Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4071–4075, June 1986
Neurobiology

Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes

(isolated brain vesicles/γ-aminobutyric acid receptor complex/chloride uptake/barbiturates)

PETER D. SUZDAK*, ROCHELLE D. SCHWARTZ*, PHIL SKOLNICK†, AND STEVEN M. PAUL*‡

*Section on Molecular Pharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, and †Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, 9000 Rockville Pike, Bethesda, MD 20892

Communicated by Louis B. Flexner, January 13, 1986

ABSTRACT **The effects of ethanol on Cl^- uptake were studied using a cell-free subcellular preparation from brain that contains a γ-aminobutyric acid (GABA)/barbiturate receptor-sensitive Cl^- transport system. In isolated vesicles prepared from rat cerebral cortex, ethanol, at concentrations that are present during acute intoxication (20–50 mM), stimulated $^{36}Cl^-$ uptake in a concentration-dependent and biphasic manner. The ethanol-stimulated uptake of $^{36}Cl^-$ was markedly inhibited by the GABA antagonists picrotoxin and bicuculline but not by a variety of other neurotransmitter receptor antagonists. The effects of ethanol in stimulating $^{36}Cl^-$ uptake in isolated brain vesicles were qualitatively and quantitatively similar to that of pentobarbital. Ethanol also markedly potentiated both muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake at concentrations below those that directly stimulate $^{36}Cl^-$ uptake. Under our incubation conditions, ethanol did not release GABA, suggesting that it interacts with the postsynaptic GABA/barbiturate receptor complex. The ability of ethanol to stimulate GABA/barbiturate receptor-mediated Cl^- transport may explain many of its pharmacological properties and provides a mechanism for the common psychopharmacological actions of ethanol, barbiturates, and benzodiazepines.**

Ethanol is one of the oldest and most commonly used of all psychotropic drugs (1). Repeated exposure to ethanol produces both psychological and physical dependence and its abuse potential constitutes a major public health problem (2). The neurochemical mechanism(s) underlying the depressant effects of ethanol on the central nervous system (CNS) is poorly understood (3) despite numerous studies demonstrating effects of ethanol on several neurotransmitter systems (4, 5). Ethanol shares many pharmacologic actions with both barbiturates and benzodiazepines. For example, ethanol, like barbiturates and benzodiazepines, possesses anxiolytic and sedative/hypnotic activity in both laboratory animals (6, 7) and humans (8). Moreover, previous studies have documented the development of behavioral cross-tolerance between ethanol, barbiturates, and benzodiazepines (9, 10). Benzodiazepines and barbiturates, which also show cross-dependence with each other, are effective in alleviating the withdrawal symptoms that occur after chronic ethanol administration, suggesting that all three drugs may share a common mechanism of action (11).

It is now generally accepted that both benzodiazepines and barbiturates produce their major pharmacological effects by augmenting the actions of the principal inhibitory neurotransmitter of brain, γ-aminobutyric acid (GABA) (12–15). Further, both benzodiazepines and barbiturates bind to specific recognition sites associated with the postsynaptic GABA receptor with affinities that are highly correlated with their behavioral potencies as anxiolytic and sedative/hypnotic agents (13–15). Recent work indicates that the GABA/benzodiazepine/barbiturate receptor complex is an oligomeric protein consisting of several subunits with multiple allosteric binding sites that are associated with a Cl^- channel (14, 15). Benzodiazepines and barbiturates bind to this complex and indirectly regulate GABA receptor-mediated Cl^- conductance, resulting in membrane hyperpolarization (15–18).

Despite the many pharmacological similarities between benzodiazepines, barbiturates, and ethanol, it is as yet unclear whether ethanol has a similar action on GABA receptor function. While behavioral (19, 20) and electrophysiological (21, 22) studies suggest that ethanol potentiates GABAergic neurotransmission, there is conflicting evidence for a direct action at the GABA receptor. The addition of ethanol to brain membranes *in vitro* has been reported to have no effect on either [^{3}H]diazepam binding to the benzodiazepine receptor (23) or [^{3}H]muscimol binding to the GABA receptor (23). However, ethanol has been reported to increase [^{3}H]diazepam binding to detergent-solubilized benzodiazepine receptors (24) and to decrease the binding of *t*-butylbicyclophosphoro[^{35}S]thionate (TBPS) to a site closely associated with the Cl^- channel in both rat and mouse brain membranes (25, 26). Unfortunately, the concentrations of ethanol used in many of these receptor binding studies exceed those observed during acute intoxication (>30 mM) and are, in fact, many times above the lethal concentration (>100 mM) (27, 28).

Recently, we have reported the use of the "synaptoneurosome," a subcellular brain preparation, to measure GABA/barbiturate receptor-mediated Cl^- transport *in vitro* (29–31). Morphologic characterization of this preparation has revealed the presence of both pre- and postsynaptic membranes that form closed vesicles (32). We now report that ethanol, like pentobarbital, stimulates $^{36}Cl^-$ uptake into these isolated brain vesicles in concentrations that are within the range observed during acute intoxication. At even lower (subthreshold) concentrations, ethanol markedly potentiates both muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake. Since the effect(s) of ethanol in stimulating $^{36}Cl^-$ uptake is selectively blocked by both the GABA receptor antagonist bicuculline and the Cl^- channel antagonist picrotoxin, our data suggest that at pharmacologically relevant concentrations, ethanol specifically interacts with the GABA/barbiturate receptor complex. These observations may explain the many behavioral and pharmacological similarities between ethanol, benzodiazepines, and barbiturates. Moreover, the ability of ethanol to stimulate GABA/barbiturate receptor-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GABA, γ-aminobutyric acid; TBPS, *t*-butylbicyclophosphoro[^{35}S]thionate.

‡To whom reprint requests should be addressed at: Section on Molecular Pharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, Bldg. 10, Room 4N214, 9000 Rockville Pike, Bethesda, MD 20892.

mediated $^{36}Cl^-$ transport may represent an important mechanism underlying the effects of ethanol on CNS function.

MATERIALS AND METHODS

Preparation of Synaptoneurosomes. Isolated membrane vesicles (synaptoneurosomes) were prepared according to the method of Hollingsworth *et al.* (32) as modified by Schwartz *et al.* (29, 30) for measurement of $^{36}Cl^-$ efflux. Cerebral cortices from adult male Sprague–Dawley rats (200–250 g) housed under diurnal lighting conditions with free access to food and water were removed, pooled, and dissected free from surrounding white matter. Brain tissue (1 g) was homogenized in 7 vol (wt/vol) of an ice-cold buffer containing 20 mM Hepes/Tris, 118 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, and 2.5 mM $CaCl_2$ (pH 7.4) using a glass-glass homogenizer (five strokes). The homogenate was diluted with 30 ml of buffer and then filtered by gravity through three layers of nylon mesh (160 μm, Tetko, Elmsford, NY) placed in a Millipore Swinex filter holder. The resulting filtrate was then gently pushed through a 10-μm Millipore filter (LCWP 047) using a 10-ml syringe. Care was taken to prevent the development of hydrostatic pressure during filtration. The filtered preparation was centrifuged at 1000 × *g* for 15 min, the supernatant was discarded, and the pellet was washed by suspension in buffer followed by centrifugation (1000 × *g* for 15 min). The resulting pellet was resuspended in buffer to a final protein concentration of 20 mg/ml.

Measurement of $^{36}Cl^-$ Uptake. $^{36}Cl^-$ uptake into synaptoneurosomes was carried out as described by Schwartz *et al.* (31). Aliquots of the membrane preparation equivalent to 2 mg of protein were incubated at 30°C for 20 min and then various concentrations of ethanol, other drugs, or buffer, and 0.5 μCi of $^{36}Cl^-$ (specific activity, 12.5 mCi/g; 1 Ci = 37 MBq; New England Nuclear) in a total incubation volume of 0.5 ml. Uptake of $^{36}Cl^-$ was terminated 5 sec later by the addition of 5 ml of ice-cold buffer followed by rapid filtration under vacuum through Whatman GF/C glass-fiber filters that had been treated with 0.05% polyethyleneimine to reduce nonspecific binding of $^{36}Cl^-$. After filtration, the filters were washed with two 5-ml portions of ice-cold buffer, air-dried, and placed in vials containing 7 ml of Readi-Solv (Beckman Instruments, Fullerton, CA). Radioactivity was determined by liquid scintillation spectrometry. A full characterization of the effects of various barbiturates and GABA receptor agonists and antagonists on $^{36}Cl^-$ efflux and uptake has been reported elsewhere (29–31, 33). All data represent mean ± SEM expressed as percent stimulation of $^{36}Cl^-$ uptake and are from a typical experiment carried out in quadruplicate and repeated three times with similar results.

Measurement of [^{3}H]GABA Release. Synaptoneurosomes (approximately 20 mg of protein per ml) were first incubated (in buffer of the composition described above) with 0.1 μM [^{3}H]GABA (specific activity, 85.4 Ci/mmol; New England Nuclear) at 30°C in the presence of 0.1 mM aminooxyacetic acid to prevent metabolism of GABA by GABA transaminase. [^{3}H]GABA release was measured by a modification of the methods of Sandoval (34) and of Rohde and Harris (35).

RESULTS

Addition of ethanol to cerebral cortical synaptoneurosomes stimulated $^{36}Cl^-$ uptake in a dose-dependent fashion (Fig. 1*A*). A significant ($P < 0.01$) stimulation of $^{36}Cl^-$ uptake was observed at ethanol concentrations as low as 20 mM and maximal stimulation (260%) was observed at concentrations between 50 and 70 mM (EC_{50}, 25–35 mM). The effects of ethanol in stimulating $^{36}Cl^-$ uptake were biphasic; higher concentrations (≥100 mM) resulted in appreciably lower

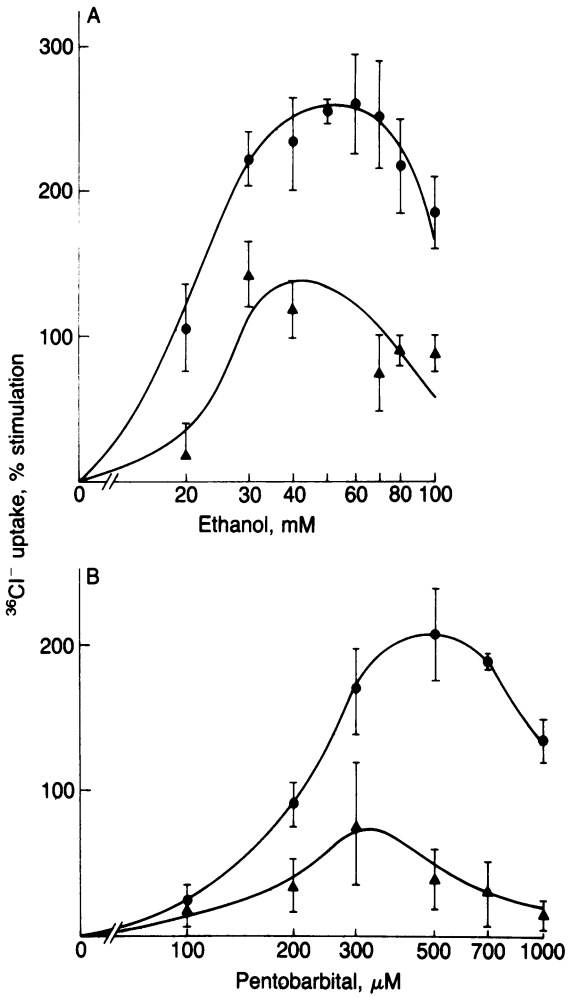

FIG. 1. (*A*) Effect of 20–100 mM ethanol alone (●) or in the presence of 100 μM picrotoxin (▲) on uptake of $^{36}Cl^-$ in cerebral cortical synaptoneurosomes. Synaptoneurosomes were incubated in assay buffer at 30°C for 15 min, picrotoxin (100 μM) was added, and incubation was continued for 5 min. Then, 0.5 μCi of $^{36}Cl^-$ and ethanol at various concentrations was added. Uptake of $^{36}Cl^-$ was terminated 5 sec later by the addition of 5 ml of ice-cold buffer followed by rapid filtration. Data represent mean ± SEM and are from a typical experiment carried out in quadruplicate and repeated three times with similar results. At each concentration of ethanol tested, picrotoxin significantly decreased ethanol-stimulated $^{36}Cl^-$ uptake ($P < 0.01$, Student's *t* test). The amount of $^{36}Cl^-$ bound to the filter in the absence of synaptoneurosomes (nonspecific filter binding) was ≈500 cpm (mean) and was subtracted from all values. In a typical experiment the basal uptake of $^{36}Cl^-$ was 700 ± 18 cpm, while in the presence of ethanol (50 mM) uptake of $^{36}Cl^-$ was 993 ± 40 cpm. (*B*) Effect of 0.1–1.0 mM pentobarbital alone (●) or in presence of 100 μM picrotoxin (▲) on $^{36}Cl^-$ uptake in cerebral cortical synaptoneurosomes. At pentobarbital concentrations >0.1 mM, picrotoxin significantly decreased pentobarbital-stimulated $^{36}Cl^-$ uptake ($P < 0.05$, Student's *t* test).

stimulation of $^{36}Cl^-$ uptake (see Figs. 1*A* and 3). The ability of ethanol to stimulate $^{36}Cl^-$ uptake was qualitatively and quantitatively similar to that of the prototypic barbiturate pentobarbital, as indicated by a similar biphasic dose–response curve and by the maximal increases in $^{36}Cl^-$ uptake (see Figs. 1*B* and 5).

We first investigated the effect of incubating synaptoneurosomes with ethanol for various times prior to the initiation of $^{36}Cl^-$ uptake. When ethanol and $^{36}Cl^-$ were added simultaneously (i.e., no preincubation time with ethanol alone) stimulation of $^{36}Cl^-$ uptake was maximal (Fig. 2). When synaptoneurosomes were preincubated with ethanol for 1

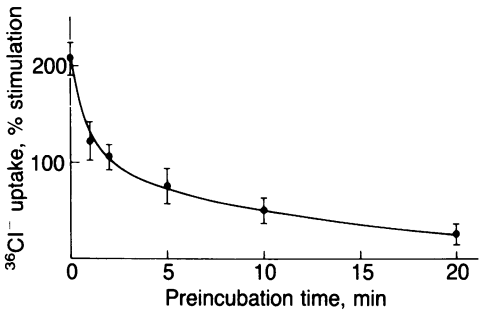

FIG. 2. Effect of preincubation time on ethanol (50 mM)-stimulated $^{36}Cl^-$ uptake. Ethanol was added 1–20 min prior to the addition of $^{36}Cl^-$ or simultaneously with $^{36}Cl^-$ (i.e., no ethanol preincubation).

min prior to the addition of $^{36}Cl^-$ the effect of ethanol was reduced by almost 40%. Preincubation for longer intervals resulted in loss of the effect of ethanol in stimulating $^{36}Cl^-$ uptake (Fig. 2). Consequently, all experiments, unless otherwise specified, were carried out without ethanol preincubation.

To determine whether the action of ethanol in stimulating $^{36}Cl^-$ uptake might be mediated by the GABA/barbiturate receptor complex, we studied the effects of the GABA antagonists picrotoxin and bicuculline on ethanol-stimulated $^{36}Cl^-$ uptake (Figs. 1*A* and 3). Prior treatment with picrotoxin (100 μM), a GABA receptor Cl^- channel antagonist, significantly decreased the ability of ethanol to stimulate $^{36}Cl^-$ uptake (Fig. 1*A*) but had no effect on basal $^{36}Cl^-$ uptake (data not shown). Similar results were observed for pentobarbital-induced $^{36}Cl^-$ uptake, although the magnitude of the inhibition by picrotoxin was somewhat greater than that for pentobarbital (Fig. 1*B*). Bicuculline, a specific $GABA_A$ receptor antagonist, also significantly antagonized ethanol-stimulated $^{36}Cl^-$ uptake (Fig. 2) at a concentration that did not decrease basal $^{36}Cl^-$ uptake. Previous studies in our laboratory (30, 31) and by others (33, 36, 37) have shown a similar antagonism of both pentobarbital and GABA-mediated $^{36}Cl^-$ uptake and/or efflux by bicuculline. The effects of bicuculline and picrotoxin in antagonizing the ethanol-stimulated uptake of $^{36}Cl^-$ appear to be selective in that a variety of neurotransmitter receptor antagonists (haloperidol, propranolol, verapamil, strychnine, clonidine, phenoxybenzamine) failed to alter ethanol-stimulated $^{36}Cl^-$ uptake.

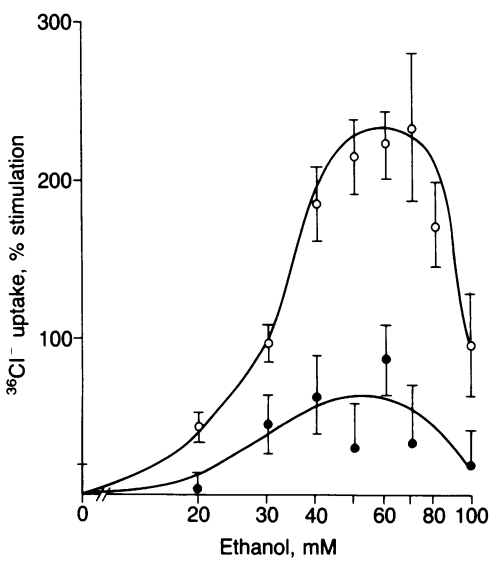

FIG. 3. Effect of 20–100 mM ethanol alone (○) or in the presence of bicuculline (●) on $^{36}Cl^-$ uptake. $^{36}Cl^-$ uptake was measured as described in Fig. 1*A*, except that bicuculline methiodide (100 μM) was added 5 min prior to the addition of $^{36}Cl^-$ and ethanol. At each concentration of ethanol tested, bicuculline significantly decreased ethanol-stimulated $^{36}Cl^-$ uptake ($P < 0.01$, Student's t test).

The ability of ethanol to stimulate $^{36}Cl^-$ uptake was similar to that of pentobarbital (Fig. 1*B*), which has been shown to potentiate GABA-mediated Cl^- conductance at concentrations below those required to directly stimulate Cl^- conductance (38, 39). Thus, the effect of low (subthreshold) ethanol concentrations on both muscimol- and barbiturate-mediated $^{36}Cl^-$ uptake was determined. Ethanol, at a concentration of 10 mM, which had no effect on basal $^{36}Cl^-$ uptake, markedly potentiated muscimol-stimulated $^{36}Cl^-$ uptake (Fig. 4). This potentiation of muscimol-stimulated $^{36}Cl^-$ uptake by ethanol appears to be the result of an increase in the V_{max} of muscimol-stimulated $^{36}Cl^-$ uptake rather than a change in the apparent K_m (Fig. 4 *Inset*). A similar potentiation of pentobarbital-stimulated $^{36}Cl^-$ uptake was also observed (Fig. 5) except that ethanol affected both the V_{max} and the apparent K_m of pentobarbital-stimulated $^{36}Cl^-$ uptake (Fig. 5 *Inset*).

To rule out that the effects of ethanol in stimulating $^{36}Cl^-$ uptake were due to the release of GABA, the effects of various concentrations of ethanol on basal and depolarized GABA release were examined. Synaptoneurosomes were preincubated with [^{3}H]GABA and then incubated for 5 sec in the presence or absence of various concentrations of ethanol and/or a depolarizing concentration of veratrine. Under these conditions ethanol failed to stimulate the release of [^{3}H]GABA or to potentiate the depolarization-induced release (data not shown). In agreement with previous reports (41), ethanol inhibited the depolarized release of [^{3}H]GABA at the highest concentrations tested (≥100 mM) (data not shown).

DISCUSSION

We have found that ethanol, when added to isolated brain vesicles *in vitro*, markedly stimulates $^{36}Cl^-$ uptake in a dose-dependent fashion. The concentrations of ethanol necessary to stimulate $^{36}Cl^-$ uptake (EC_{50}, 25–35 mM) are within the range of those observed during acute intoxication (25–50 mM) (27, 28). The ability of ethanol to stimulate $^{36}Cl^-$ uptake in synaptoneurosomes appears to be mediated by an interaction with the GABA/barbiturate receptor complex since both picrotoxin and bicuculline block the effects of ethanol whereas other neurotransmitter receptor antagonists are ineffective.

The stimulation of $^{36}Cl^-$ uptake by ethanol is qualitatively similar to the stimulation of $^{36}Cl^-$ uptake by anesthetic barbiturates such as pentobarbital (31) (Fig. 1*B*). It has been shown by electrophysiologic experiments that these barbiturates directly stimulate Cl^- conductance at high (i.e., anesthetic) concentrations and that at lower (sedative-hypnotic) concentrations they markedly potentiate GABA receptor-mediated Cl^- conductance (38, 39). Similarly, ethanol at concentrations below those that directly stimulate $^{36}Cl^-$ uptake markedly potentiates both muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake (Figs. 4 and 5). It is also interesting that ethanol, like pentobarbital, produces a biphasic effect on $^{36}Cl^-$ uptake. Ethanol has previously been reported to have a biphasic effect on both the brain concentration and turnover of GABA (42, 43). However, it is more likely that the decrease in ethanol-stimulated $^{36}Cl^-$ uptake observed at higher ethanol concentrations is due to an ethanol-induced state of receptor desensitization. In fact, in experiments in which ethanol was included in the preincubation mixture for various times prior to the measurement of $^{36}Cl^-$ uptake, a progressive diminution of ethanol-stimulated $^{36}Cl^-$ uptake was observed (Fig. 2). This time-dependent

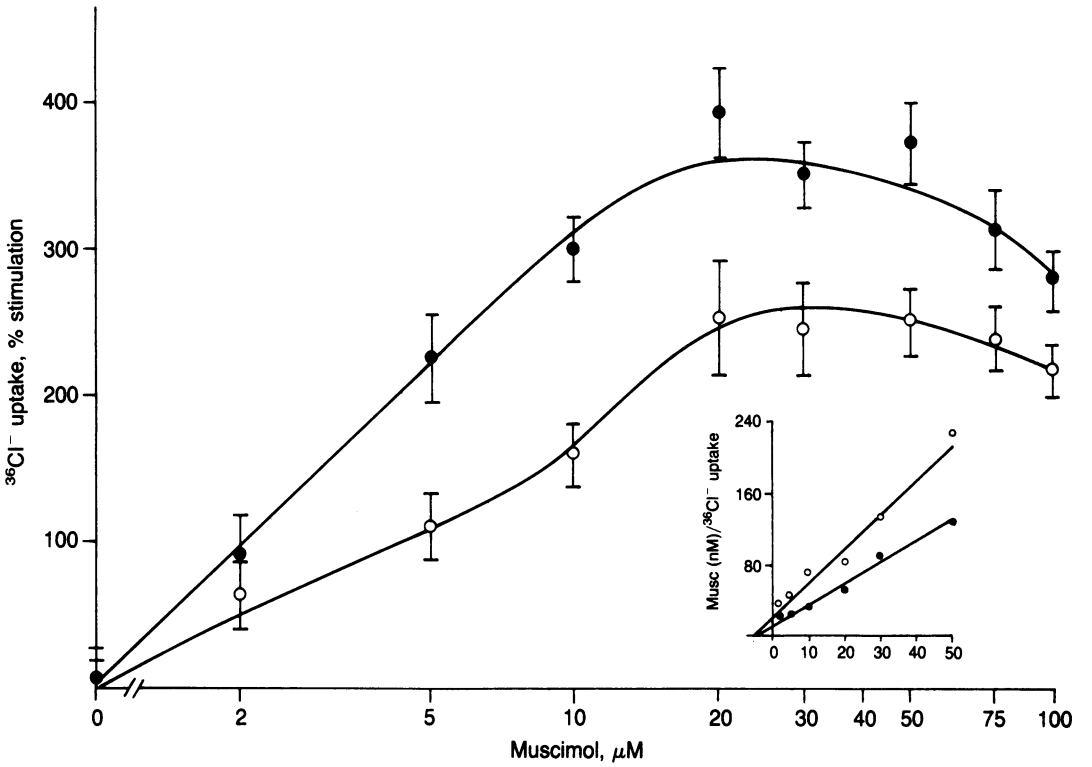

FIG. 4. The effect of a subthreshold concentration of ethanol on muscimol-stimulated $^{36}Cl^-$ uptake was determined using 2–100 μM muscimol alone (○) or in combination with ethanol (10 mM) (●), as described in Fig. 1*A*. A Hanes–Woolf plot (40) of the data (*Inset*) indicates that ethanol increased the V_{max} for muscimol (Musc)-stimulated $^{36}Cl^-$ uptake. The apparent K_m was not significantly altered (5.4 and 4.5 μM in the absence and presence of ethanol, respectively). At muscimol concentrations >2 μM, ethanol significantly increased muscimol-stimulated $^{36}Cl^-$ uptake ($P < 0.01$, Student's *t* test).

decrement in $^{36}Cl^-$ uptake has also been observed with pentobarbital and muscimol (unpublished work) and probably represents desensitization. However, at lower concentrations of ethanol (≤25 mM), no appreciable decrease in $^{36}Cl^-$ uptake was observed during a 20-min preincubation (unpublished work).

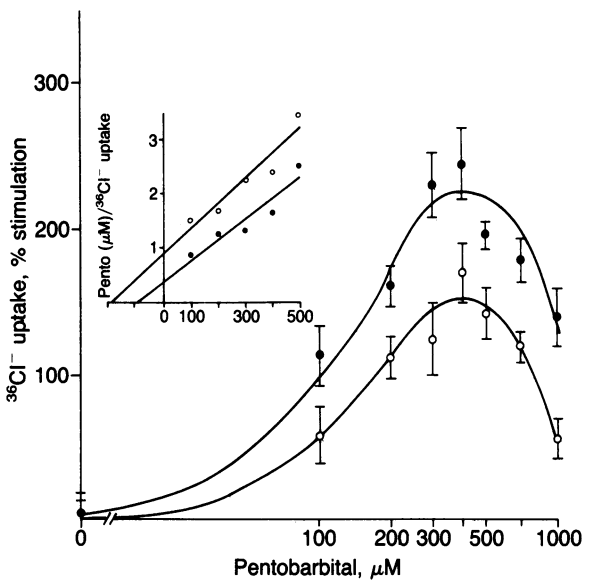

FIG. 5. The effect of a subthreshold concentration of ethanol on pentobarbital-stimulated $^{36}Cl^-$ uptake was determined using 0.1–1.0 mM pentobarbital alone (○) or in combination with ethanol (10 mM) (●), as described in Fig. 1*A*. A Hanes–Woolf plot (40) of the data (*Inset*) indicates that ethanol increased the V_{max} for pentobarbital (Pento)-stimulated $^{36}Cl^-$ uptake from 218% to 264% and decreased the apparent K_m from 0.200 to 0.103 mM. At each concentration of pentobarbital tested, ethanol significantly increased pentobarbital-stimulated $^{36}Cl^-$ uptake ($P < 0.05$, Student's *t* test).

The possible mechanism(s) responsible for the action(s) of ethanol on $^{36}Cl^-$ transport includes an ethanol-induced perturbation of membrane lipids, resulting in an increase in membrane fluidity (41, 44–46). We have recently found that pretreatment of synaptoneurosomes with low concentrations of phospholipase A_2 markedly attenuates both muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake and efflux (31). In addition, in recent experiments (unpublished work), good correlations have been found between the potencies of a series of alcohols in stimulating $^{36}Cl^-$ uptake in synaptoneurosomes and both their intoxication potencies in rats and their membrane/buffer partition coefficients. Thus, an effect of ethanol on membrane lipids may alter the microenvironment of the GABA/barbiturate receptor-coupled Cl^- channel, resulting in an increase in Cl^- conductance. Alternatively, ethanol may act directly at the recognition site associated with the GABA/benzodiazepine/barbiturate receptor complex. Several groups have, in fact, reported that ethanol inhibits the specific binding of [^{35}S]TBPS, a radioligand that has been shown to label a site close to or on the Cl^- channel itself (25, 26). However, the concentrations of ethanol required for inhibition of [^{35}S]TBPS binding (IC_{50}, ≈300 mM) (26) are substantially higher than those required to stimulate $^{36}Cl^-$ uptake. Nevertheless, the very rapid decrease in ethanol-stimulated $^{36}Cl^-$ uptake as a function of incubation time (Fig. 2) suggests that radioreceptor binding studies carried out under equilibrium conditions at room temperature (e.g., 90 min for [^{35}S]TBPS) may underestimate the affinity of ethanol for these binding sites.

Although the exact mechanism(s) responsible for the ability of ethanol to stimulate $^{36}Cl^-$ uptake is unknown, it apparently does not involve the release of GABA. Previous

studies have failed to show an effect of ethanol in stimulating either basal or depolarized release of GABA from synaptosomes (35). Using our membrane preparation and incubation conditions, ethanol had no effect on the release of [^{3}H]GABA under either basal or depolarized conditions. Moreover, at higher ethanol concentrations (≥100 mM) we observed significant inhibition of the depolarized release of [^{3}H]GABA which is also in agreement with previous findings (41). Finally, examination of the kinetics of the potentiation of muscimol-stimulated $^{36}Cl^-$ uptake by low concentrations of ethanol (Fig. 4 *Inset*) revealed an increase in the apparent V_{max} of $^{36}Cl^-$ uptake, an effect that would not be expected by the release of endogenous GABA. Nevertheless, it is conceivable that at higher concentrations (≥20 mM) ethanol may be releasing GABA from a presynaptic pool not labeled by [^{3}H]GABA under our assay conditions.

Regardless of the mechanisms responsible for the ability of ethanol to stimulate $^{36}Cl^-$ transport, our results show that pharmacologically relevant concentrations of ethanol stimulate the GABA/barbiturate receptor complex in a manner similar to that of the barbiturates; at low concentrations ethanol potentiates GABA receptor-mediated Cl^- permeability and at higher (i.e., intoxicating) concentrations ethanol appears to stimulate Cl^- permeability directly. The actions of ethanol on GABA/barbiturate receptor-mediated Cl^- transport may explain the anxiolytic, sedative-hypnotic, and intoxicating properties of this drug.

1. Ritchie, J. M. (1980) in *The Pharmacological Basis of Therapeutics*, eds. Gilman, A. G., Goodman, L. S. & Gilman, A. (MacMillan, New York), pp. 376–390.
2. Jaffe, J. H. (1980) in *The Pharmacological Basis of Therapeutics*, eds. Gilman, A. G., Goodman, L. S. & Gilman, A. (MacMillan, New York), pp. 535–584.
3. Ticku, M. K., Burch, T. P. & Davis, W. C. (1983) *Pharmacol. Biochem. Behav.* **18,** 15–18.
4. Hunt, W. A. & Majchrowicz, E. (1983) *Pharmacol. Biochem. Behav.* **18,** 371–374.
5. Tabakoff, B. & Hoffman, P. L. (1980) in *Alcohol Tolerance and Dependence*, eds. Rigter, H. & Crabbe, J. C. (Elsevier/North-Holland, Amsterdam), pp. 201–226.
6. Koob, G. F., Strecker, R. E. & Bloom, F. (1980) *Subst. Alcohol Actions Misuse* **1,** 447–457.
7. Liljeqvist, S. & Engel, J. A. (1984) *Pharmacol. Biochem. Behav.* **18,** 521–525.
8. Cole, J. O. & Davis, J. M. (1975) in *American Handbook of Psychiatry*, eds. Freedman, D. X. & Dyrud, J. E. (Basic Books, New York), pp. 427–440.
9. Belleville, R. E. & Fraser, H. F. (1957) *J. Pharmacol. Exp. Ther.* **120,** 409–474.
10. Boisse, N. N. & Okamoto, M. (1980) in *Alcohol Tolerance and Dependence*, eds. Rigter, H. & Crabbe, J. C. (Elsevier/North-Holland, Amsterdam), pp. 265–292.
11. Goldstein, D. B. (1978) *J. Pharmacol. Exp. Ther.* **186,** 1–9.
12. Guidotti, A., Corda, M. G., Wise, B. C., Vaccarino, F. & Costa, E. (1983) *Neuropharmacology* **22,** 1471–1479.
13. Olsen, R. W. (1981) *J. Neurochem.* **37,** 1–13.
14. Olsen, R. W. (1982) *Annu. Rev. Pharmacol. Toxicol.* **22,** 245–277.
15. Skolnick, P. & Paul, S. M. (1982) in *International Review of Neurobiology*, eds. Smythies, J. R. & Bradley, R. J. (Academic, New York), Vol. 23, pp. 103–140.
16. Haefely, W., Polc, P., Schaffner, R., Keller, H. H., Pieri, L. & Mohler, H. (1979) in *GABA-Neurotransmitters*, eds. Krogsgaard-Larsen, P., Scheel-Kruger, J. & Kofod, H. (Munksgaard, Copenhagen), pp. 357–375.
17. Simmonds, M. A. (1981) *Br. J. Pharmacol.* **73,** 739–747.
18. Costa, E., Guidotti, A., Mao, C. C. & Suria, A. (1975) *Life Sci.* **17,** 167–186.
19. Cott, J., Carlsson, A., Engel, J. & Lindqvist, M. (1976) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **297,** 203–209.
20. Liljeqvist, S. & Engel, J. A. (1984) *Pharmacol. Biochem. Behav.* **21,** 521–525.
21. Davidoff, R. S. (1973) *Arch. Neurol. (Chicago)* **28,** 60–63.
22. Nestoros, J. N. (1980) *Science* **209,** 708–710.
23. Greenberg, D. A., Cooper, E. C., Gordon, A. & Diamond, I. (1984) *J. Neurochem.* **42,** 1062–1068.
24. Davis, W. C. & Ticku, M. K. (1981) *Mol. Pharmacol.* **20,** 287–294.
25. Ramanjaneyulu, R. & Ticku, M. K. (1984) *J. Neurochem.* **42,** 221–229.
26. Squires, R. F., Casida, J. E., Richardson, M. & Saederup, E. (1983) *Mol. Pharmacol.* **23,** 329–336.
27. Majchrowicz, E. (1977) in *Alcohol Intoxication and Withdrawal—IIb: Studies in Alcohol Dependence*, ed. Gross, M. M. (Plenum, New York), pp. 15–23.
28. Majchrowicz, E. (1975) *Psychopharmacologia* **43,** 245–254.
29. Schwartz, R. D., Skolnick, P., Hollingsworth, E. B. & Paul, S. M. (1984) *FEBS Lett.* **175,** 193–196.
30. Schwartz, R. D., Jackson, J. A., Weigert, D., Skolnick, P. & Paul, S. M. (1985) *J. Neurosci.* **5,** 2963–2970.
31. Schwartz, R. D., Skolnick, P., Seale, T. W. & Paul, S. M. (1986) in *Advances in Biochemical Psychopharmacology: GABA-ergic Transmission and Anxiety*, eds. Biggio, G. & Costa, E. (Raven, New York), in press.
32. Hollingsworth, E. B., McNeal, E. T., Burton, J., Williams, R. W., Daly, J. W. & Creveling, C. R. (1985) *J. Neurosci.* **5,** 2240–2253.
33. Harris, A. R. & Allan, A. M. (1985) *Science* **228,** 1108–1110.
34. Sandoval, M. E. (1980) *J. Neurochem.* **35,** 915–921.
35. Rohde, B. H. & Harris, R. A. (1983) *Neuropharmacology* **22,** 721–727.
36. Wong, E. H. F., Leeb-Lundberg, F. L. M., Teichberg, V. I. & Olsen, R. W. (1984) *Brain Res.* **303,** 267–275.
37. Thampy, K. G. & Barnes, E. (1984) *J. Biol. Chem.* **259,** 1753–1757.
38. Barker, J. L. & Ransom, B. R. (1978) *J. Physiol.* **280,** 331–354.
39. Barker, J. L. & Ransom, B. R. (1978) *J. Physiol.* **280,** 355–372.
40. Segel, I. H. (1976) in *Biochemical Calculations*, ed. Segel, I. H. (Wiley, New York), 2nd Ed., pp. 262–264.
41. Strong, R. & Wood, G. W. (1984) *J. Pharmacol. Exp. Ther.* **229,** 726–730.
42. Systinsky, I. A., Guzikov, B. M., Gomanko, M. V., Eremin, V. R. & Konovalova, N. N. (1975) *J. Neurochem.* **25,** 43–48.
43. Wixon, H. N. & Hunt, W. A. (1980) *Subst. Alcohol Actions Misuse* **1,** 481–491.
44. Michaelis, E. K., Chang, H. H., Roy, S., McFaul, J. A. & Zimbrick, E. (1983) *Pharmacol. Biochem. Behav.* **18,** 1–6.
45. Chin, J. H. & Goldstein, D. B. (1977) *Mol. Pharmacol.* **13,** 435–441.
46. Goldstein, D. B. & Chin, J. H. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 2073–2076.