Pharmacology Biochemistry and Behavior, Vol. 57, Nos. 1/2, pp. 73–80, 1997
Copyright © 1997 Elsevier Science Inc.
Printed in the USA. All rights reserved
0091-3057/97 $17.00 + .00

PII S0091-3057(96)00130-X

The Interaction of Dopamine, Cocaine, and Cocaethylene With Ethanol on Central Nervous System Depression in Mice

DAVID M. WILSON, ANDREW P. FERKO,[1] EDWARD J. BARBIERI, G. JOHN DiGREGORIO, EMIL BOBYOCK AND ROBERT McMICHAEL

Department of Pharmacology, Division of Toxicology, School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19102

Received 19 June 1995; Revised 15 February 1996; Accepted 20 March 1996

WILSON, D. M., A. P. FERKO, E. J. BARBIERI, G. J. DiGREGORIO, E. BOBYOCK AND R. MCMICHAEL. *The interaction of dopamine, cocaine, and cocaethylene with ethanol on central nervous system depression in mice.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 73–80, 1997.—The interactions between dopamine, cocaine, cocaethylene, and ethanol were studied in Swiss–Webster mice. The loss of the righting reflex (LORR) was used as a measure of CNS depression. Animals were injected intraperitoneally (IP) with ethanol (4.0 g/kg), which caused a LORR. Immediately upon regaining of the righting reflex, mice were injected intracerebroventricularly (ICV) with saline, dopamine (0.1, 0.5, or 1.0 μmol/kg), cocaine (1, 15, or 25 μmol/kg), or cocaethylene (1, 15, or 25 μmol/kg). In the presence of systemic ethanol, all three compounds produced CNS depression in a dose-dependent manner. The dopamine D_2-receptor antagonist sulpiride and the D_1-receptor antagonist fluphenazine were given acutely ICV with dopamine in the presence of systemic ethanol to examine whether these antagonists could block the return to the LORR produced by dopamine. Sulpiride, however, actually enhanced the interaction between ethanol and dopamine in a dose-dependent manner as measured by the LORR; fluphenazine neither blocked nor enhanced the effect of dopamine in the presence of systemic ethanol. In addition, these antagonists had no effect on cocaine- and cocaethylene-induced CNS depression in the presence of systemic ethanol. The results of this study showed that the neurotransmitter dopamine and both cocaine and cocaethylene can promote further CNS depression in the presence of systemic ethanol, and that dopamine was significantly more potent than cocaine and cocaethylene as measured by the return to the LORR. © 1997 Elsevier Science Inc.

Ethanol Cocaine Cocaethylene Dopamine Benzoylecgonine Sulpiride Fluphenazine CNS depression Loss of righting reflex

PREVIOUS studies have shown that various neurotransmitters and putative neurotransmitters such as GABA (14,15), glycine (67), glutamate (16), cysteine sulfinic acid (15), and taurine (17) interact with ethanol to promote CNS depression as measured by the loss of the righting reflex (LORR) in mice. Researchers have suggested that ethanol potentiates $GABA_A$-receptor function and that this effect of ethanol on the $GABA_A$ receptor may be responsible for the depressant and anxiolytic properties of ethanol (1,2,28,38,59). In addition, ethanol can affect the dopaminergic system in the CNS (8,26,60,66), and it has been shown that dopamine can stimulate the release of GABA from GABAergic interneurons (43,52–54). In the present study, the effect of the neurotransmitter dopamine in the presence of systemic ethanol was examined in a mouse model for its effect on CNS depression.

Cocaine was used in this study because its mechanism of action in the CNS appears to involve predominantly the dopaminergic system (7,16,31,55) and because cocaine abuse is often associated with the use of ethanol (25,37). When cocaine is combined with ethanol an active product, cocaethylene, is formed in vivo. Ethanol inhibits the carboxylesterase-catalyzed hydrolysis of cocaine to benzoylecgonine in the liver and simultaneously induces the ethyl transesterification of cocaine to cocaethylene (11,27,33). Cocaethylene is as potent as cocaine in binding to dopamine reuptake sites and in inhibiting neuronal dopamine reuptake (30). Cocaethylene appears to

[1]Requests for reprints should be addressed to A. P. Ferko, Department of Pharmacology, Division of Toxicology, School of Medicine, Allegheny University of the Health Sciences, Broad and Vine Streets, Philadelphia, PA 19102.

be more euphorigenic and rewarding than cocaine (33), and the compound may be more addictive (50). Furthermore, it has been suggested that the blockade of dopamine reuptake into neurons by cocaethylene may be responsible for the enhanced euphoria associated with combined ethanol and cocaine use (27). Therefore, it was interesting to study the activity of cocaethylene in this investigation.

Little research has been performed with intracerebroventricular (ICV) injection of dopamine, cocaine, and cocaethylene in the presence of ethanol. The ICV route of drug administration was selected in this study because it allows the drug readily to get to its site of action and provides a rapid onset of action, and this route eliminates the initial peripheral biotransformation of the drug (14–16). The hypothesis of this study is that dopamine, cocaine, and cocaethylene can cause CNS depression in the presence of systemic ethanol as measured by the LORR.

In addition, in the presence of systemic ethanol the interactions between dopamine, cocaine, and cocaethylene with sulpiride (a D_2-receptor antagonist) and fluphenazine (a D_1-receptor antagonist) were examined.

METHOD

Male Swiss–Webster mice, ranging in weight from 27 to 35 g, were obtained from Taconic Laboratory (New York, NY). Mice were housed for 1 week in a standard environment that consisted of a light cycle from 0600 to 1800 h, a temperature of 21 ± 1°C, and free access to water and Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO). The ethanol solution for IP injection was prepared from 95% (v/v) ethanol and diluted to 20% (w/v) with saline (0.9% NaCl) (14). Cocaine hydrochloride was obtained from Mallinckrodt Specialty Chemicals Co. (St. Louis, MO); cocaethylene fumarate was obtained from NIDA; and dopamine hydrochloride, benzoylecgonine hydrate, fluphenazine dihydrochloride, and racemic sulpiride were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were obtained from commercial sources and were of analytic grade.

Cocaine solutions for injection were adjusted to pH 7.0 with NaOH solution (15,67). Dopamine solution was prepared with 0.04 mg/ml ascorbic acid to prevent the oxidation of dopamine, and adjusted to pH 7.0 with NaOH solution. Fluphenazine solution for injection was adjusted to pH 6.7 with NaOH solution. Benzoylecgonine was prepared in saline. Sulpiride was adjusted to pH 7.0 with HCl solution. These solutions were prepared immediately before each experiment.

Acute Ethanol Administration and LORR as an Index of CNS Depression

All experiments were started between 0900 and 1000 h. External noises were minimized, and the animals were kept from other animal contact during the LORR stages of the experiments. LORR was defined as the inability of the mouse to right itself by rolling onto its feet after being placed on its back three times in 15 s (15,67). An animal was considered to have regained the righting reflex when the animal was also able to right itself on all four legs three times in 15 s after being placed on its back. The length of time that the animal experienced the LORR was used as a measure of CNS depression, as described by Bignall (6) and used previously by Ferko and colleagues (14–17,67).

After the IP injection of ethanol (4.0 g/kg), all animals experienced a LORR, referred to as an ethanol-induced LORR. The time from the IP injection to the onset of ethanol-induced LORR was recorded in seconds and termed the onset of the LORR. The duration of the ethanol-induced LORR was recorded as well. Following ICV injection of a drug or saline, some of the animals experienced a second LORR, which was referred to as the drug-induced return to the LORR, or simply, drug-induced LORR.

ICV Drug Administration

The ICV injection procedure used was according to Pedigo et al. (42) and is described briefly. Twenty minutes after the animal lost the righting reflex following IP ethanol administration, a sagittal incision was made on the dorsal aspect of the head, exposing the skull sutures. A preparatory puncture 3 mm deep from the outer surface of the cranium was made 2 mm caudal and 2 mm to the right of bregma using a 26-ga needle. The needle was inserted and immediately removed; no drug or saline was administered at this time (15,67). This step was performed on ethanol-anesthetized mice (10,21,32) to gain access to the lateral ventricle of the brain for later ICV drug administration.

Immediately after the animals regained the righting reflex following IP ethanol injection, they were given an ICV injection of saline or drug solution in a total volume of 5 μl/30 g body wt. This step was performed on ethanol-analgetic animals (39,48,70). Upon ICV injection of saline or drug, a return to LORR was observed and recorded in minutes. Once regaining the righting reflex after drug-induced LORR (ICV injection), a 20-μl blood sample was taken from the orbital sinus to determine the concentration of ethanol circulating in the blood. The correct placement of the drug solution by ICV administration was verified at the end of the experimental procedures by an ICV injection of methylene blue dye and subsequent dissection (14,17).

LORR Experiments with Ethanol (IP) and Dopamine (ICV)

The purpose of these experiments was to determine whether a drug-induced return to the LORR could be caused by the ICV administration of dopamine in the presence of systemic ethanol. Dopamine concentrations of 0.1, 0.5, or 1.0 μmol/kg were injected ICV in a volume of 5 μl immediately after the animals regained the righting reflex following the ethanol-induced LORR. Because the dopamine solutions were prepared with ascorbic acid to prevent oxidation of dopamine, ascorbic acid controls were run as well. Ascorbic acid (0.04 mg/ml in saline) was delivered ICV in a volume of 5 μl immediately after the mice regained the LORR following ethanol injection.

LORR Experiments with Ethanol (IP) and Cocaine, Cocaethylene, or Benzoylecgonine (ICV)

The purpose of these experiments was to assess whether an ICV injection of cocaine, cocaethylene, or benzoylecgonine could cause CNS depression in the presence of systemic ethanol. The previously mentioned procedure was used. The ICV injections used were either saline (control), or 1, 15, or 25 μmol/kg of cocaethylene, cocaine, or benzoylecgonine following the regaining of the ethanol-induced LORR. Benzoylecgonine was once considered to be an inactive metabolite of cocaine; however, benzoylecgonine can enter the brain following peripheral administration of the compound (3) and can cause vasoconstriction of central arteries (11,13).

Effects of Fluphenazine and Racemic Sulpiride on the Dopamine-Induced LORR in the Presence of Systemic Ethanol

These experiments were performed to determine the effect of two selective dopaminergic antagonists, fluphenazine racemic sulpiride, on the dopamine induced-LORR. Although dopamine receptor blockers can reduce the effect of exogenously administered dopamine, it has been shown that the acute administration of a dopaminergic antagonist can enhance the release of endogenous dopamine (4,35,41,57,69). These experiments were performed to note whether acute ICV injection of fluphenazine or sulpiride would reduce or enhance the effect of dopamine in the presence of systemic ethanol. A solution of fluphenazine (0.1 or 0.001 μmol/kg) or sulpiride (1.0, 0.1, or 0.001 μmol/kg) admixed with 0.5 μmol/kg of dopamine was administered by ICV injection following regaining of the ethanol-induced LORR.

LORR Experiments with Ethanol and the Dopamine Antagonists Fluphenazine and Sulpiride

These experiments were conducted to determine whether the ICV administration of the dopamine receptor antagonists would cause CNS depression when they were administered in the presence of systemic ethanol. The concentrations of fluphenazine and sulpiride used were 0.001 and 0.1 μmol/kg.

Effects of Fluphenazine and Sulpiride on the Cocaine- and Cocaethylene-Induced LORR

The purpose of these experiments was to note whether fluphenazine and sulpiride could alter the cocaine- or cocaethylene-induced LORR in the presence of systemic ethanol. Once the animals regained the ethanol-induced LORR, an ICV injection was immediately given consisting of 0.001 μmol/kg of either antagonist and 12.5 μmol/kg of cocaine or cocaethylene following the regaining of the ethanol-induced LORR.

LORR Experiments in the Absence of Ethanol

All of the previously used compounds (i.e., dopamine, cocaine, cocaethylene, benzoylecgonine, fluphenazine, and sulpiride) were given by ICV injection in the absence of systemic ethanol to determine whether any of these drugs could in themselves induce an LORR (14,67). Mice were injected with saline (0.02 ml/g, IP) to simulate the same volume of ethanol that would have been administered. Twenty minutes later, the mice were anesthetized lightly with methoxyflurane so the preparatory ICV procedure could be performed. The average time of the ethanol-induced LORR and regaining the righting reflex was about 50 min. Hence, this was the time used to give the ICV drug injection, so as to attempt to mimic an ethanol treatment trial. At this point, the mice were sedated with methoxyflurane, but not to the point where they lost the righting reflex. They were then injected with one of the previously mentioned drugs; the doses used were as follows: dopamine (1 μmol/kg), cocaine (1, 15, and 25 μmol/kg), cocaethylene (25 μmol/kg), benzoylecgonine (25 μmol/kg), sulpiride (0.1 μmol/kg), and fluphenazine (0.1 μmol/kg). Observations were made for the next 2 h, except with the dopamine trial in which observations were made for 1 h, owing to time constraints.

Blood Ethanol Analysis

Blood drawn from the orbital sinus of each mouse was assayed for the concentration of ethanol according to Lundquist (36). A Milton-Roy Spectronic Model 601 Spectrophotometer (Rochester, NY) was used to read the optical densities at 340 nm (17,67).

Data Analysis

Statistically significant differences were determined by analysis of variance (ANOVA). All multiple comparisons with a control set and comparison among experimental sets were done by ANOVA followed by the posthoc Scheffé's test. All data are reported as the mean ± standard error.

RESULTS

Acute Ethanol Administration and LORR as an Index of CNS Depression

An IP injection of 4.0 g/kg of ethanol resulted in an onset to the ethanol-induced LORR that averaged 83.5 s (range 78.3–97.5); the duration of the LORR averaged 51.5 min (range 47.0–62.3). In all experiments performed in this investigation, neither the onset nor the duration of the ethanol-induced LORR were significantly different as presented in the individual tables. Blood ethanol concentrations following the ethanol-induced LORR were approximately 3.5 mg/ml in all experiments.

Effect of Dopamine on LORR in the Presence and Absence of Systemic Ethanol

After mice regained the ethanol-induced LORR, the ICV administration of dopamine (at 0.1, 0.5, and 1.0 μmol/kg) produced a return to LORR (Table 1). The response seemed to reach a maximum after 1.0 μmol/kg, because 25 μmol/kg of dopamine ($n = 4$; data not shown in Table 1) manifested a return to LORR with a duration of 31.3 ± 2.4 min. Dopamine itself caused no significant LORR in this animal model; in the absence of systemic ethanol, ICV injection of dopamine (1.0 μmol/kg) produced an LORR of 0.2 ± 0.2 min ($n = 5$). Ascorbic acid controls were run because the dopamine solutions were prepared with ascorbic acid to prevent their oxidation. Animals injected ICV with 0.04 mg/ml ascorbic acid in saline immediately upon regaining the ethanol-induced LORR showed no significant drug-induced return to LORR (2.3 ± 1.2 min) (Table 1) comparable to saline controls without ascorbic acid (0.3–0.8 min) (Tables 2–4).

Effects of Cocaine and Cocaine Metabolites Cocaethylene and Benzoylecgonine on LORR in the Presence of Systemic Ethanol

The ICV injection of either cocaine or cocaethylene (at 1, 15, or 25 μmol/kg) enhanced CNS depression in the presence of systemic ethanol. Both cocaine (Table 2) and cocaethylene (Table 3) produced a return to LORR in a dose-dependent manner. In both cases, there was an inverse relationship between blood ethanol concentrations and duration of the drug-induced return LORR (Tables 2 and 3). Therefore, higher micromolar amounts of both cocaine and cocaethylene caused longer drug-induced return LORRs, the blood ethanol concentrations decreased as the length of drug-induced return to LORR increased, and these blood ethanol values were lower compared with the controls.

Benzoylecgonine (at 1, 15, and 25 μmol/kg) produced only a mild degree of CNS depression in the presence of systemic ethanol (Table 4). Only the 15-μmol/kg dose caused a signifi-

TABLE 1

EFFECT OF DOPAMINE ON THE RETURN TO THE LORR WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE ETHANOL (ETOH)-INDUCED LORR*

Group	*n*	Onset to LORR (s)	ETOH LORR (min)	Drug-Induced Return to LORR (min)†	Blood ETOH (mg/ml)
ETOH‡ + saline§	9	87.8 ± 8.5	47.0 ± 3.0	2.3 ± 1.2	3.56 ± 0.06
ETOH + dopamine (0.1 μmol/kg)	8	86.3 ± 3.8	51.9 ± 2.8	17.5 ± 4.0¶	3.16 ± 0.10¶
ETOH + dopamine (0.5 μmol/kg)	8	82.5 ± 6.3	49.0 ± 3.5	15.8 ± 3.1¶	3.33 ± 0.06
ETOH + dopamine (1.0 μmol/kg)	8	78.8 ± 2.5	48.1 ± 2.7	29.6 ± 3.4#**	3.25 ± 0.10

* All values are expressed as the mean ± standard error.
† Dopamine (μmol/kg, ICV) was injected immediately after regaining ETOH-induced LORR.
‡ ETOH (ethanol) was given at 4.0 g/kg, IP. Saline was given ICV.
§ Saline was injected ICV with 0.04 g/ml ascorbic acid.
¶ Significantly different from controls at $p < 0.05$.
# Significantly different fron controls at $p < 0.01$.
** Significantly different from dopamine (0.5 μmol/kg) group at $p < 0.05$.

cant return to the LORR. At 15 and 25 μmol/kg, benzoylecgonine caused convulsions after the drug-induced return to LORR. At 15 μmol/kg, all mice exhibited some degree of convulsive activity for up to 60 min; at 25 μmol/kg, all mice convulsed and died. Convulsions were not observed in animals injected with cocaine or cocaethylene in the presence of systemic ethanol.

Effects of Cocaine, Cocaethylene, and Benzoylecgonine in the Absence of Ethanol

Cocaine (1, 15, or 25 μmol/kg), cocaethylene (25 μmol/kg), and benzoylecgonine (25 μmol/kg) were given by ICV injection in the absence of prior ethanol administration to assess whether these compounds had depressant effects by themselves. Cocaine (at 25 μmol/kg) induced an LORR of 12.4 ± 1.4 min ($n = 5$); however, as shown in Table 2, the cocaine-induced return to LORR was much longer in the presence of systemic ethanol (i.e., 30.9 ± 3.5 min). Unlike its effect in the presence of systemic ethanol, cocaine caused the mice to exhibit some convulsive activity for about 9 min at this dose. Cocaine alone, at 1 and 15 μmol/kg, produced smaller LORR values of 6.6 ± 0.7 ($n = 5$) and 2.2 ± 1.0 ($n = 5$) min, respectively, in the absence of ethanol. The 15-μmol/kg dose caused convulsions for about 3 min in two animals, and one died from the ICV injection of the drug; cocaine at the 1-μmol/kg dose caused no convulsant effects. Saline control values were 0.3 ± 0.1 min ($n = 8$) for the duration of the LORR.

In the absence of ethanol, cocaethylene (25 μmol/kg) when injected ICV cause an LORR of 13.1 ± 2.9 min ($n = 8$); this compound also caused some convulsions in the absence of systemic ethanol for about 6 min, and these seemed to be mostly of the tonic variety. When benzoylecgonine was injected ICV at 25 μmol/kg ($n = 5$) in the absence of systemic ethanol, it caused no LORR (0.0 ± 0.0 min), but it did produce convulsions for a short time (minutes) ending in the animals' death. The presence of systemic ethanol in the animals suppresses the convulsive activity exhibited by cocaine (15 and 25 μmol/kg) and cocaethylene (25 μmol/kg).

Effects of the Dopamine Receptor Antagonists Fluphenazine and Sulpiride

The results in Table 5 show that sulpiride antagonist enhanced the dopamine-induced return to LORR in a dose-dependent manner. Fluphenazine had no significant effect on the dopamine-induced return to LORR.

These two dopamine antagonists were each administered alone by ICV injection following regaining of ethanol-induced LORR to examine whether either drug would enhance CNS depression because they did not attenuate the dopamine-

TABLE 2

EFFECT OF COCAINE ON THE RETURN TO THE LORR WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE ETHANOL (ETOH)-INDUCED LORR*

Group	*n*	Onset to LORR (s)	ETOH LORR (min)	Drug-Induced Return to LORR (min)†	Blood ETOH (mg/ml)
ETOH‡ + saline	9	83.3 ± 2.6	47.8 ± 3.1	0.8 ± 0.5	3.52 ± 0.03
ETOH + cocaine (1 μmol/kg)	10	81.0 ± 2.4	54.7 ± 4.0	11.6 ± 1.4§	3.36 ± 0.04
ETOH + cocaine (15 μmol/kg)	6	90.0 ± 6.7	47.3 ± 6.1	21.3 ± 2.1¶§	3.33 ± 0.07
ETOH + cocaine (25 μmol/kg)	8	78.8 ± 2.5	53.0 ± 4.1	30.9 ± 3.5¶#**	3.06 ± 0.05¶#**

* All values are expressed as the mean ± standard error.
† Cocaine (μmol/kg, ICV) was injected immediately after regaining ETOH-induced LORR.
‡ ETOH (ethanol) was given at 4.0 g/kg, IP. Saline was given ICV.
§ Significantly different from controls at $p < 0.01$.
¶ Significantly different from cocaine (1 μmol/kg) group at $p < 0.05$.
# Significantly different fron cocaine (1 μmol/kg) group at $p < 0.01$.
** Significantly different from cocaine (15 μmol/kg) group at $p < 0.05$.

TABLE 3

EFFECT OF COCAETHYLENE (CE) ON THE RETURN TO THE LORR WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE ETHANOL (ETOH)-INDUCED LORR*

Group	*n*	Onset to LORR (s)	ETOH LORR (min)	Drug-Induced Return to LORR (min)†	Blood ETOH (mg/ml)
ETOH‡ + saline	9	85.0 ± 2.5	48.7 ± 2.7	0.8 ± 0.5	3.53 ± 0.04
ETOH + CE (1 μmol/kg)	8	82.5 ± 2.8	59.1 ± 3.9	18.5 ± 3.8§	3.27 ± 0.06
ETOH + CE (15 μmol/kg)	9	83.3 ± 2.6	49.0 ± 4.3	33.8 ± 2.9¶#	3.25 ± 0.05¶
ETOH + CE (25 μmol/kg)	6	82.5 ± 3.4	52.1 ± 2.6	49.0 ± 6.8¶**	3.15 ± 0.06¶

* All values are expressed as the mean ± standard error.
† Cocaethylene (μmol/kg, ICV) was injected immediately after regaining ETOH-induced LORR.
‡ ETOH (ethanol) was given at 4.0 g/kg, IP. Saline was given ICV.
§ Significantly different from controls at $p < 0.05$.
¶ Significantly different from cocaethylene (1 μmol/kg) group at $p < 0.05$.
# Significantly different fron controls at $p < 0.01$.
** Significantly different from cocaethylene (1 μmol/kg) group at $p < 0.01$.

induced return to LORR. Fluphenazine alone (at 0.001 and 0.1 μmol/kg) induced a return to LORR. The lower concentration of fluphenazine produced a statistically significant return to LORR ($n = 8$; LORR = 15.1 ± 3.8 min), which was similar to the return to LORR values of fluphenazine (0.001 μmol/kg) in combination with dopamine (Table 5). Fluphenazine (0.1 μmol/kg) in the presence of systemic ethanol produced a return to the LORR of 11.6 ± 1.9 min. As was observed with fluphenazine, sulpiride (at 0.001 and 0.1 μmol/kg) significantly produced CNS depression in the presence of systemic ethanol. Sulpiride (at 0.1 μmol/kg) produced a return to LORR for 28.8 ± 5.4 min ($n = 8$) and the 0.001-μmol/kg dose caused a return to LORR for 21.4 ± 3.0 min. Finally, when used alone (i.e., in the absence of systemic ethanol) neither fluphenazine nor sulpiride had any effect in inducing an LORR.

Effects of Fluphenazine and Sulpiride on the Cocaine- and Cocaethylene-Induced Return to LORR

Sulpiride and fluphenazine (at 0.001 μmol/kg) were injected ICV in combination with either cocaine or cocaethylene (at 12.5 μmol/kg) after mice regained of the ethanol-induced LORR, in an attempt to block the return to LORR induced by these compounds. The low dose of dopamine antagonists was used because it seemed to cause little if any additive effects on the dopamine-induced return to LORR in the presence of systemic ethanol. Neither sulpiride nor fluphenazine significantly antagonized the cocaine- or cocaethylene-induced return to LORR (data not shown).

DISCUSSION

The present study showed that the neurotransmitter dopamine can cause acute depressant effects on the CNS in the presence of systemic ethanol. Fowler et al. (18,19) cited the ability of cocaine and ethanol to increase synaptic concentrations of dopamine as a reason for the behavioral enhancement involved with their concomitant use. A dose–response relationship occurred between cocaine or cocaethylene and the drug-induced return to an LORR in the presence of systemic ethanol. There was an inverse relationship between blood ethanol concentrations and the length of cocaine- or cocaethylene- induced return to LORR, suggesting that these drugs did not inhibit the biotransformation of ethanol to obtain the observed central depressant effects. Cocaethylene induced a longer drug-induced return to LORR (Table 3) than cocaine (Table 2) at equivalent doses. This may be explained by two factors: (a) cocaethylene does not leave the CNS as readily as does cocaine (18,19), allowing cocaethylene to block reuptake sites for a longer period of time; and (b) cocaethylene is more resistant to biotransformation by plasma cholinesterase than cocaine (18,19,34,50), and hence can be expected to accumulate, resulting in a longer return to LORR as compared to equimolar amounts of cocaine.

TABLE 4

EFFECT OF BENZOYLECGONINE (BE) ON THE RETURN TO THE LORR WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE ETHANOL (ETOH)-INDUCED LORR*

Group	*n*	Onset to LORR (s)	ETOH LORR (min)	Drug-Induced Return to LORR (min)†	Blood ETOH (mg/ml)
ETOH‡ + saline	8	81.4 ± 2.7	49.0 ± 3.2	0.3 ± 0.2	3.51 ± 0.03
ETOH + BE (1 μmol/kg)	5	79.0 ± 4.0	62.2 ± 9.5	3.3 ± 1.3	3.15 ± 0.08
ETOH + BE (15 μmol/kg)	7	83.9 ± 3.3	51.4 ± 3.3	12.7 ± 3.6§¶	3.00 ± 0.07§
ETOH + BE (25 μmol/kg)	8	89.9 ± 3.8	52.6 ± 3.4	6.4 ± 0.5	3.00 ± 0.08§

* All values are expressed as the mean ± standard error.
† Benzoylecgonine (μmol/kg, ICV) was injected immediately after regaining ETOH-induced LORR.
‡ ETOH (ethanol) was given at 4.0 g/kg, IP. Saline was given ICV.
§ Significantly different from controls at $p < 0.05$.
¶ Significantly different from benzoylecgonine (1 μmol/kg) group at $p < 0.05$.

TABLE 5

EFFECT OF DOPAMINE ANTAGONISTS SULPIRIDE AND FLUPHENAZINE ON THE DOPAMINE-INDUCED RETURN TO THE LORR WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE ETHANOL (ETOH)-INDUCED LORR*

Group	*n*	Onset to LORR (s)	ETOH LORR (min)	Drug-Induced Return to LORR (min)†	Blood ETOH (mg/ml)
Control					
ETOH‡ + dopamine†	8	97.5 ± 13.0	52.5 ± 4.0	22.6 ± 1.7	3.19 ± 0.09
With sulpiride					
ETOH + dopamine + sulpiride (0.001 μmol/kg)	9	78.8 ± 3.2	47.2 ± 2.2	14.0 ± 1.9¶§	3.25 ± 0.12
ETOH + dopamine + sulpiride (0.1 μmol/kg)	5	81.0 ± 3.7	62.3 ± 6.1	40.0 ± 9.5§	2.98 ± 0.18
ETOH + dopamine + sulpiride (1.0 μmol/kg)	4	78.8 ± 3.8	59.5 ± 6.2	70.0 ± 6.5#	3.02 ± 0.18
With fluphenazine					
ETOH + dopamine + fluphenazine (0.001 μmol/kg)	5	79.0 ± 2.9	59.4 ± 6.8	17.8 ± 5.3	3.17 ± 0.05
ETOH + dopamine + fluphenazine (0.1 μmol/kg)	6	78.3 ± 4.0	51.3 ± 5.3	26.3 ± 5.0	3.33 ± 0.11

* All values are expressed as the mean ± standard error.
† Dopamine (0.5 μmol/kg, ICV) was injected ICV alone or with antagonist (μmol/kg) immediately after regaining ETOH-induced LORR.
‡ ETOH (ethanol) was given at 4.0 g/kg, IP.
§ Significantly different from dopamine (0.5 μmol/kg) + sulpiride (1.0 μmol/kg) group at $p < 0.01$.
¶ Significantly different from dopamine (0.5 μmol/kg) + sulpiride (0.1 μmol/kg) group at $p < 0.05$.
# Significantly different from controls at $p < 0.05$.

Another metabolite of cocaine, benzoylecgonine, produced a small but significant drug-induced return to LORR, but only at the 15-μmol/kg concentration. The 15- and 25-μmol/kg concentrations caused extensive convulsions in an apparent dose-dependent fashion; the convulsions caused by the 25-μmol/kg dose resulted in death of the animals. Benzoylecgonine is a noted vasoconstrictor of cerebral arteries (11,13); thus, it is possible that ischemia caused the convulsant activity. This may also be why cocaine and cocaethylene at higher doses caused some convulsions in the absence of systemic ethanol. Cocaine has a local anesthetic effect on neuronal conduction, which may be the factor causing cocaine's convulsions in the absence of systemic ethanol. If cocaethylene and benzoylecgonine have some local anesthetic properties, then this may be the cause of the convulsions; however, the exact mechanism is not known. The convulsive activity of cocaine, cocaethylene, and benzoylecgonine, especially at higher doses, may make the interpretation of the interaction between ethanol and these drugs more complex.

Dopamine may play a role in the effects observed with cocaine and cocaethylene. In addition, dopamine also produced a drug-induced return to LORR. Although it is possible that the observed central depressant effects of cocaine and cocaethylene may be due to local anesthetic activity, as similar results were observed with dopamine (which has no local anesthetic activity), it is reasonable to assume that alterations in neurotransmitter activity were responsible for the observed effects of cocaine and cocaethylene, especially at low doses.

There are complex interactions between neurotransmitters in the CNS that regulate motor and behavioral activity, such as between the dopaminergic and GABAergic neurotransmitter systems (49). Studies have shown that dopamine receptors are involved in the regulation of the activity of GABAergic neurons (9,46,49). The major inhibitory neurotransmitter in the CNS, γ-aminobutyric acid (GABA), has been shown to be involved in the central depressant effects of ethanol as measured by the LORR (15,16). Dopamine may cause LORR by stimulating the release of GABA. It is known that dopamine stimulates the release of GABA from GABAergic interneurons in the prefrontal cortex (43,52–54). There is some controversy as to which subtypes of dopamine receptors are activated in causing the release of GABA. The prevailing belief seems to be that D_2 activation causes the release of GABA in the prefrontal cortex (43,47,53). The striatum demonstrates a D_1-receptor–mediated increase in GABA release (5,23,43,51). Activation of the D_1 receptor has even been postulated to enhance D_2-receptor–stimulated release of GABA (52). A similar mechanism may be operating to induce an LORR with the administration of dopamine, cocaine, and cocaethylene.

The dopaminergic receptor antagonists sulpiride and fluphenazine did not antagonize the drug-induced LORR by dopamine, cocaine, or cocaethylene; however, sulpiride enhanced the CNS depression produced by dopamine. Moreover, both sulpiride and fluphenazine in the presence of systemic ethanol produced significant drug-induced LORR similar to the effects of dopamine, cocaine, and cocaethylene. A possible explanation for the effects of these drugs is that they released dopamine when administered acutely. It has been shown that sulpiride, when given acutely, can block a D_2 autoreceptor (4,57,69) and thereby can increase dopamine release into the synaptic cleft. Also, fluphenazine at high doses has been shown to increase dopamine in the synaptic cleft (4,41). The dopaminergic blocker haloperidol, when administered acutely, also has been shown to block mainly D_2 autoreceptors and can increase the synaptic release of dopamine (35). The effect of cocaine and haloperidol, when administered together acutely, produced an additive effect by increasing synaptic concentrations of dopamine; when haloperidol is used for its antipsychotic effect, chronic administration is necessary to establish dopaminergic blockade (35).

It is known that ethanol can interact with a number of neurotransmitter systems in the CNS. Although the exact mechanism of action is not known for the enhanced CNS depression caused by dopamine, cocaine, and cocaethylene in the presence of systemic ethanol, it may be related to dopamine's ability to release GABA. In addition, the CNS depression produced by the dopamine blockers sulpiride and fluphenazine, as measured by LORR, may be due to the release of dopamine, which then promotes enhanced GABAergic activity as stated previously. As interesting as this explanation may be, more work is necessary to fully understand the nature of these drug interactions between ethanol, dopamine, cocaine, and cocaethylene.

REFERENCES

1. Allan, A. M.; Harris, R. A. Gamma-aminobutryic acid and alcohol actions: Neurochemical studies of long sleep and short sleep mice. Life Sci. 39:2005–2015; 1986.
2. Allen, A. M.; Harris, R. A. Acute and chronic ethanol treatment alters GABA receptor-operated chloride channels. Pharmacol. Biochem. Behav. 27:665–670; 1987.
3. Barbieri, E. J.; Ferko, A. P.; DiGregorio, G. J.; Ruch, E. K. The presence of cocaine and benzoylecgonine in rat cerebrospinal fluid after the intravenous administration of cocaine. Life Sci. 51:1739–1746; 1992.
4. Barnes, J. M.; Barnes, N. M.; Costall, B.; Naylor, R. J. The actions of (−)N-n-propylnorapomorphine and selective dopamine D_1 and D_2 receptor agonists to modify the release of [^{3}H] dopamine from the rat nucleus accumbens. Neuropharmacology 29:327–336; 1990.
5. Beauregard, M.; Ferron, A. Dopamine modulates the inhibition induced by GABA in rat cerebral cortex: An iontophoretic study. Eur. J. Pharmacol. 205:225–231; 1991.
6. Bignall, K. E. Ontogeny of levels of neural organization: The righting reflex as a model. Exp. Neurol. 42:566–573; 1974.
7. Bradberry, C. W.; Nobiletti, J. B.; Elsworth, J. D.; Murphy, B.; Jatlow, P.; Roth, R. A. Cocaine and cocaethylene: Microdialysis comparison of brain drug levels and effects on dopamine and serotonin. J. Neurochem. 60:1429–1435; 1993.
8. Brodie, M. S.; Shefner, S. A.; Dunwiddie, T. V. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res. 508:65–69; 1990.
9. Caboche, J.; Vernier, P.; Rogard, M.; Julien, J. P.; Mallet, J.; Besson, M. J. Role of dopaminergic D_2 receptors in the regulation of glutamic acid decarboxylase messenger RNA in the striatum of the rat. Eur. J. Neurosci. 4:438–447; 1992.
10. Daniel, L. C. Alteration of general anesthetic potency by agonists and antagonists of the polyamine binding site of the N-methyl-D-aspartate receptor. J. Pharmacol. Exp. Ther. 261:304–310; 1992.
11. Dean, R. A.; Harper, E. T.; Dumaual N.; Stoeckel, D. A.; Bosron, W. F. Effects of ethanol on cocaine metabolism: Formation of cocaethylene and norcocaethylene. Toxicol. Appl. Pharmacol. 117:1–8; 1992.
12. DiPalma, J. R.; DiGregorio, G. J. (eds.) Basic pharmacology in medicine. 3rd edition. New York: McGraw-Hill; 1990.
13. Erickson, B.; Konkol, R. J.; Madden, J. A. A comparison of the epileptogenic potential of cocaine and benzoylecgonine following intraventricular administration in nonanesthetised rats. FASEB J. 4:A746; 1990.
14. Ferko, A. P. The interaction between ethanol and cysteine on the central depressant effects of ethanol in mice. Pharmacol. Biochem. Behav. 36:619–624; 1989.
15. Ferko, A. P. Cysteine sulfinic acid can enhance the central depressant effect of ethanol in mice. Pharmacol. Biochem. Behav. 39:653–757; 1991.
16. Ferko, A. P. NMDA enhances the central depressant properties of ethanol in mice. Pharmacol. Biochem. Behav. 43:297–301; 1992.
17. Ferko, A. P.; Bobyock, E. Effect of taurine on ethanol-induced sleep time in mice geneticaly bred for differences in ethanol sensitivity. Pharmacol. Biochem. Behav. 31:667–673; 1988.
18. Fowler, J. S.; Volkow, N. D.; MacGregor, R. R.; Logan, J.; Dewey, S. L.; Gatley, S. J. Comparative PET studies of the kinetics and distribution of cocaine and cocaethylene in baboon brain. Synapse 12:220–227; 1992.
19. Fowler, J. S.; Volkow, N. D.; Logan, J.; MacGregor, R. R.; Wang, G. J.; Wolf, A. P. Alcohol intoxication does not change [^{11}C] cocaine pharmacokinetics in human brain and heart. Synapse 12:228–235; 1992.
20. Fuchs, V.; Coper, H.; Rommelspacher, H. The effects of ethanol and haloperidol on dopamine receptor (D_2) density. Neuropharmacology 26:1231–1233; 1987.
21. Gessner, P. K.; Shakarjian, M. P. Interactions of paraldehyde with ethanol and chloral hydrate. J. Pharmacol. Exp. Ther. 235: 32–36; 1985.
22. Gilman, A. G.; Rall, T. W.; Niles, A. S.; Taylor, P. (eds.) Goodman and Gilman's the pharmacological basis of therapeutics. 8th edition. New York: Pergamon; 1990:118–153.
23. Girault, J. A.; Spampinato, U.; Glowinski, J.; Besson, M. J. In vivo release of [^{3}H]-aminobutyric acid in the rat neostriatum—II. Opposing effects of D_1 and D_2 dopamine receptor stimulation in the dorsal caudate putamen. Neuroscience 19:1109–1117; 1986.
24. Godbout, R.; Mantz, J.; Pirot, S.; Glowinski, J; Thierry, A. M. Inhibitory influence of the mesocortical dopaminergic neurons on their target cells: Electrophysiological and pharmacological characterization. J. Pharmacol. Exp. Ther. 258:728–738; 1991.
25. Grant, B. F.; Harford, T. C. Concurrent and simultaneous use of alcohol with cocaine: Results of a national survey. Drug Alcohol Depend. 25:97–104; 1990.
26. Harris, R. A.; Brodie, M. S.; Dunwiddie, T. V. Possible substrates of ethanol reinforcement: GABA and dopamine. Ann. NY Acad. Sci. 654:61–69; 1992.
27. Hearn, W. L.; Flynn, D. D.; Hime, G. W.; Rose, S.; Cofino, J. C.; Mantero-Antienza, E.; Wetli, C. V.; Mash, D. C. Cocaethylene: A unique cocaine metabolite displays high affinity for the dopamine transporter. J. Neurochem. 56:698–701; 1991.
28. Hunt, W. The effect of ethanol on GABAergic transmission. Neurosci. Behav. Dev. 7:87–95; 1983.
29. Hunt, W. A. Neuroscience research: How has it contributed to our understanding of alcohol abuse and alcoholism? Alcohol. Clin. Exp. Res. 15:1055–1065; 1993.
30. Jatlow, P.; Elsworth, J. D.; Bradberry, C. W.; Winger, G.; Taylor, J. R.; Russell, R.; Roth, R. H. Cocaethylene: A neuropharmacologically active metabolite associated with concurrent cocaine-ethanol ingestion. Life Sci. 48:1787–1794; 1991.
31. Jatlow, P.; Hearn, W. L.; Elsworth, J. D.; Roth, R. H.; Bradberry, C. W.; Taylor, J. R. Cocaethylene inhibits uptake of dopamine and can reach high plasma concentrations following combined cocaine and ethanol use. National Institute Drug Abuse research monograph series 105. Washington, DC: U.S. Government Printing Office; 1991:572–573.
32. Korneyev, A. Y.; Costa, E.; Guidotti, A. During anesthetic-induced activation of hypothalamic pituitary adrenal axis, blood-borne steroids fail to contribute to the anesthetic effect. Neuroendocrinology 57:559–565; 1993.
33. Landry, M. J. An overview of cocaethylene, an alcohol-derived, psychoactive, cocaine metabolite. J. Psychoact. Drugs 24:273–276; 1992.
34. Lau, C. E. Determination of cocaethylene, cocaine and their metabolites in rat serum microsamples by high-performance liquid-chromatography, and its application to pharmacokinetic studies in rodents. J. Chromatogr. 582:167–172; 1992.
35. Lidsky, T. I.; Banerjee, S. P. Acute administration of haloperidol enhances dopaminergic transmission. J. Pharmacol. Exp. Ther. 265:1193–1198; 1993.
36. Lundquist, F. The determination of ethyl alcohol in blood and tissues. Methods Biochem. Anal. 7:217–248; 1959.
37. McCance-Katz, E. F.; Price, L. H.; McDougle, C. J.; Kosten, J. R.; Black, J. E.; Jatlow, P. I. Concurrent cocaine-ethanol Ingestion in humans: Pharmacology, physiology, behavior, and the role of cocaethylene. Psychopharmacology 111:39–46; 1993.
38. Mehta, A. K.; Ticku, M. K. Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves gamma-aminobutyric acid gated chloride channels. J. Pharmacol. Exp. Ther. 246:558–564; 1988.
39. Mogil, J. S.; Marek, P.; Yirmiya, R.; Balian, H.; Sadowski, B.; Taylor, A. N.; Liebeskind, J. C. Antagonism of the non-opioid component of ethanol-induced analgesia by the NMDA receptor antagonist MK-801. Brain Res. 602:126–130; 1993.
40. Moises, H. C.; Woodward, D. J. Potentiation of GABA inhibitory action in cerebellum by locus coeruleus stimulation. Brain Res. 182:327–344; 1980.
41. Nawak, J. Z.; Arbilla, S.; Dahl, S. G.; Langer, S. Z. Antagonism of presynaptic dopamine receptors by phenothiazine drug metabolites. Life Sci. 46:327–336; 1990.
42. Pedigo, N. W.; Dewey, W. L.; Harris, L. S. Determination and

characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. J. Pharmacol. Exp. Ther. 193:845–852; 1975.
43. Penit-Soria, J.; Audinat, E.; Crepei, F. Excitation of rat prefrontal cortical neurons by dopamine: An in vivo electrophysiological study. Brain Res. 425:263–274; 1987.
44. Petersen, R. C.; Stillman, R. C. (eds.) National Institute on Drug Abuse research monograph series 13. Washington, DC: U.S. Government Printing Office; 1977.
45. Piercey, M. F.; Lum, J. T.; Hoffmann, W. E.; Carlsson, A.; Jung E.; Svensson, K. Antagonism of cocaine's pharmacological effects by dopaminergic antagonists, (+)-AJ76 and (+)-UH232. Brain Res. 588:217–222; 1992.
46. Pilo, A.; Vetulani, J.; Nomura, S. J. An in vivo Procedure for the selective inactivation of rat brain cerebral cortical α-adrenoceptors using N-ethoxycarboxyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Brain Res. 493:8–13; 1989.
47. Pirot, S.; Godbout, R.; Mantz, J.; Tassin, J. P.; Glowinski, J.; Thierry, A. M. Inhibitory effects of ventral tegmental area stimulation on the activity of prefrontal cortical neurons: Evidence for the involvement of both dopaminergic and GABAergic components. Neuroscience 49:857–865; 1992.
48. Pohorecky, L. A.; Shah, P. Ethanol-induced analgesia. Life Sci. 41:1289–1295; 1987.
49. Qin, Z.-P.; Zhang, S.-P.; Weiss, B. Dopaminergic and glutamatergic blocking drugs differentially regulate glutamic acid decarboxylase mRNA in mouse brain. Mol. Brain Res. 21:293–302; 1994.
50. Randall, T. Cocaine, alcohol mix in body to form even longer lasting, more lethal drug. JAMA 267:1043–1044; 1992.
51. Reid, M. S.; O'Connor, W. T.; Herrera-Marschitz, M.; Ungerstedt, U. The effects of intranigral GABA and dynorphin A injections on striatal dopamine and GABA release: Evidence that dopamine provides inhibitory regulation of striatal GABA neuron via D_2 receptors. Brain Res. 519:255–260; 1990.
52. Retaux, S.; Besson, M. J.; Penit-Soria, J. D_2 Dopaminergic receptor activation enhances the spontaneous release of ^{3}H-GABA in the prefrontal cortex of rats, in vitro. The facilitation role of D_1 dopaminergic receptors. Comptes Rendus de l'Academie des Sciences 311:295–300; 1990.
53. Retaux, S.; Besson, M. J.; Penit-Soria, J. Opposing effects of dopamine D_2 receptor stimulation on the spontaneous and the electrically evoked release of [^{3}H] GABA on rat prefrontal cortex. Neuroscience 42:31–71; 1991.
54. Retaux, S.; Besson, M. J.; Penit-Soria, J. Synergism between D_1 and D_2 dopamine receptors in the inhibit of the evoked release of [^{3}H] GABA on rat prefrontal cortex. Neuroscience 43:323–329; 1991.
55. Ritz, M. C.; Cone, E. J.; Kuhar, M. J. Cocaine inhibition of ligand binding at dopamine, norepinephrine, and serotonin transporters: A structure-activity study. Life Sci. 46:635–645; 1990.
56. Sanna, E.; Concas, A.; Serra, M.; Biggio, G. In vivo administration of ethanol enhances the function of the gamma-aminobutyric acid-dependent chloride channel in the rat cerebral cortex. J. Neurochem. 54:696–698; 1990.
57. Santiago, M.; Machado, A.; Cano, J. Regulation of prefrontal cortical dopamine release by dopamine receptor agonists and antagonists. Eur. J. Pharmacol. 239:83–91; 1993.
58. Sawaguchi, T.; Matsumura, M.; Kubota, K. Effects of dopamine antagonists on neuronal activity related to a delayed response task in monkey prefrontal cortex. J. Neurophysiol. 63:1401–1412; 1990.
59. Suzdak, D. P.; Glowa, J. R.; Crowley, J. N.; Schwartz, R. D.; Skolnick, P.; Paul, S. M. A selective imadazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243–1247; 1986.
60. Tabakoff, B.; Hoffman, P. L. Recent advances in alcohol research—1990. Alcohol Alcoholism (Suppl 1):1–7; 1991.
61. Ticku, M. K. Ethanol interaction at the gamma-aminobutyric acid receptor complex. Ann. NY Acad. Sci. 625:136–144; 1991.
62. Ticku, M. K.; Mhatre, M.; Mehta, A. K. Modulation of GABAergic transmission by ethanol. Adv. Biochem. Psychopharmacol. 47:255–268; 1992.
63. Waterhouse, B. D.; Stowe, Z. N.; Jimenez-Revera, C. A.; Sessler, F. M.; Woodward, D. J. Cocaine actions in central noradrenergic circuit: Enhancement of cerebellar Purkinje neuron responses to ionotophoretically applied GABA. Brain Res. 546:297–309; 1991.
64. Weiss, D. S. Actions of ethanol on γ-aminobutyric acid-activated chloride channels. JAMA 79:473–475; 1992.
65. Weiss, F.; Hud, Y. L; Ungerstedt, U.; Markov, A.; Plotsky, P. M.; Koob, G. F. Neurochemical correlates of cocaine and ethanol self-administration. Ann. NY Acad. Sci. 654:220–241; 1992.
66. Weiss, F.; Lorang, M. T.; Bloom, F. E.; Koob, G. F. Oral alcohol self-administration stimulates dopamine release in rat nucleus accumbens: Genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267:250–258; 1993.
67. Williams, K.; Ferko, A. P.; Barbieri, E. J.; DiGregorio, G. J. Glycine enhances the central depressant properties of ethanol in mice. Pharmacol. Biochem. Behav. 50:199–205; 1995.
68. Wozniak, K. M.; Pert, A.; Mele, A.; Linnoila, M. Focal application of alcohols elevates extracellular dopamine in rat brain: A microdialysis study. Brain Res. 519:31–40; 1991.
69. Yamada, S.; Yokoo, H.; Nishi, S. Modulation of (−)-sulpiride-induced increase in electrically evoked release of dopamine from rat striatal slices. J. Pharm. Pharmacol. 45:479–481; 1993.
70. Yirmiya, R.; Taylor, A. N. Genetic differences in opiate receptor concentration and sensitivity to ethanol's effects. Pharmacol. Biochem. Behav. 33:793–796; 1989.