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Role of GABA in the Actions of Ethanol in Rats Selectively Bred for Ethanol Sensitivity

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LIU, Y. AND R. A. DEITRICH. *Role of GABA in the actions of ethanol in rats selectively bred for ethanol sensitivity*. PHARMACOL BIOCHEM BEHAV **60**(4) 793–801, 1998.—Rats from the N/Nih heterogeneous stock have been selectively bred for high (HAS) or low (LAS) initial sensitivity to injected ethanol as measured by duration of the loss of the righting reflex. The selection for ethanol sensitivity in these lines apparently has reached a maximum. These lines are useful to elucidate the central nervous system mechanisms of the genetic differences between the lines and also provide clues to the mechanisms of ethanol's action. We have found that: 1) ethanol, etomidate, and ketamine but not propofol produce different sleep times and brain levels of the drug on awakening between these two lines; 2) only ethanol, etomidate, and ketamine produced significant differences between the HAS and LAS rats in GABA-mediated stimulation of chloride uptake into brain microsacs; 3) GABA, propofol, and etomidate decreased the K_d for flunitrazepam binding to whole-brain membranes but equally in both lines. Neither ethanol nor ketamine had an effect; 4) only GABA, ethanol, and etomidate increased the K_d for TBPS binding and only GABA decreased B_{max} of TBPS binding. As with the previous selection for ethanol sensitivity in mice (short and long sleep) these lines of rats have very marked line differences in GABA-mediated events, and these are correlated with the sedative effects of ethanol. From these and previous studies we know that the major differences between selected lines of mice and rats are that the mouse lines are not differentially sensitive to halothane or pentobarbital while the rat lines are. However, the mouse lines are differentially sensitive to propofol and the rat lines are not. These data should be useful in dissecting the actions of ethanol at the $GABA_A$ receptor. © 1998 Elsevier Science Inc.

Ethanol Select lines Behavioral response $GABA_A$ receptor Chloride uptake Flunitrazepam TBPS Binding

SELECTIVE breeding for differences in drug-related behavior has been widely used in studies aimed at establishing the genetic basis for behavioral drug sensitivity (12). Many rodent lines have been selectively bred for differences in sensitivity to the effect of ethanol. "Sleep time," measured by the interval from loss to recovery of the righting response following an hypnotic dose of ethanol, is an accepted method for determining ethanol sensitivity (13,19). Recently, high and low alcohol sensitivity (HAS and LAS) lines of rats have been selectively bred for differences in ethanol-induced sleep time to be analogous with selectively bred long-sleep (LS) and short-sleep (SS) mice (16,20). HAS and LAS rat lines have been produced by repeated genetic selection under pressure of differential response to ethanol administration for 24 generations (20). These rat lines should now carry fixed alleles enriched for an increased or decreased response to hypnotic effects of ethanol. The characteristic of different sensitivity to ethanol is not due to ethanol metabolism, but to a difference in CNS function, as it is with SS And LS mice (16).

Studies of HAS and LAS rats have shown a measurable response difference that extends to some but not to all, drugs possessing CNS depressant actions (6,39), including barbiturates (17,21), benzodiazepines (39), halothane, isoflurane, and enflurane (17). The findings suggest that the behavioral response to sedative-hypnotic drugs may not have a common site of action, and may be due to different mechanisms of action in the CNS (44).

The mechanism of action of drug-induced behavioral sensitivity response to anesthetics is not well understood at the molecular level. Ethanol has a similar pharmacological profile to barbiturates and benzodiazepines in that they potentate the inhibitory effects of gamma-aminobutyric acid (GABA) via an increase in conductance through the chloride channel coupled to $GABA_A$ receptor complex (34,50,62). By correlating

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genetic differences between rat and mouse lines, behavioral sensitivity to ethanol (2–4,27–29,33,46) with $GABA_A$ subunit composition (30), it may be possible to provide clues as to which subunits are responsible for ethanol sedative effects. For example, brain membranes prepared from HAS rats were more sensitive to ethanol-induced increases in muscimol activated chloride flux compared with membranes prepared from LAS rats (3). A similar finding was made with flunitrazepam, except GABA was used in place of muscimol (4). A great deal of evidence has also accumulated concerning the differences between SS and LS mice with regard to behavioral and molecular mechanisms in the $GABA_A$ system (2–4,28,29,33). However, it is unknown if the parallel between the two ethanol selected lines (LS/SS mice and HAS/LAS rats) extends to other agents acting at the $GABA_A$ receptor complex.

Propofol, etomidate, and ketamine are agents that are structurally unrelated to each other but all may have actions at the $GABA_A$ receptor–chloride ion channel. These agents are widely used as intravenous anesthetics, and have been the subject of many studies investigating the mechanism of action of anesthetics and sedative-hypnotic drugs in the CNS (40, 63,64). Etomidate and propofol mimic the actions of GABA and barbiturates (11,22) at concentrations similar to those measured in the brain during anesthesia (36). Ketamine is neither benzodiazepine-like nor barbiturate-like and, at present, there are no indications as to a possible mechanism for the potentiation of GABA function (41,58). Ketamine is normally considered to act at the NMDA receptor (35,38). To further investigate the differences in the behavioral, molecular, and conformational characteristics of GABA receptors in HAS and LAS rats, we measured etomidate and ketamine-induced sleep time, the issue levels of the drugs at awakening, and evaluated the effects of several different agents on GABA-mediated chloride flux in HAS and LAS rats. This was done to determine if these differences in behavioral response were correlated with the differences in ethanol induced CNS depression and if these correlations extended to chloride flux measurements in brain microsacs. Although ethanol is known to interact with a variety receptors in ways that correlate with behavioral actions, this article is devoted to analysis of the interaction with GABA receptor (18).

METHOD

Animals

Experimental protocols were approved prior to work by the Animal Care & Use Committee of the University of Colorado Health Sciences Center. HAS and LAS rats of both sexes of the 21st generation of the selection were used. Both replicate lines were used, with no differences noted between the replicate lines. They were bred at the University of Colorado Health Sciences Center and were 3 months of age, weighing 225 to 325 g at the time they were used. They were all drug naive at the start of the experiments.

Chemicals

Drugs and chemicals used and their respective sources were as follows: injection solution for propofol (Diprivan, Stuart Pharmaceuticals, Sweden), etomidate (Amidate, Abbott Labs., N. Chicago, IL), and ketamine (Ketalar, Parke-Davis, Morris Plains, NJ); standard reagent etomidate (Abbott) and ketamine (Sigma Chemical Co., St. Louis, MO); $^{36}Cl^-$(specific activity, 12.8 mCi/g of Cl, from ICN, Irvine, CA); [^{3}H] flunitrazepam (specific activity, 86 Ci/mmol from Amersham Life Science Inc., Arlington Heights, IL) and [^{35}S]TBPS (*t*-butylbicyclo[2.2.2]phosphorothionate, specific activity, 88.7 Ci/mmol from NEN, Boston, MA); TBPS (RBI, Natick, MA), Picrotoxin, GABA, (γ-amino-n-butyric acid), flunitrazepam and diazepam (Sigma).

Behavioral Response

A single dose of 7.5 mg/kg of etomidate or 20 mg/kg of ketamine was injected intravenously in the tail vein of the test rats (the calculation of IV dose was based on that for humans and was calculated on the method of body surface area) (61). Ethanol 15% w/v in saline was administered intraperitoneally (IP) (3.6 g/kg for male and female LAS rats, 2.45 g/kg for the HAS male rats, and 2.55 g/kg for HAS female rats). The sleep-time interval was measured as the time (minutes) between loss and recovery of righting reflex after administration of a single dose of drug. After loss of the righting reflex, the animals were placed immediately in Plexiglas V-shaped troughs on their backs. An animal was identified as recovered when it was capable of righting itself in the trough three times within a 1-min period. Ethanol, etomidate, and ketamine levels at awakening were determined in whole brain and blood samples. Ethanol determinations were based on the method of enzyme analysis based on NADH production in the presence of alcohol dehydrogenase. The method of gas chromatographic/mass spectrometry was used for etomidate analysis (24). Ketamine concentration was measured by a gas chromatographic procedure (32).

$^{36}Cl^-$ uptake

The procedure was carried out according to Allan and Harris (2). Animals were killed by decapitation, and their brains removed and placed in ice-cold buffer (NaCl 145 mM, KCl 5 mM, $MgCl_2$, 1 mM, D-glucose 10 mM, $CaCl_2$ 1 mM, and HEPES 10 mM; adjusted to pH 7.5 with Tris base). The brain was homogenized by hand (10–12 strokes) 10 ml of ice-cold assay buffer,using a glass-Teflon homogenizer (Thomas, size C). The homogenate was centrifuged at 900 × *g* for 15 min. The supernatant was decanted and the pellet washed with 40 ml of assay buffer, and centrifuged at 900 × *g* for 15 min. The final pellet was suspended in ice-cold assay buffer. Aliquots (0.2 ml) of membrane vesicles (0.6–0.8 mg of protein) were incubated for 5 min in a shaking water bath at 34°C. After this incubation, uptake was initiated by the addition of 0.2 ml of $^{36}Cl^-$ (2 mCi/ml assay buffer) containing 3–100 μM GABA (final concentration). Three seconds after the addition $^{36}Cl^-$, influx was terminated by the addition of 4 ml of ice-cold assay buffer containing 100 μM picrotoxin and rapid filtration under vacuum (10 mmHg) onto a Whatman GF/C glass microfiber filter using a Hoefer manifold (Hoefer Scientific, San Francisco, CA). The filters were washed with an 8-ml assay buffer containing 100 μM picotoxin with the manifold towers removed. The amount of radioactivity on the filters was determined by liquid scintillation spectrometry. The amount of $^{36}Cl^-$ bound to the filter in the absence of membranes (no tissue blank) was subtracted from all values. GABA-stimulated $^{36}Cl^-$ flux was determined as the amount of $^{36}Cl^-$ bound in the presence of GABA minus nonspecific uptake ($^{36}Cl^-$ bound in the absence of GABA). The apparent potency (EC_{50}) and efficacy (E_{max}) of ethanol (5–200 mM), propofol (1–100 μM), etomidate (0.5–50 μM), and ketamine (1–100 μM) were determined by construction of dose–response curves using eight concentrations of the drug in the $^{36}Cl^-$ solution containing 5 μM of GABA. Net uptake was defined as the amount of

$^{36}Cl^-$ uptake in the presence 5 μM of GABA plus the drug tested minus the $^{36}Cl^-$ uptake in the presence of GABA alone. Protein content was determined by the method of Lowry et al. (43).

[^{3}H]Flunitrazepam Binding and [^{35}S]TBPS Binding

Binding was measured by a filtration method modified from Gee et al. (23) for flunitrazepam, and from Ramanjaneyulu and Ticku (53) for TBPS. After the animals were killed, the brains were immediately removed and homogenized in 20 vol of ice-cold 50 mM Tris-citrate buffer (pH 7.4, 25°C) using a polytron (setting 10, 10 s). The homogenate was centrifuged at 1,000 × *g* for 10 min, the pellet discarded, and the supernatant was centrifuged at 20,000 × *g* for 20 min. The pellet was resuspended and centrifuged three times in ice-cold 50 mM Tris-citrate buffer containing 100 mM NaCl. Then the pellet was suspended in ice-cold mM Tris-citrate buffer and centrifuged once at 20,000 × *g* for 20 min. The final pellet was suspended in ice-cold 50 mM Tris-citrate buffer. Aliquots of membrane solution (0.1–0.6 mg of protein) were incubated with 1 nM [^{3}H]flunitrazepam, or 5 nM [^{35}S]TBPS in a total volume of 0.3 ml of 50 mM Tris-citrate buffer for 60 min at 4°C for flunitrazepam and for 120 min at room temperature for TBPS. The reaction was terminated by rapid filtration through glass-fiber filter strips (Whatman GF/B), which were rinsed twice with 4 ml of ice-cold Tris-citrate buffer using a Cell Harvester filtration manifold (model M-24, Brandel). The amount of radioactivity on the filters was determined by liquid scintillation spectrometry. The affinity (K_d) and number of B_{max} of [^{3}H]flunitrazepam binding and [^{35}S]TBPS binding sties were determined by indirect Scatchard analysis of saturation binding data using 10 concentrations of non-radioactive flunitrazepam (0.05–300 nM) and TBPS (2.5–640 nM). The effect of GABA, ethanol, propofol, etomidate, and ketamine on TBPS binding was determined by competition analysis with a single concentration of the agent to displace binding. Nonspecific binding for the individual binding assays was defined as follows: [^{3}H]flunitrazepam, in the presence of 10 μM diazepam [^{35}S]TBPS, in the presence of 100 μM picrotoxin. Nonspecific binding was subtracted from the total binding to obtain specific binding.

Data Analysis

The results are expressed as mean ± SE. The data are reported as μg/ml (blood) or μg/g (brain) etomidate and ketamine for the blood or brain levels at awakening and as minutes for sleep time. For $^{36}Cl^-$ uptake studies, the apparent efficacy (E_{max}) was determined from the dose–response data as the change in $^{36}Cl^-$ flux at the maximally effective concentration, and the apparent potency (EC_{50}) was determined by linear transformation of the data (sigmoid curve analysis). For receptor binding studies, calculation of the receptor affinity (K_d) and number (B_{max}) was performed using the EBDA computer program (48). Statistical comparisons of parameters were made using the Student's *t*-test or by two-way ANOVA where appropriate.

RESULTS

Behavioral Response

Table 1 presents results of ethanol, propofol, etomidate, and ketamine on sleep time in HAS and LAS rats. The HAS rats clearly displayed a greater sensitivity to both etomidate and ketamine than did the LAS rats, as manifested by approximately twofold longer sleep times in HAS than LAS rats ($p < 0.001$). Blood and brain levels of etomidate and ketamine at awakening were both different between HAS and LAS rats ($p < 0.001$) and reciprocally related to sleep time. The LAS rats had twofold greater tissue levels than did the HAS rats. Propofol has been shown to produce no difference in sleep time, but a small difference in tissue levels at awakening between HAS and LAS rats (42). Ethanol produced the characteristic difference between HAS and LAS rats in blood and brain levels at awakening. The sleep time values are not useful because the HAS and LAS rats received different doses in an attempt to give pharmacologically equal doses. These doses are those used for selection.

$^{36}Cl^-$ Uptake

GABA-stimulated $^{36}Cl^-$ uptake was compared in HAS and LAS membrane vesicles of prepared from whole brain. The apparent efficacy (E_{max}: HAS 27.10 ± 0.59, LAS 26.02 ± 0.51) and potency (EC_{50}: HAS 5.94 ± 0.47, LAS 6.10 ± 0.48) of GABA-stimulated $^{36}Cl^-$ uptake was not different between the two lines of rat ($p > 0.05$) (Fig. 1.). Ethanol produced a clear augmentation of GABA-stimulated $^{36}Cl^-$ uptake in HAS membranes but not in LAS membranes. The concentrations of ethanol ranging from 5–100 mM produced a significant increased changed in GABA-stimulated $^{36}Cl^-$ uptake by 5 μM GABA in HAS rats. None of the ethanol concentra-

TABLE 1

SLEEP TIMES AND AWAKENING TISSUE LEVELS OF ETHANOL AND ANESTHETIC IN HAS/LAS RATS

	Sleep time (min ± SE)		Brain (μg/g ± SE)		Blood (μg/ml ± SE)	
Measure	HAS	LAS	HAS	LAS	HAS	LAS
Ethanol	201 ± 12	62 ± 6	1515 ± 66†	3272 ± 89	1711 ± 59†	3563 ± 76
Propofol	19.9 ± 0.8	19.9 ± 1.3	4.57 ± 0.16*	5.29 ± 0.17	4.88 ± 0.19*	5.68 ± 0.18
Etomidate	48.5 ± 7.5†	29.3 ± 4.2	1.54 ± 0.21†	2.67 ± 0.18	0.47 ± 0.08†	0.91 ± 0.17
Ketamine	23.3 ± 3.6†	11.9 ± 1.5	2.89 ± 0.53†	5.28 ± 0.74	9.95 ± 2.45†	17.7 ± 2.38

Each value represents the mean ± SE, $n = 12$ to 18 for each group and data are expressed as μg/ml (g) drugs for the blood or brain levels at awakening and as minutes for sleep time. Comparison of sleep times with ethanol is not useful because different doses of ethanol were given to HAS and LAS rats; see the Method section.

*$p < 0.05$, †$p < 0.001$ significant difference between means of HAS and LAS lines with Student's *t*-test (*from 38 presented here for completeness).

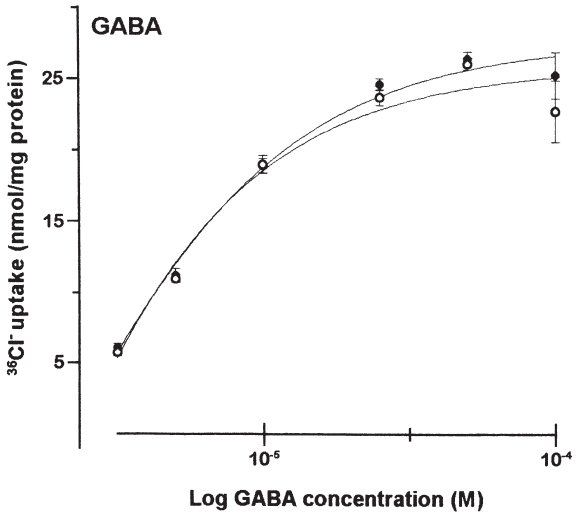

FIG. 1. GABA-stimulated $^{36}Cl^-$ uptake was measured in the membrane vesicles of brain from HAS and LAS rats. The abscissa represents GABA concentration in molar, ranging from 3–100 μM. HAS = closed circles, LAS = open circles. Each point represents mean ± SE, $n = 8$. For each group data are expressed as μM for the apparent potency (EC_{50}) and as nmol of Cl^-/mg of protein/3 s for the apparent efficacy (E_{max}). comparisons between HAS and LAS lines with Student's *t*-test.

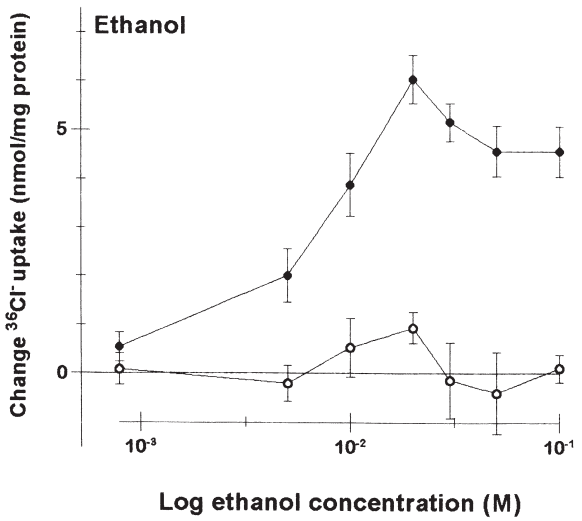

FIG. 2. Effect of ethanol on GABA (5 μM)-stimulated $^{36}Cl^-$ uptake was measured in the membrane vesicles of brain from HAS and LAS rats. The ordinate represents the change in GABA-stimulated chloride uptake produced by ethanol (nanomoles of Cl^-/mg of protein/3 s). The abscissa represents ethanol concentration in molar, ranging from 5–100 mM. HAS = closed circles, LAS = open circles. Each point represents mean ± SE, $n = 8$.

tions tested produced any significant change in chloride uptake elicited by 5 μM GABA in LAS rats (Fig. 2).

The effect of propofol, etomidate, and ketamine on $^{36}Cl^-$ uptake in brain membranes produced by 5 μM GABA was examined. The abscissa represents drugs concentration ranging from 0.5–50 μM for etomidate, 1–100 μM for propofol or ketamine. The agents potentiated GABA-stimulated $^{36}Cl^-$ uptake in both HAS and LAS rats (Fig. 3). Propofol augmented the GABA-stimulated chloride influx equally in both lines of rats. The $_{max}$ (nmol of Cl^-/mg) and EC_{50} (μM) are 14.3 ± 0.39 and 6.80 ± 0.31 for HAS rats, and 14.15 ± 0.45 and 6.32 ± 0.24 for LAS rats. These were not different between two rat lines ($p > 0.05$). However, etomidate and ketamine were found to have a significant difference in potentiating GABA-stimulated chloride influx between HAS and LAS rats. The E_{max} (nmol of Cl^-/mg) and EC_{50} (μM) of etomidate are 14.11 ± 0.53 and 2.34 ± 0.20 for HAS rats, and 9.74 ± 0.67 and 3.88 ± 0.25 for LAS rats. The E_{max} (nmol of Cl^-/mg) and EC_{50} (μM) of ketamine are 9.95 ± 2.45 and 5.39 ± 0.34 for HAS rats, and 4.08 ± 0.28 and 8.04 ± 0.53 for LAS rats. The HAS rats had a higher E_{max} and lower EC_{50} than the LAS rats (both $p < 0.001$).

[^{3}H]Flunitrazepam Binding

[^{3}H]flunitrazepam binds with a high affinity to the benzodiazepine site, which modulates the function of the $GABA_A$ receptor chloride channel complex (8). [^{3}H]flunitrazepam binding was examined in buffer-washed whole-brain membranes of HAS and LAS rats. The binding parameters (K_d and B_{max}) are shown in Table 2. There were no differences in either K_d or B_{max} of [^{3}H]flunitrazepam binding between the rats lines ($p > 0.05$). To assess the effects of various drugs on the modulation of benzodiazepine binding, a single concentration was selected for [^{3}H]flunitrazepam binding. The ability of 20 μM GABA, 25 μM propofol, or 10 μM etomidate to modulate [^{3}H]flunitrazepam binding was reflected in a twofold increase in affinity in both rat lines ($p < 0.0001$), but the number of receptors (B_{max}) was not changed. Furthermore, there was no effect on either the K_d or the B_{max} by 20 mM ethanol and 20 μM ketamine. These data were analyzed by two-way ANOVA.

[^{35}S]TBPS Binding

[^{35}S]TBPS is a selective, high-affinity conversant at the picrotoxinin site that modulates the function of $GABA_A$ receptor chloride channel complex (9). [^{35}S]TBPS binding was examined from buffer-washed whole-brain membranes of HAS and LAS rats. The binding parameters (K_d and B_{max}) are shown in Table 3. Analysis was by two-way ANOVA. No differences were revealed in either the affinity or number of [^{35}S]TBPS binding sites between the rat lines ($p > 0.05$). To assess the effects of various drugs at the picrotoxinin site, we selected a low concentration of GABA (1 μM), propofol (1 μM), or etomidate (0.5 μM) and a dose of ethanol (20 μM) or ketamine (20 μM) to be added to the [^{35}S]TBPS binding assays. The affinity ($p < 0.0001$) and number ($p < 0.0001$) of binding sites of [^{35}S]TBPS both were changed by the addition of GABA in both lines. However, there was a significant difference between the lines ($p = 0.0455$) in the effect of ethanol on K_d values, due to the marked increase in the HAS line. There was significant interaction between line and ethanol ($p = 0.0011$). The number (B_{max}) of binding sites were not changed in either rat line ($p > 0.05$). There was also a significant ($p = 0.0031$) interaction between etomidate and line. There was no effect on either the K_d or the B_{max} by propofol or ketamine, nor were there any differences between rat lines ($p > 0.05$).

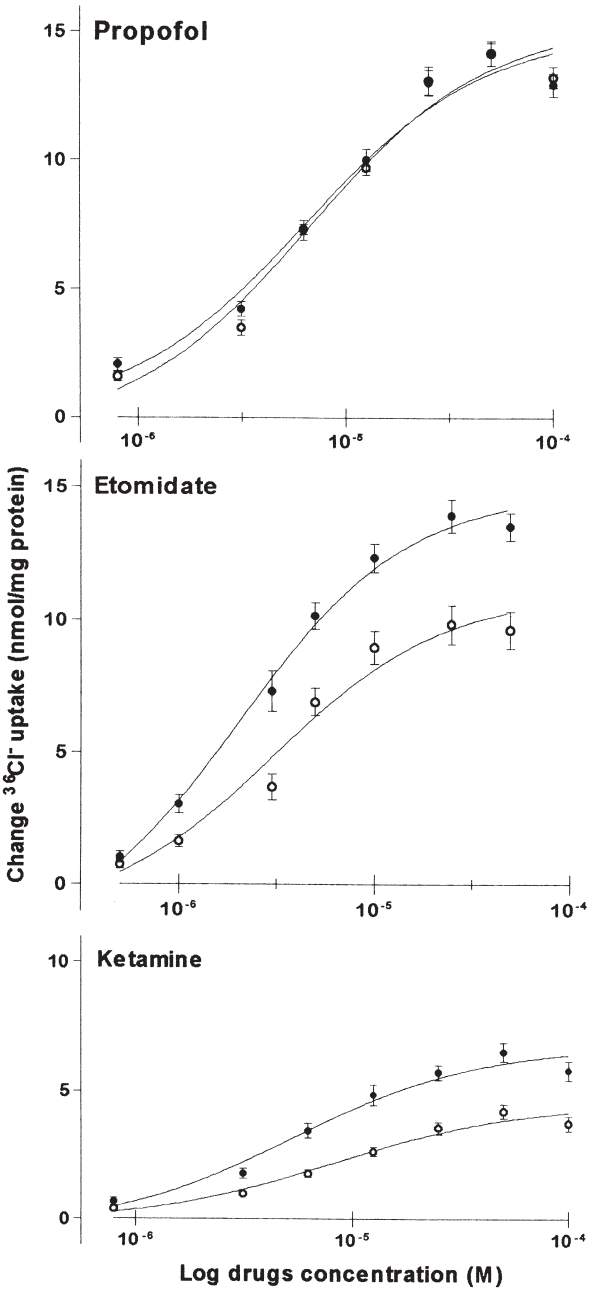

FIG. 3. Effect of propofol, etomidate, and ketamine on GABA (5 μM)-stimulated $^{36}Cl^-$ uptake were measured in the membrane vesicles of brain from HAS and LAS rats. The ordinate represents the change in GABA-stimulated chloride uptake produced by propofol, etomidate, or ketamine. The abscissa represents drug concentration in molar, ranging from propofol 1–100 μM, etomidate 0.5–50 μM, or ketamine 1–100 μM. HAS = closed circles, LAS = open circles. Each point represents mean ± SE, $n = 8$. For each group data are expressed as μM for the apparent potency (EC_{50}) and as nmol of Cl^-/mg of protein/3 s for the apparent efficacy (E_{max}). *$p <$ 0.001 significant difference between means of HAS and LAS lines with Student's *t*-test.

DISCUSSION

Two replicate lines of rats (HAS 1 and 2 and LAS 1 and 2) have been selectively bred through 24 generations for their differential sensitivity to hypnotic doses of ethanol. There is no longer any response to selection, and the selection process has been suspended. The studies reported here were designed to dissect the role that GABA-mediated events contribute to the behavioral effects of ethanol and other drugs in these animals. The experiments replicate and extend results obtained with these rats at earlier generations. It is instructive to compare the magnitude of the responses noted in this study with those earlier studies (3,4) as well as to compare with the results obtained from selectively bred short- and long-sleep mice (SS/LS). With both mice and rats it has been found that differential sensitivity to some anesthetic and sedative-hypnotic drugs, but not others, parallels that found with ethanol (19).

It is now generally accepted that the many different types of anesthetic and sedative-hypnotic agents produce their major pharmacological effects in part by augmenting the actions of the $GABA_A$ receptor chloride ionophore system (1,14, 28,57). The $GABA_A$ benzodiazepine chloride channel is made up of various combinations of numerous subunits. There is a large number of combinations of GABA subunits that have been expressed in frog oocytes and tissue culture cells (30). The interactions of numerous drugs with these receptors give a correspondingly large number of possible targets for their actions. This is undoubtedly due to the strong conservation of amino acid sequence between the various receptor subunits. Consequently, binding sites with reasonable affinities for a variety of drugs exist on numerous receptors and are made up of different subunits. However, it is unlikely that all of these receptor combinations exist in vivo or that they are responsible for the actions of all the drugs that have been tested upon them in vitro. Thus, one way to study in vivo situation is to utilize selectively bred animals to discover what behavioral effects these drugs have on selected lines. Then, by analysis of the receptor subunit composition that actually exist in these animals, reasonable conclusions can be drawn as to which particular subunit configurations are important for the behavioral effects produced by these drugs.

Ethanol markedly augments chloride uptake in the HAS rats (this article) (3,4) and LS mice (2,28). It should be noted that the essentially identical results with HAS and LAS rats were obtained in generation 7, suggesting that this response was selected for very early in the breeding process (3,4). Although there was continued separation of sleep time from generation 7 to generation 24, this must have been due to recruitment of other mechanisms. The importance of a functional assay for the $GABA_A$ receptor complex is apparent in that competitive binding studies with flunitrazepam and TBPS did not reveal the genetic differences for any compound except for ethanol on TBPS binding, whereas the chloride flux assay revealed genetic differences for ethanol, ketamine, and etomidate. Studies in the mice have failed to reveal an effect of ethanol on TBPS binding to brain membranes (33); however, studies in rats revealed a marked effect on TBPS binding, even when ethanol is given in vivo (54,55). In vitro, Peris et al. (52) found about a 15% inhibition of TBPS binding at 20 mM ethanol.

Propofol also is postulated to act at the GABA receptor (10,11,25,31,51). Although propofol exhibits a marked difference in brain levels at awakening between SS and LS mice (51), this was not the case in rats (42). On the other hand, there is no line difference in propofol-stimulated GABA-

TABLE 2

SATURATION BINDING OF [^{3}H]FLUNITRAZEPAM T0 WHOLE BRAIN MEMBRANES FROM HAS AND LAS RATS

	K_d		B_{max}	
	HAS	LAS	HAS	LAS
Basal	2.61 ± 0.11	2.66 ± 0.09	1108 ± 128	1322 ± 118
GABA (20 μM)*	1.25 ± 0.08	1.19 ± 0.03	1207 ± 101	1391 ± 67
Ethanol (20 mM)	2.48 ± 0.19	2.46 ± 0.16	1251 ± 90	1437 ± 87
Propofol (25 μM)*	1.49 ± 0.08	1.43 ± 0.10	1249 ± 97	1448 ± 71
Etomidate (10 μM)*	1.38 ± 0.13	1.40 ± 0.07	1192 ± 112	1432 ± 75
Ketamine (20 μM)	2.62 ± 0.27	2.64 ± 0.22	1205 ± 101	1338 ± 109

Each value represents the mean ± SE, n = 5–6 for each group and data are expressed as nanomolar for the affinity (K_d) at each binding and as femtomoles per milligram of protein for the number (B_{max}) at each binding. Comparisons between means of binding in the presence of the listed compound and basal binding within each line.

* K_d significantly different from basal binding, GABA: $F = (1,20) = 293.31$, $p < 0.0001$; propofol: $F(1,20) = 157.74$, $p < 0.0001$; etomindate: $F(1,20) = 146.55$, $p < 0.001$.

mediated chloride uptake in either rats (this article) at concentrations from 1 to 100 μM, or in mice at concentrations from 0.5 to 50 μM (59). In fact, propofol may also have an effect on the glycine receptor in addition (47). Sanna et al. (56) studied the effects of propofol on recombinant human $GABA_A$ receptors expressed in frog oocytes. They found that at low levels of propofol (10 to 50 μM), there was no subunit requirement for propofol enhancement of GABA-stimulated chloride uptake. However, at higher concentrations, propofol directly stimulated the $GABA_A$ receptor only if the beta subunit was expressed. Other studies also indicate direct effect of propofol on the $GABA_A$ receptor (26). It would be tempting to speculate that neither the selectively bred rats nor mice have any functionally important polymorphism in the beta subunit of the $GABA_A$ receptor.

Etomidate does produce a behavioral difference between HAS and LAS and also in GABA-mediated chloride uptake on both EC_{50} and E_{max} values. It has a ratio of brain levels in the two lines of rats virtually identical to that of ethanol. Brain levels for etomidate have not yet been completed in mice, but the sleep-time differences suggest that there should be a marked difference between brain levels at awakening in the SS and LS mice as well.

In comparison, ketamine is much more effective in causing antagonism of the *N*-methyl-D-aspartate (NMDA) receptor (5). Nevertheless, one of the pharmacological effects of ketamine is that it potentiates the effect of GABA (41,58). In the present study, the sleep-time response and GABA-mediated chloride flux of ketamine are in same direction as that of ethanol in HAS and LAS rats. Ketamine brain levels at awakening

TABLE 3

SATURATION BINDING OF [^{3}S]TBPS TO WHOLE BRAIN MEMBRANES FROM HAS AND LAS RATS

	K_d		B_{max}	
	HAS	LAS	HAS	LAS
Basal	46.05 ± 2.21	55.76 ± 4.03	1458 ± 32	1486 ± 82
GABA (1 μM)*¶	83.28 ± 5.34	97.46 ± 6.29	454 ±112	556 ± 73
Ethanol (20 mM)†‡	75.97 ± 10.01	43.85 ± 1.96	1590 ± 52	1608 ± 49
Propofol (1 μM)	48.21 ± 6.76	46.72 ± 3.13	1530 ± 73	1588 ± 40
Etomidate (0.5 μM)*§	69.58 ± 0.13	54.61 ± 4.88	1560 ± 80	1645 ± 43
Ketamine (20 μM)	51.02 ± 2.58	51.55 ± 7.95	1648 ± 58	1562 ± 48

Each value represents the mean ± SE, n = 5–6 for each group and data are expressed as nanomolar for the affinity (K_d) at each binding and as femtomoles per milligram of protein for the number (B_{max}) at each binding. Comparisons between means of binding in presence of listed compound and basal binding within each line.

* K_d significantly different from basal binding, GABA: $F = (1,16) = 80.63$, $p < 0.0001$; etomidate $F(1,16) = 9.97$, $p = 0.0061$.

† Significantly different between lines ethanol: $F(1,16) = 4.7$, $p < 0.0455$.

‡ Interaction between ethanol and line $F(1,16) = 15.79$, $p < 0.0011$.

§ Interaction between etomidate and line $F(1,16) = 12.12$, $p = 0.0031$.

¶ B_{max}: significantly different from basal binding, GABA: $F(1,16) = 142.62$, $p < 0.0001$. Propofol and ketamine, no differences.

show a marked difference between LAS and HAS rats. Likewise, there was a significant difference in the effect of ketamine on GABA-stimulated chloride uptake between HAS and LAS in both EC_{50} and E_{max} values.

Differences between the lines were not obtained in either number or affinity of [^{3}H]flunitrazepam binding sites. This is in concert with results from SS and LS mice by Harris and Allan (29). However, a difference was seen between the lines in the effect of ethanol on the binding of [^{35}S]TBPS to the convulsant site on GABA complex; a decrease in affinity of the binding was displayed in HAS rats. Ketamine affected neither the [^{3}H]flunitrazepam binding nor the [^{35}S]TBPS binding in either rat line.

Etomidate and propofol brought about approximately a twofold increase in affinity of [^{3}H]flunitrazepam binding in both lines but propofol had no significant effect on [^{35}S]TBPS binding in either line of rats.

Marley et al. (45) and De Fiebre et al. (15) imply that hypnotic sensitivity is related to the water solubility of anesthetic and sedative-hypnotic agents. Differential drug sensitivities were found with most water-soluble agents between selected lines, and as the lipid solubility of agent increased, the difference in drug sensitivity decreased (15). Etomidate and ketamine (water-soluble agents) showed a differential sensitivity between HAS and LAS rats, but propofol, which is a lipid-soluble anesthetic, did not. On the other hand, the gaseous anesthetics, which are lipid soluble, show even greater differences between the lines.

It can been seen in Table 4 that the ratio of ethanol brain or blood levels at the time of regaining the righting reflex is approximately twofold between the HAS and LAS rats, as it is for the SS and LS mice. To avoid the potential problem of differential metabolism of the compounds in the HAS and LAS lines, we examined the brain levels of compounds at the time of regaining the righting reflex. Although these ratios will vary slightly from experiment to experiment, it is clear that the selection for ethanol sensitivity has been of the same magnitude in rats as in mice. With results available for a number of anesthetic compounds that have been tested in both the selected mouse and rat lines, some interesting results appear. Lines of mice and rats are both differentially sensitive to the gaseous anesthetic, isoflurane (17,60). Mice are not differentially sensitive to halothane while HAS and LAS rats are. The selected mouse lines respond differentially to propofol, but the rat lines differ only marginally. On the other hand, the selected rat lines differ significantly with pentobarbital but the mice lines do not differ, or differ in the opposite direction (17,37,49).

TABLE 4

COMPARISON OF THE RATIO OF AWAKENING TISSUE LEVELS IN RATS (HAS/LAS) AND MICE (LS/SS) TO VARIOUS HYPNOTIC AGENTS

Compound	Level (LAS/HAS)	Reference	Level (SS/LS)	Reference
Ethanol	1.82, 2.08	(8), This article	1.82	(7)
Halothane	1.92	(8)	0.74	(60)
Enflurane	1.78	(8)	—	
Isoflurane	2.36	(8)	1.93	(60)
Pentobarbital	1.24	(8)	1.0, 0.83	(37, 49)
Propofol	1.17	(41)	1.99	(59)
Etomidate	1.72	This article	—	
Ketamine	1.78	This article	—	

In summary, selective breeding for initial ethanol sensitivity in rats has been completed. The high alcohol-sensitive and low alcohol-sensitive lines (HAS and LAS) underwent 24 generations of selection. During the selection process a number of studies were carried out targeting the GABA receptor as a potential mechanism for the behavioral differences. The current studies extend and complement these earlier studies and allow for more definitive conclusions than previously possible. In addition, comparison to the similar selection in mice (the short- and long-sleep mice) has allowed us to pinpoint similarities in reactions to many compounds (ethanol, etomidate, most gaseous anesthetics) and important differences (propofol, pentobarbital, halothane) in the response of the $GABA_A$ system between the species.

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