Pharmacology Biochemistry and Behavior, Vol. 46, pp. 179–182, 1993
Printed in the U.S.A

0091-3057/93 $6.00 + 00

Bidirectional Effects of GABAergic Agonists and Antagonists on Maintenance of Voluntary Ethanol Intake in Rats

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BOYLE, A. E., R. SEGAL, B. R. SMITH AND Z. AMIT. *Bidirectional effects of GABAergic agonists and antagonists on maintenance of voluntary ethanol intake in rats.* PHARMACOL BIOCHEM BEHAV **46**(1) 179–182, 1993.—The effects of THIP ($GABA_A$ agonist) and picrotoxin (GABA antagonist) on the maintenance of voluntary ethanol ingestion were examined. Thirty-three male Long-Evans rats were initially exposed to a screening procedure in which increasing concentrations of ethanol (from 2% to 9%) were presented in a free choice with water, on an alternate day schedule. Following the screening procedure, the rats were exposed to five ethanol presentations at a concentration of 9%, which constituted the baseline period, and five additional ethanol presentations during which the effects of the GABAergic manipulations were determined (test period). During the test period, the animals received IP injections of either 16 mg/kg of THIP, 2 mg/kg of picrotoxin or saline. The results suggested that the differential GABA manipulations resulted in bidirectional effects on the consumption of ethanol. More specifically, the $GABA_A$ agonist THIP increased the intake of ethanol as compared to baseline measures, while the GABA antagonist picrotoxin decreased ethanol intake. Similarly, the administration of THIP increased ethanol preference. In contrast, preference for ethanol over water was decreased following the administration of picrotoxin. It appears that the effects of these GABAergic manipulations are specific to ethanol, since total fluid intake was not influenced by the administration of either drug (i.e., THIP or picrotoxin). In light of the literature suggesting that THIP and picrotoxin are active at different sites within the $GABA_A$ chloride-ionophore receptor complex, the present findings would suggest that the $GABA_A$ receptor may play a role in regulating the voluntary intake of ethanol.

THIP Picrotoxin GABA Ethanol Self-administration

ALTHOUGH the precise mechanisms mediating the actions of ethanol remain to be identified, there is increasing evidence to suggest that the major inhibitory neurotransmitter GABA may play a role in mediating some of the behavioral effects of ethanol (3,7,8,15,16).

Furthermore, progress has been made in identifying a putative role for specific GABA receptor subtypes. Two pharmacologically and functionally unique GABA receptor subsystems have been identified, $GABA_A$ and $GABA_B$ (9). The $GABA_A$ receptor subtype has been described as a complex unit in which a chloride (Cl^-)-ionophore is controlled by the GABA receptor and interacts with binding sites for benzodiazapine, picrotoxin, and barbiturates. In addition, the $GABA_A$ receptor subsystem produces inhibition (pre- or postsynaptically) by modulating Cl^- conductance (12,13,16).

An examination of the relationship between GABA and the effects of ethanol suggest that the various receptor subsystems may be differentially involved (2,11,16). Although the $GABA_B$ receptor has been implicated in some of the effects of ethanol, such as physical intoxication (2,11), it is the $GABA_A$ receptor that has been most closely associated with the actions of ethanol. Specifically, ethanol both directly and indirectly has been demonstrated to interact and enhance the Cl^- channel flux of the $GABA_A$ receptor (12,13). In addition, the behavioral effects of ethanol administration have been suggested to be a function of the relative sensitivity of the $GABA_A$ receptor system to ethanol (1,10,17).

Furthermore, a body of research has suggested that the $GABA_A$ receptor in particular may act to regulate voluntary ethanol intake (4,14). Specifically, it has been reported that the administration of the $GABA_A$ agonist THIP enhanced the acquisition of ethanol ingestion in a voluntary intake paradigm (14), while the $GABA_B$ agonist baclofen was found to have nonspecific effects, in that increased ethanol intake was associated with a generalized increase in total fluid intake.

The role of the $GABA_A$ receptor in influencing ethanol intake has also been extended to include effects on voluntary ethanol intake in a maintenance paradigm. It has been reported (4) that ethanol consumption in a free-choice maintenance paradigm was decreased as a result of the administration of the GABA agonist calcium-acetyl-homotaurine. Also demonstrated within the same study, the decrease in ethanol intake induced by calcium-acetyl-homotaurine was attenuated by the administration of the $GABA_A$ antagonist bicuculline.

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While calcium-acetyl-homotaurine represented a nonspecific GABA agonist, the observed attenuation of its effect by bicuculline, a $GABA_A$ antagonist, clearly suggested that the $GABA_A$ subsystem at least in part mediated the effects of calcium-acetyl-homotaurine on ethanol intake.

However, in contrast to these earlier research findings indicating a role for the $GABA_A$ receptor system in the mediation of the maintenance of voluntary ethanol intake (4), others have suggested (6) that pharmacological manipulations of the $GABA_B$, but not the $GABA_A$, system result in specific influences on ethanol intake within a maintenance paradigm. In particular, it has been suggested (6) that benzodiazapines and the $GABA_A$ agonist muscimol failed to influence ethanol intake, whereas the $GABA_B$ agonist baclofen significantly decreased ethanol intake.

Thus, while the literature suggests that there are findings to support a role for the $GABA_A$ receptor system in the mediation of the acquisition of voluntary ethanol intake, the findings regarding the role of the $GABA_A$ system in the maintenance of voluntary ethanol intake are equivocal. Therefore, in an attempt to further clarify the role of the $GABA_A$ system in the maintenance of voluntary ethanol intake, the present study will examine the influence of the specific $GABA_A$ agonist THIP and $GABA_A$ Cl^- channel blocker picrotoxin on ethanol intake within a maintenance paradigm.

METHOD

Subjects

Thirty-three male Long-Evans new colony rats (Charles Rivers Canada Inc.) weighing between 175–200 g were individually housed in stainless steel cages in a room controlled for constant temperature, humidity, and 12L : 12D schedule. Food and water were freely available throughout the test period.

Procedure

Following an acclimatization period, the rats were exposed to a screening procedure during which a sequence of increasing concentrations of ethanol solutions was presented, within glass richter tubes mounted on the front of the home cages, in a free choice with water on an alternate day schedule. Beginning with a 2% ethanol solution, the concentrations were increased after every second ethanol presentation until a 9% concentration was achieved. The position of the ethanol-filled tube, in relation to the water-filled tube, was altered on successive ethanol presentation days to avoid the potential of a position bias. During the intervening days both tubes were filled with water.

Following the screening procedure and a subsequent stabilization period, the rats were exposed to five ethanol presentations at a concentration of 9%, in a free choice with water, on 2 alternate days. This interval constituted the baseline period.

Beginning with the first alternate day following the baseline period, the rats were exposed to a test period in which the effects of the GABAergic manipulations were determined. The test period consisted of five additional ethanol presentations on alternate days, in a free choice with water.

During the test period, rats were assigned to groups that received IP injections of either 16 mg/kg of THIP (a $GABA_A$ agonist), 2 mg/kg of picrotoxin (a $GABA_A$ chloride-ionophore channel blocker), or saline on the ethanol presentation days. Group selections were made in such a manner that the amount of absolute ethanol consumed during the baseline period was approximately equal for each group. The dose of THIP utilized was selected on the basis of literature reporting that it significantly increased ethanol intake in an acquisition paradigm (14). Throughout the baseline and test periods, ethanol and water scores, in addition to body weight, were recorded.

RESULTS

In the present experiment, the effects of THIP and picrotoxin on the intake of ethanol, water, and body weight were examined using multiple three-way ANOVAs (the variables consisting of drug, trial periods, and days).

The analysis of the effects of the GABAergic manipulations on absolute ethanol intake indicated that a bidirectional effect was produced. The results indicated that there was a significant interaction between trial periods and drug groups, $F(2, 30) = 15.44\ p < 0.0001$. In light of the significant two-way interaction, a test of simple main effects and simple interactions was performed holding drug group variable constant. As can be seen in Fig. 1, THIP treatment resulted in an increase in the intake of absolute ethanol during the test trials when compared to those values observed during the baseline period, $F(1, 30) = 27.57, p < 0.001$. In contrast, picrotoxin produced a decrease in absolute ethanol intake relative to its baseline values, $F(1, 30) = 4.88, p < 0.035$. Saline-treated rats failed to show any significant differences across trial periods, $F(1, 30) = 0.21, p < 0.64$.

Presented in Fig. 2 are the preference ratios for THIP-, picrotoxin-, and saline-treated rats during baseline and test trials. The analysis indicated a significant interaction, $F(1, 30) = 27.64, p < 0.0001$, between trial periods and drug groups. Subsequent tests of simple main effects and interaction, holding drug groups constant, indicated that consistent with what had been observed with absolute ethanol intake, THIP treatment increased preference ratios, $F(1, 30) = 40.92, p < 0.0001$, during the test trial period relative to the baseline period, whereas picrotoxin decreased preference, $F(1, 30) = 14.43, p < 0.0007$. No differences in preference ratios between baseline and test trials were observed with saline treatment, $F(1, 30) = 71.71, p < 0.40$.

An analysis of the data indicated that while the groups did differ in terms of their levels of total fluid intake, $F(2, 30) =$

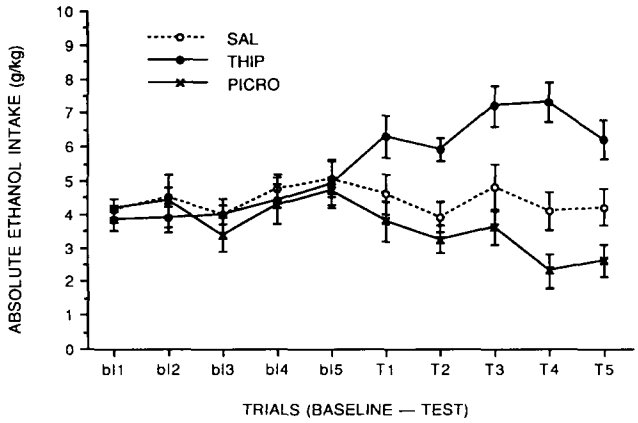

FIG. 1. The effects of THIP and picrotoxin treatment on the intake of absolute ethanol intake across baseline and treatment trials. Vertical lines represent the SEM.

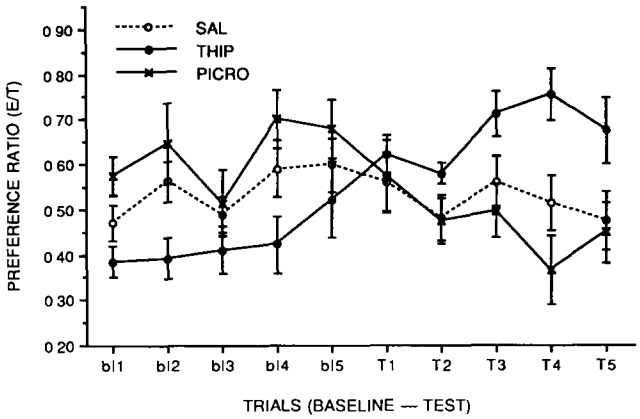

FIG. 2. The effects of THIP and picrotoxin treatment on preference levels across baseline and treatment trials. Vertical lines represent the SEM.

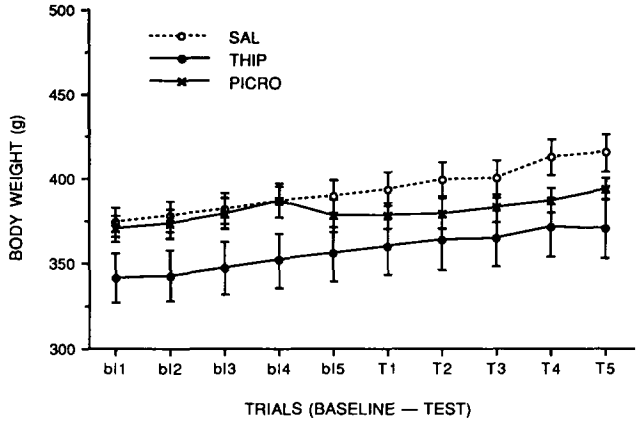

FIG. 4. The effects of THIP and picrotoxin treatment on body weight across baseline and treatment trials. Vertical lines represent the SEM.

4.69, $p < 0.016$, there were no significant changes in intake as a function of drug treatment (Fig. 3).

Finally, the analysis of body weight values, as presented in Fig. 4, indicated a significant three-way interaction between drug treatment, trial period, and days, $F(8, 120) = 2.58$, $p < 0.0124$. However, the main effect for drug treatment was not significant. An analysis of simple main effects and simple interactions holding the drug group variable constant indicated that both THIP-, $F(1, 30) = 19.26$, $p < 0.001$, and saline-, $F(1, 30) = 52.09$, $p < 0.00001$, treated rats exhibited an increase in body weight across trial periods. In contrast, picrotoxin-treated rats failed to demonstrate any significant change in body weight across trial periods, $F(1, 30) = 2.32$, $p < 0.138$.

DISCUSSION

Results of the present study indicate that within a maintenance paradigm, the $GABA_A$ receptor agonist THIP acted to enhance the voluntary intake of ethanol while the functional $GABA_A$ antagonist picrotoxin decreased intake. These findings are consistent with previous data that suggested that THIP facilitates ethanol intake within an acquisition paradigm (14). Overall, the results indicated that GABAergic manipulations, which are known to produce opposing effects on Cl^- flux, also produced directionally similar effects on ethanol intake/preference. It is worth noting that reports on bidirectional effects of agonists and antagonists of the same system, as observed in the present study, are quite rare. Nonetheless, the present findings argue strongly in favor of a role for the $GABA_A$ receptor in regulating voluntary ethanol intake.

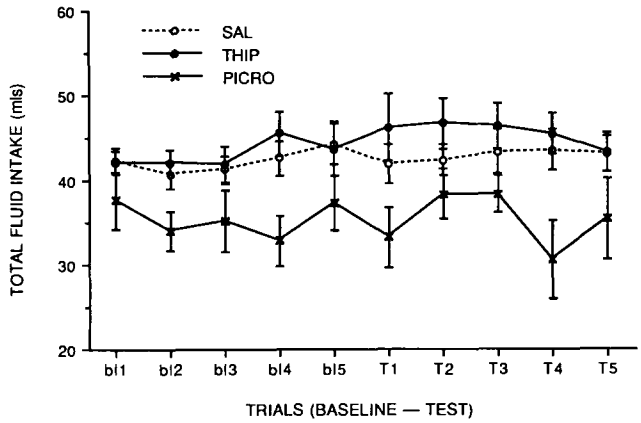

FIG. 3. The effects of THIP and picrotoxin treatment on the total intake of fluids across baseline and treatment trials. Vertical lines represent the SEM.

While the GABAergic manipulations produced changes in ethanol intake, the results suggest that the effects on ethanol were not a function of a generalized fluid effect. Total fluid intake for all three treatment conditions was unchanged as a function of drug administration.

In contrast, changes in body weight were not consistent across treatment groups. THIP-treated subjects exhibited a rate of increase in body weight equivalent to that of saline controls. Changes in the pattern of body weight gain due to drug treatment were observed only in the picrotoxin-treated subjects. Specifically, these subjects failed to demonstrate the increase in body weight across trial periods that was observed in both the THIP and saline controls. Furthermore, a review of the data revealed that deviations in the body weight of picrotoxin-treated rats preceded the drug treatment period and as a consequence occurred prior to the onset of a decrease in ethanol intake. Thus, there would appear to be a dissociation between the effects of the GABAergic manipulations on ethanol intake and body weight. The notion of a dissociation between the effects of GABAergic manipulations on ethanol and food intake (as inferred from a change in body weight) is consistent with previous findings from this laboratory (5).

While the present findings support the notion that the $GABA_A$ receptor may contribute to the regulation of the voluntary intake of ethanol, they are inconsistent with a report in the literature that suggests the $GABA_A$ receptor agonist muscimol was ineffective in modulating ethanol intake (6). It is unlikely that the discrepancy between these studies is related to the differential use of acquisition or maintenance paradigms, since the $GABA_A$ agonist THIP has now been demonstrated to influence ethanol intake in both maintenance and acquisition (15) studies. Therefore, the failure of the $GABA_A$

agonist muscimol to influence ethanol intake (6) may be attributable to methodological issues such as that pertaining to the dose of the drug administered. In particular, the dose of muscimol administered in this particular study (6) was relatively low. Thus, the failure to observe an effect on ethanol intake could be argued to reflect the use of a dose of muscimol insufficient to produce alterations in behavioral responding. Support for this suggestion is obtained from the failure of the authors (6) to report any effect of the administered dose on indices of consummatory behavior, such a changes in the pattern of body weight gain or total fluid intake, despite the fact that muscimol, like THIP, is reported to be an anorectic.

Furthermore, while the results of the present study are consistent with findings that suggested the $GABA_A$ receptor may mediate voluntary ethanol intake (4,14), the increased intake observed in the present study with the $GABA_A$ agonist THIP is at odds with the report (4) that suggested the $GABA_A$ agonist calcium-actyl-homotaurine decreases ethanol consumption. While this discrepancy is not readily explained, it is suggested that methodological differences between the studies may be relevant in this regard. In particular, Boismare et al. (4) made use of groups of subjects that consisted exclusively of high ethanol-preferring rats as opposed to the more heterogeneous group of drinkers used in the present study. It is possible that the discrepancy in the effects observed with these two $GABA_A$ agonists may be a reflection of the interaction of the agonists with groups of rats of different overall mean ethanol consumption levels.

Thus, the results of the present study provide a further indication that the $GABA_A$ receptor system plays a role in regulating the voluntary intake of ethanol. However, further research is required to determine the extent to which the mechanism mediating the relationship between the $GABA_A$ system and the intake of ethanol is a function of changes in the reinforcing efficacy of ethanol or is attributable to other mechanisms. To achieve this end, an experimental approach incorporating multiple behavioral parameters, in addition to simple preference measures, may be warranted.

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