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Effect of GABAergic Drugs on Motor Impairment From Ethanol, Barbital and Lorazepam in Rat Lines Selected for Differential Sensitivity to Ethanol

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HELLEVUO, K., K. KIIANMAA AND E. R. KORPI. *Effect of GABAergic drugs on motor impairment from ethanol, barbital and lorazepam in rat lines selected for differential sensitivity to ethanol.* PHARMACOL BIOCHEM BEHAV **34**(2) 399–404, 1989.—The effect of GABAergic drugs on the motor-impairing effects of ethanol, barbital, and lorazepam were studied in the ethanol-sensitive ANT (Alcohol Nontolerant) and ethanol-insensitive AT (Alcohol Tolerant) rat lines, selected for differential ethanol-induced motor impairment on the tilting plane. The basic population from which these rat lines were derived, the mixed (M) line, was also included in the study. The ANT rats were more sensitive to the intoxicating effects of ethanol, barbital, and lorazepam than the AT and M rats at the dose ranges tested. Picrotoxin antagonized motor impairment from all three drugs. Flumazenil (Ro 15-1788) antagonized only the effects of lorazepam, and isoniazid did not modify motor impairment induced by any of the three drugs. These results confirm that the selection of AT and ANT lines has not been specific to ethanol, and that it has increased sensitivity to ethanol, barbital, and lorazepam in the ANT rats rather than decreasing it in the AT rats relative to the M rats. The finding that picrotoxin counteracted motor impairment from ethanol, barbital, and lorazepam support the view that the $GABA_A$ receptor complex is important in mediating the intoxicating effects of these drugs. These results also suggest that the genetically-determined difference in sensitivity to ethanol between the rat lines involves GABAergic mechanisms, but it remains to be determined whether any part of the $GABA_A$ receptor itself has been affected by the selection program.

Ethanol Benzodiazepine Barbiturate GABA Motor impairment Selected lines Ethanol antagonism Picrotoxin Flumazenil (Ro 15-1788) Isoniazid

THE ethanol-sensitive ANT (Alcohol Nontolerant) rats show a large performance decrement on the tilting plane after ethanol (2 g/kg), while the ethanol-insensitive AT (Alcohol Tolerant) rats show little motor impairment (6,19). These lines provide a valuable tool for studying the involvement of different neuronal mechanisms in ethanol sensitivity. The ANT rats are also more sensitive than the AT rats to the motor-impairing effects of barbital and lorazepam (12,40), but do not differ in impairment from morphine (40). The line difference in sensitivity cannot be explained by differences in blood ethanol or drug concentrations (6,12). The selection of the AT and ANT rats thus has not been specific to ethanol, but rather has produced changes in the mechanisms of neuronal sensitivity common to ethanol, barbiturates, and benzodiazepines.

Ethanol, barbiturates, and benzodiazepines also share a common pharmacological profile, being anxiolytic, anticonvulsant, muscle relaxant, and sedative (2). The primary target of barbiturates and benzodiazepines is probably the γ-aminobutyric acid (GABA)/benzodiazepine receptor/chloride-ionophore complex ($GABA_A$ receptor) [see (4, 9, 14, 35, 36, 39)]. In vitro binding studies suggest that this complex has recognition sites for GABA, benzodiazepines, and barbiturates.

As the major inhibitory neurotransmitter, GABA is also susceptible to several effects of ethanol [see (17, 21, 44, 45)]. GABA-mediated neurotransmission is potentiated by ethanol in the cerebral cortex and substantia nigra in vivo (30, 32, 33). Behaviorally, GABA agonists have been found to suppress the stimulation of locomotor activity produced by ethanol in mice (5) and GABA antagonists to reduce the motor impairment and the duration of the loss of righting reflex caused by a high dose of ethanol in rats and mice (8, 10, 22, 26).

In this study we wanted to clarify the role of GABAergic mechanisms in the genetically-determined differences in sensitivity to ethanol, barbital, and lorazepam in the AT and ANT rats and also in the mixed (M) line of rats from which they were derived (6). Dose-response curves of the motor impairing effects of

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ethanol, barbital, and lorazepam were first constructed to find doses that cause marked motor impairment in all three rat lines. Then, the impairment from these three drugs was investigated in rats pretreated with picrotoxin (a blocker of the $GABA_A$ receptor-associated chloride channel), flumazenil (Ro 15-1788) (a benzodiazepine receptor antagonist), and isoniazid (a GABA synthesis inhibitor). In an additional experiment, antagonism by different doses of picrotoxin was studied in ethanol- and barbital-treated AT and ANT rats.

METHOD

Female AT, ANT (F_{27}, F_{28}, F_{33}, and F_{35}) and M (F_{28} and F_{29}) rats were used in all experiments. The mean body weight ($\pm$ SEM) was 287 ± 2 g ($n = 271$) in the AT rats, 280 ± 1 g ($n = 269$) in the ANT rats, and 284 ± 3 g ($n = 100$) in the M rats. The ambient temperature was 22 ± 2°C, relative humidity 50–55% and light-dark cycle 12/12 hr (lights on at 6:00 a.m.). All animals were housed in group cages of 5–6 rats with free access to standard rat chow (R3 Rat Feed, Ewos Ab, Södertälje, Sweden) and tap water.

Tilting Plane Test

Motor impairment was measured in the rats with the tilting plane test (1,12), conducted on a blind basis between 8 and 11 a.m. The animal was placed on a wire-cloth covered plane, which was tilted at a constant speed from horizontal to vertical position in 5 sec. The sliding angle of the rat was recorded. In the trial, the rat was given a predrug test. The effect of the drug treatment was assessed by testing the same animal after injection. The change in the sliding angle was taken as the measure of motor impairment.

Motor Impairing Effects of Ethanol, Barbital and Lorazepam

Motor impairment was induced by intraperitoneal injections of ethanol (0.5, 1.25, 2.0,and 2.75 g/kg), barbital (40, 80, 120, and 160 mg/kg) or lorazepam (1, 3, 5, and 7 mg/kg) in the naive AT, ANT, and M rats, and measured on the tilting plane 30 min after injection. Ethanol was administered as 6% or 12% (w/v) solution in saline, barbital as 5 mg/ml or 10 mg/ml in saline, and lorazepam as 0.24 mg/ml or 0.6 mg/ml in 5% propyleneglycol/1% Macrogol 400/saline.

Antagonism by Picrotoxin, Isoniazid and Flumazenil

The effects of picrotoxin (2 mg/kg, IP), isoniazid (250 mg/kg, IP), and flumazenil (10 mg/kg, IP) on the motor-impairing effects of ethanol (2.75 g/kg, IP) and barbital (160 mg/kg, IP) were studied in the naive AT, ANT, and M rats, and on those of lorazepam (3 mg/kg, IP) in the ANT rats. Ethanol, barbital, and lorazepam were administered in doses which were found to cause marked impairment. The doses of picrotoxin and isoniazid were chosen on the basis of pilot experiments so, that given alone, the drugs caused convulsions in less than 25% of the animals. The 250 mg/kg dose of GABA synthesis inhibitor isoniazid produced in 45 min a 19% (frontal cortex)–30% (hypothalamus) decrease of brain GABA concentrations when measured after microwave irradiation (data not shown). Picrotoxin (0.4 mg/ml in saline) was administered 10 min before ethanol or lorazepam and 5 min before barbital. Isoniazid (50 mg/ml in saline) was injected 30 min before ethanol or lorazepam and 15 min before barbital. Flumazenil (2 mg/ml in 2% Tween 80/saline) was administered 15 min before ethanol, lorazepam, or barbital. The control groups were treated with the corresponding vehicle at the respective times. The tilting plane tests were performed 30 min after administration of ethanol, barbital, or lorazepam.

The Effect of Different Doses of Picrotoxin

The effect of picrotoxin (0.5, 1.25, or 2 mg/kg, IP) on motor impairment from ethanol (2.75 g/kg, IP) and barbital (160 mg/kg, IP) was studied in the naive AT and ANT rats. Saline or picrotoxin (0.2 mg/ml or 0.4 mg/ml in saline) was administered 10 min before ethanol and 5 min before barbital. The tilting plane test was performed 30 min after administration of ethanol or barbital.

Drugs

Barbital (sodium salt or 5,5-diethylbarbituric acid) was purchased from Merck, Darmstadt, F.R.G., lorazepam (Temesta®) from Wyeth/Huhtamäki Pharmaceuticals, Turku, Finland, and picrotoxin and isoniazid (isonicotinic acid hydrazide) from Sigma Chemical Co., St. Louis, MO. Flumazenil was kindly donated by Hoffmann-La Roche & Co. Ltd, Basel, Switzerland.

Statistical Analysis

Differences between groups were studied with analysis of variance followed by the Student-Newman-Keuls test or Student's *t*-test.

RESULTS

Motor Impairing Effects of Ethanol, Barbital, and Lorazepam

Figure 1a shows the dose-response curves of the motor-impairing effects of ethanol in the AT, ANT, and M rats. A two-way analysis of variance revealed a significant rat line $\times$ dose interaction, $F(8,95) = 5.49$, $p = 0.0001$, the motor performance of the ANT rats being significantly more impaired than that of the AT and M rats at doses of 1.25–2.75 g/kg of ethanol.

The ANT rats were also significantly more sensitive than the AT and M rats to 80–160 mg/kg of barbital, which was proved by a significant rat line $\times$ dose interaction, $F(8,67) = 2.89$, $p = 0.0079$ (Fig. 1b). Similarly, a significant rat line $\times$ lorazepam dose interaction was found, $F(8,78) = 7.42$, $p = 0.0001$, the ANT rats being significantly more impaired than the AT and M rats which, although sedated, did not show any impairment in the motor task at 1–7 mg/kg (Fig. 1c). The AT rats did not show any impairment of motor performance, even after a dose of 14 mg/kg of lorazepam (data not shown).

Antagonism by Picrotoxin, Isoniazid and Flumazenil

The level of motor impairment from ethanol, barbital, and lorazepam varied within the same rat line in different experiments (Figs. 1, 2 and 3). This may be due to the use of different generations of rats, which show variation from generation to generation in the level of motor impairing effect of ethanol (6). An attempt to reduce this effect was made by using animals of similar body weights (and age).

Picrotoxin significantly reduced ethanol-induced motor impairment in the ANT, AT, and M lines as shown in Fig. 2a. However, neither isoniazid nor flumazenil could modify ethanol-induced motor impairment in any of the three rat lines.

Motor impairment caused by barbital was also significantly reduced by picrotoxin in all three rat lines (Fig. 2b). There was a significant rat line $\times$ drug effect interaction, $F(1,50) = 6.76$, $p = 0.0100$, suggesting statistically that picrotoxin antagonized barbital more in the ANT than AT rats. This interaction was augmented by the significant difference in the scores of the saline

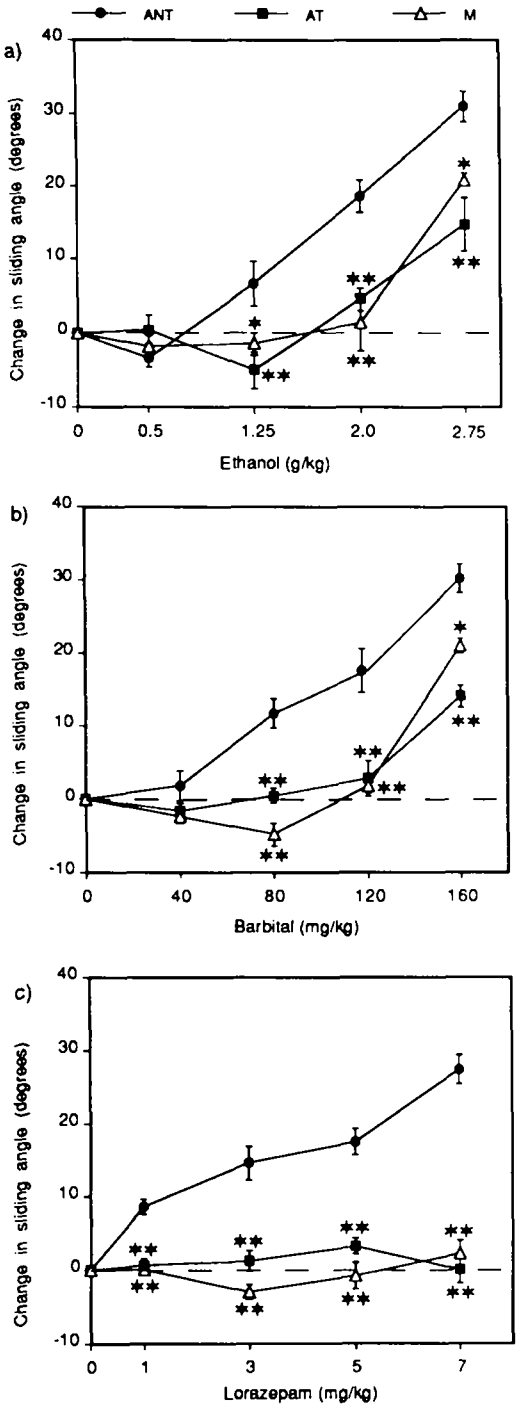

FIG. 1. The motor-impairing effect of intraperitoneally administered ethanol (panel a), barbital (panel b), and lorazepam (panel c) in the ANT, AT, and M rats on the tilting plane. The points represent the mean ± SEM of 6–8 rats. *$p<0.05$; **$p<0.01$ difference from the ANT line, Student-Newman-Keuls test.

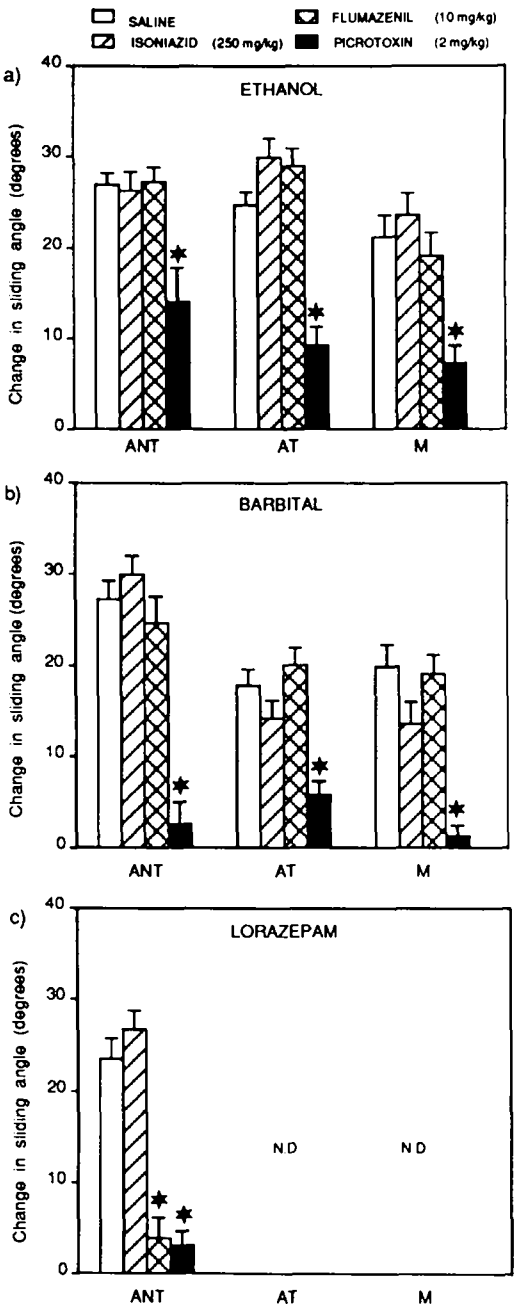

FIG. 2. The effect of intraperitoneally administered isoniazid, flumazenil, and picrotoxin on the motor impairment induced by (a) ethanol (2.75 g/kg, IP) and (b) barbital (160 mg/kg, IP) in the ANT, AT, and M rats and on the motor impairment induced by lorazepam (3 mg/kg, IP) (c) in the ANT rats on the tilting plane. The columns represent the mean ± SEM of 6–20 rats. A significant rat line × picrotoxin effect interaction was found in barbital-induced motor impairment (see the Results section). *$p<0.01$ difference from the control group, Student-Newman-Keuls test. N.D. = not determined.

groups of the AT and ANT lines ($p<0.01$). Barbital-induced motor impairment could not be modified by either isoniazid or flumazenil in any of the rat lines.

Since lorazepam did not impair the performance of the AT and M rats in the dose-response experiment on the tilting plane, only ANT rats were tested. Both picrotoxin and flumazenil significantly antagonized the motor-impairing effects of lorazepam in the ANT rats, whereas isoniazid had no effect (Fig. 2c).

The Effect of Different Doses of Picrotoxin

As in the previous experiment, picrotoxin antagonized signif-

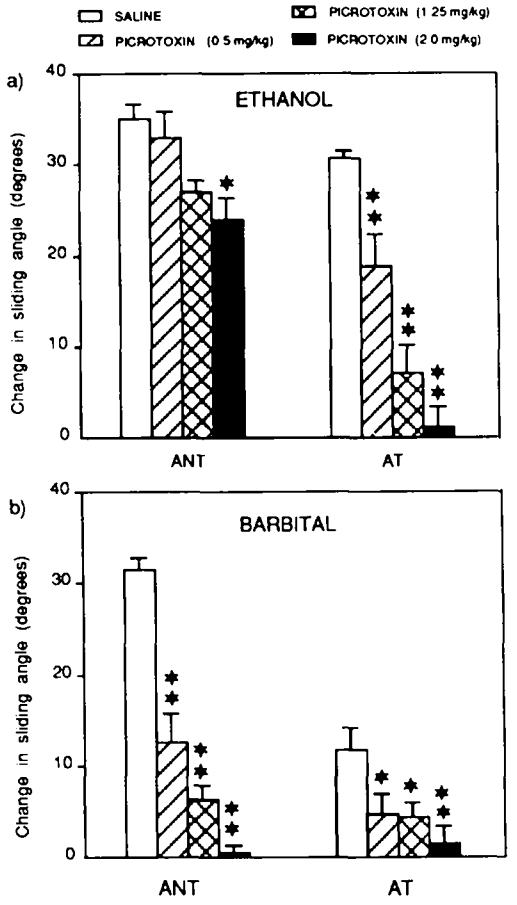

FIG. 3. The effect of different doses of intraperitoneally injected picrotoxin on the motor impairment induced by (a) ethanol (2.75 g/kg, IP) and (b) barbital (160 mg/kg, IP) in the ANT and AT rats on the tilting plane. A significant rat line × picrotoxin dose interaction was found both in ethanol- and barbital-induced motor impairment (see the Results section). The columns represent the mean ± SEM of 6–11 rats. *$p<0.05$, **$p<0.01$ difference from the control group, Student-Newman-Keuls test.

icantly motor impairment from ethanol in the AT and ANT rats. A significant rat line × picrotoxin dose interaction revealed that the pictoroxin-induced antagonism of the motor impairment from ethanol was significantly greater in the AT than in the ANT rats, $F(3,45)=5.33$, $p=0.0031$ (Fig. 3a). All doses of picrotoxin reduced significantly ethanol-induced motor impairment in the AT rats, whereas the performance of the ANT rats was modified only by the highest dose of picrotoxin.

Motor impairment caused by barbital was significantly antagonized by all doses of picrotoxin in both AT and ANT rats (Fig. 3b). A significant rat line × picrotoxin dose interaction, $F(3,69)=10.45$, $p=0.0001$, was found and as in the previous experiment (Fig. 2b), the significant line difference in motor impairment of the saline groups of the AT and ANT lines ($p<0.01$) may have augmented the statistical interaction.

DISCUSSION

The results of this study confirm the earlier findings that barbital and lorazepam impair the motor performance of the ethanol-sensitive ANT rats more than that of the ethanol-insensitive AT rats on the tilting plane (6, 12, 40). Furthermore, it was evident that the lines differ in motor impairment over different doses of each drug. As suggested by earlier studies, it is unlikely that the differential sensitivity of the rat lines to ethanol, barbital, and lorazepam can be explained in terms of differential concentration profiles of the drugs in the blood (6,12). The results of this study furthermore indicate that the selection of the AT and ANT rat lines has produced an increase in sensitivity to the motor-impairing effects of ethanol, barbital, and lorazepam in the ANT rats rather than a decrease in sensitivity in the AT rats.

In the present study, motor impairment from ethanol and barbital was counteracted by picrotoxin, but not flumazenil or isoniazid, and motor impairment from lorazepam by picrotoxin and flumazenil, but not isoniazid. Statistical analysis suggested that picrotoxin antagonized ethanol intoxication less and barbital intoxication more in the ANT than AT rats. This result is confounded by the differential motor impairment between various experiments within the lines and 'ceiling effects.' In the last experiment it is possible that the ethanol intoxication was so strong in the ANT rats that it could not be counteracted by picrotoxin. In the case of barbital, the drug caused only a slight impairment of motor performance in the AT rats, leaving little to be counteracted by picrotoxin. These results do not allow us to conclude that genetic modification has taken place in the picrotoxin site of the $GABA_A$ receptor associated chloride channels in the ethanol-sensitive ANT rats during the selective outbreeding. In vitro studies have not shown differences between ethanol-naive AT and ANT rats in the characteristics of the picrotoxin-sensitive binding of [^{3}H]t-butylbicycloorthobenzoate, a ligand binding to the chloride ionophores in brain cell membranes (25).

Our results suggest, however, the involvement of the enhanced GABAergic neurotransmission in the motor impairing effects of ethanol, barbital, and lorazepam. Ethanol, depressant barbiturates, and benzodiazepines have been described to enhance the $GABA_A$ receptor-associated chloride fluxes in brain vesicles at physiologically relevant concentrations in a picrotoxin-sensitive manner (34, 38, 42). Picrotoxin antagonizes GABAergic neurotransmission by blocking the chloride ionophore (43), which is coupled to the $GABA_A$ receptor (11). In line with the present findings, picrotoxin has been shown in rodents to antagonize the intoxicating effects of ethanol (26), barbiturates (18,29) and benzodiazepines (7,41) in various tests. The duration of the loss of the righting reflex induced by ethanol, amobarbital, or chlordiazepoxide has been shown to be shortened by picrotoxin (20, 22, 24, 26). Another chloride-ionophore blocker, isopropylbicyclophosphate, also reduces the duration of ethanol-induced loss of righting reflex and mortality from pentobarbital (29).

The imidazobenzodiazepine, flumazenil, was a specific benzodiazepine antagonist reversing only the effect of lorazepam. This accords with numerous earlier findings showing flumazenil-induced antagonism of several behavioral, neurochemical, biochemical, and electrophysiological changes produced by benzodiazepines, but not those produced by ethanol or barbiturates (3, 16, 27, 31, 37).

Isoniazid inhibits the GABA synthesizing enzyme glutamate decarboxylase (GAD) (15, 23, 28). Administration of isoniazid is followed by depletion of brain GABA, which results in convulsions (15,23). In the present study, isoniazid decreased the concentration of GABA (see the Method section) to the same extent as found earlier by others (15), and was convulsive in less than 25% of the animals. When administered together with ethanol, barbital, or lorazepam, neither convulsions nor improvement in motor performance could be seen. Thus, the decrease in the concentration of GABA produced here was not sufficient enough to alter the CNS sensitivity to depressants.

The results of this study confirm that the selection of the ethanol-sensitive ANT rat line and ethanol-insensitive AT rat line has also produced a difference in sensitivity to barbital and

lorazepam between the lines. The fact that these drugs act on the GABA system in conjunction with the earlier finding that the rat lines do not differ in the motor impairment produced by morphine (40), suggests that the genetically-determined difference in these rats lines involves specifically GABA and not some general facility to overcome all forms of motor impairment. These results also suggest that sensitivity to ethanol, barbital, and lorazepam seems to have developed in the ANT rats rather than insensitivity in the AT rats.

The results that picrotoxin antagonized motor impairment from ethanol, barbital, and lorazepam support the idea that the $GABA_A$ receptor complex is important in mediating the intoxicating effects of these drugs. Line differences found in the number of muscimol binding sites (25) and GABA turnover (13), encourage further studies to clarify whether pre- and/or postsynaptic GABAergic mechanisms underlie the differential sensitivity to ethanol between the AT and ANT rats.

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