversed by the specific BDZ antagonist RO 15-1788 (see also [2, 20, 34, 46]). The specific GABA receptor antagonist, bicuculline, or the GABAergic antagonist, picrotoxin, was unable to reduce this effect of diazepam. Thus, these data suggest that the anti-conflict action of diazepam is due to a specific activation of central BDZ receptors, and the bicuculline or picrotoxin-sensitive mechanisms are not involved in the anti-conflict action of diazepam in an unconditioned conflict paradigm. Similar results have been obtained by Lippa *et al.* [27], who found that in a licking conflict test, picrotoxin did not reverse the anti-conflict effect of diazepam, but reduced the ataxic effects of diazepam. On the other hand, using the Geller-Seifter conflict, an antagonism of the anti-conflict effect of chlordiazepoxide [1] or oxazepam [37,39] by picrotoxin has been reported. In the experiments by Sepinwall and Cook [37], a reversal of the anti-conflict effect was observed only when picrotoxin was administered in doses which, by themselves, decreased unpunished responding. Moreover, a partial bicuculline-produced reversal of the anti-conflict effects of diazepam was demonstrated by Zakusov *et al.* [49]. A consistent feature in the various studies reviewed above appears to be that a slight reversal of the anti-conflict properties of various BDZ's is observed after administration of GABAergic antagonists in the Geller-Seifter conflict situations with no corresponding findings from studies where Vogel's conflict paradigm was employed.

There is considerable evidence that ethanol also exerts an anti-conflict action in the Geller-Seifter test [22,29], whereas the anticonflict properties of ethanol appear to be more difficult to demonstrate with the Vogel's conflict test paradigm ([33,48], the present study). In our study, an anti-conflict effect of ethanol could be demonstrated only by using a low shock intensity (0.16 mA). This is in agreement with earlier observations that the anti-conflict of ethanol is more easily demonstrated in situations, i.e. Geller-Seifter test situations, where a smaller degree of punishment-produced behavioral suppression is present [22,38]. However, in those test situations, the anti-conflict properties of ethanol were observed following doses of ethanol, which by themselves decreased also unpunished responding.

It should be noted that there is a qualitative difference in these two forms of experimentally induced "anxiety." The Geller-Seifter test represents a conditioned suppression of responding with a well defined behavioral baseline, whereas the Vogel procedure is an example of unconditioned behavioral suppression ("frustration"), utilizing a one-session test with naive experimental animals. Thus, it may be suggested that the former type of behavior, while based on prolonged periods of training and learning experiences, in all probability is mediated via different, picrotoxin-sensitive neurochemical mechanisms and/or different neuroanatomical structures than the latter one. Furthermore, it cannot be excluded that some BDZ receptors which mediate the anti-conflict actions of the BDZ's are connected to GABAergic mechanisms insensitive to bicuculline and/or picrotoxin [13]. Since different subunits at the GABA-BDZ receptor complex interact with each other in a complex and, at present, unknown manner [14, 32, 45], direct biochemical experiments will be required to elucidate the site at which ethanol produces its anti-conflict action.

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