ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 971-977, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02146-9

Effects of Ventral Tegmental Microinjections of the $GABA_A$ Agonist Muscimol on Self-Administration of Ethanol and Sucrose

CLYDE W. HODGE,[1] MIKI HARAGUCHI,[2] ANN M. CHAPPELLE AND HERMAN H. SAMSON

Department of Physiology and Pharmacology, The Bowman Gray School of Medicine, Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157-1083

Received 3 May 1995; Revised 18 August 1995; Accepted 4 September 1995

HODGE, C. W., M. HARAGUCHI, A. M. CHAPPELLE AND H. H. SAMSON. *Effects of ventral tegmental microinjections of the $GABA_A$ agonist muscimol on self-administration of ethanol and sucrose.* PHARMACOL BIOCHEM BEHAV **53**(4) 971-977, 1996.—Two groups of Long-Evans rats were trained to lever press on a fixed-ratio 4 (FR4) schedule of reinforcement with ethanol (10% v/v) or sucrose (75% w/v) presented as the reinforcer. After implantation of guide cannulae aimed at the ventral tegmental area (VTA), weekly bilateral injections of muscimol (10, 30, and 100 ng) were tested. During control conditions, response patterns for both groups were characterized by high rates that began shortly after the start of the session and terminated after approximately 10 min. Muscimol (10 ng) administration in the VTA increased the number of sucrose- but had no effect on the total number of ethanol-reinforced responses. Muscimol (30 ng) shifted the response patterns of both groups from high initial rates with early termination to slow initial rates with delayed termination, suggesting the possibility of nonspecific locomotor effects. These data suggest that ethanol- and sucrose-reinforced response totals are differentially sensitive to changes in GABAergic transmission in the VTA. The similar muscimol-induced changes in response patterns with the two reinforcers supports the hypothesis that $GABA_A$ receptors in the VTA are involved similarly in the maintenance of ethanol- and sucrose-reinforced responding. However, the failure of muscimol to increase ethanol-reinforced responding suggests that GABAergic systems in other brain regions may also be involved in the changes in ethanol intake seen following peripheral administration of GABAmimetic drugs.

Ethanol Sucrose Self-administration Reinforcement Muscimol $GABA_A$ Ventral tegmental area (VTA) Rats

BRAIN γ-aminobutyric acid (GABA) is hypothesized to mediate some of the behavioral and pharmacological effects of ethanol (5,13,20,31,38). For example, low concentrations of ethanol enhance $GABA_A$ Cl channel flux (23). Muscimol stimulation of $^{36}Cl^-$ uptake, in cortical and cerebellar membranes, is potentiated by ethanol in rats selectively bred for high acute sensitivity (HAS) to the hypnotic effects of ethanol, but not in low acute sensitive (LAS) rats (1). Behavioral studies show that GABA mimetic drugs potentiate some of the intoxicating effects of ethanol (21), which are reversed by $GABA_A$ antagonists (22,36).

With regard to ethanol self-administration, GABAmimetic drugs have been shown to decrease voluntary ethanol intake (3,7,8). The partial inverse benzodiazepine agonist Ro 15-4513 dose dependently decreases ethanol self-administration in an operant paradigm (30,34) and reverses some of the other behavioral effects of ethanol (36). However, pentobarbital and diazepam have been shown to increase ethanol intake (6,24). More recently, however, peripheral administration of the $GABA_A$ agonist THIP (16.0 mg/kg, IP) as shown to increase voluntary 24-h consumption of ethanol (2.0–10%) during acquisition by increasing the size, duration, and frequency of drinking bouts (4,35). These data suggest that GABA receptors are involved in the neurobehavioral regulation of ethanol intake and reinforcement. However, due to the ubiquitous CNS distribution of GABA receptors, peripheral administra-

[1] To whom requests for reprints should be addressed.
[2] Present address: Syntex, 3401 Hillview Ave., P.O. Box 10850, R2-101, Palo Alto, CA 94303.

tion studies do not reveal specific neural pathways that might be involved in ethanol reinforcement.

Recent research, utilizing direct central nervous system (CNS) administration, in combination with operant models of self-administration, has revealed that mesolimbic systems are involved in ethanol reinforcement [see (32) for a review], as well as reinforcement by other drugs of abuse including the opiates and psychomotor stimulants (19,20). Central administration of drugs that enhance DA transmission in the nucleus accumbens increase ethanol-reinforced responding, whereas decreasing nucleus accumbens DA transmission reduces responding. For example, administration of the nonspecific agonist *d*-amphetamine (4.0–20.0 μg) or the more selective D_2/D_3 agonist quinpirole (1.0–10 μg) increases the number of ethanol (10% v/v) reinforced lever presses in free-feeding and -drinking rats (11,32,33). Accordingly, nucleus accumbens administration of the D_2 antagonist raclopride (32), or ventral tegmental (VTA) injections of quinpirole (10), decrease ethanol reinforced lever pressing.

Infusion of low concentrations of the $GABA_A$ agonist muscimol in the A10 region of the VTA has been shown to dose dependently increase dopamine levels in the nucleus accumbens and locomotor activity, both of which were inhibited by haloperidol (15,25). Higher doses of muscimol (0.5 μg) decrease dopamine levels in the nucleus accumbens (9). Thus, $GABA_A$ agonist-induced prolongation of ethanol intake (4) may be partly due to dopamine activation in the terminal fields of the nucleus accumbens. Support for this conclusion comes from anatomical data showing a 50% greater density of GABAergic terminals in the nucleus accumbens of alcohol preferring (P) rats as compared to the ethanol nonpreferring (NP) rats (14). Furthermore, microinjections of muscimol in the VTA induce intense feeding and drinking (18). Thus, the mesoaccumbens pathways represent potential sites of action whereby GABA transmission may modulate the reinforcement function of ethanol.

The present study was designed to test the role of GABAergic transmission in the VTA in ethanol reinforcement. The $GABA_A$ agonist muscimol was administered centrally in the VTA of two groups of animals responding on fixed-ratio 4 (FR4) schedules of reinforcement. One group received ethanol (10% v/v) as the reinforcer and the other group received sucrose (75% w/v) reinforcement as a control to test the selectivity of the $GABA_A$ agonist on ethanol reinforcement.

METHODS

Animals

Eighteen male Long-Evans rats, weighing between 300–350 g at the start of the experiment, were housed individually with food (Harlan TKLAD 8604, Madison, WI) always available. Water access was restricted to 1 h per day during the first 3 days of lever-press training, but was available continuously thereafter. The colony room was maintained on a 12 L : 12 D cycle, with the lights on at 0700 h. Temperature and humidity were maintained within NIH guidelines. All experimental sessions were run during the light portion of the cycle.

Apparatus

Operant sessions were conducted in Plexiglas chambers (27 × 37 × 21 cm) located in sound-attenuating cubicles. Exhaust fans helped to mask external noise. The left wall of each chamber was equipped with a liquid dispenser (Ralph Gerbrands Corp., model B-LH, Arlington, MA) and two response levers. Responses on a lever located to the left of the liquid dispenser activated a dipper that presented fluid (0.1 ml) for 3 s during each operation. Apple microcomputers controlled the sessions and collected data. Microinjections were conducted through cannulae (33 gauge stainless steel tubing coupled to 26 gauge tubing) that were connected with PE-20 plastic tubing to two 1.0 μl syringes (Hamilton, Reno, NV) mounted on a microinfusion pump (Harvard Apparatus, Model 22). Pumps were programmed to deliver 0.5 μl/min/syringe.

Procedure

After 1 week of daily handling and adaptation to individual housing conditions, fluid access was restricted to 1 h per day and the rats were trained to lever press by reinforcing successive approximations with sucrose (20% w/v) presented as the reinforcer. When responding occurred reliably on a fixed-ratio 1 (FR1) schedule, the response requirement was gradually increased to a final value of FR4. The rats were then divided randomly into two groups of $n = 9$ each. One group was trained to orally self-administer ethanol (10% v/v) using a sucrose substitution procedure (29). Briefly, the sucrose concentration was decreased gradually to 2% and then the ethanol concentration was slowly increased from 2 to 10% over a period of approximately 3 weeks. At this time, the sucrose was removed from the solution, and 10% ethanol maintained lever pressing. The other group of rats was trained to lever press with 75% sucrose as the reinforcer, by changing the concentration from 20 to 50% and then to 75% w/v, to match response rate and pattern with the ethanol reinforcement group. Operant sessions were 30 min in duration and were conducted 5 days per week (M–F).

When responding was stable for a minimum of 2 weeks, bilateral stainless steel guide cannulae (26 gauge) were surgically implanted. Daily sessions were resumed following a 1-week recovery period. Microinjections began after approximately 4 weeks when response rates and patterns stabilized and matched presurgery values. Microinjections were conducted once per week on Thursday, with sham control injections on Wednesday. Data from Tuesday were used as no-injection controls.

Following completion of the injections, the rats were sacrificed and their brains were removed for histological verification of injection sites. Data were only used from those animals whose injection sites were determined to be bilateral in the VTA.

Surgery

Rats were anesthetized with Equithesin (3.0 ml/kg, IP) and placed in a stereotaxic device (David Kopf Instruments, model 1204 with rodent adapter) with the incisor bar −3.3 mm below the interaural line. Cannulae were aimed to terminate 1 mm dorsal to the VTA and secured to the skull with cranial screws and dental cement. Removable wire obturators (33 gauge) were inserted the full length of the guide cannulae to limit obstruction. Plastic cylinders were affixed around the cannulae to prevent disruption. Stereotaxic coordinates for the VTA were +3.7 mm from the interaural line, +1.6 mm lateral to the midline, and −7.0 mm ventral to the cortical surface at 10° lateral to the vertical plane (27).

Microinjection Procedure

Unanesthetized animals were placed in round plastic tubs (30 cm in diameter by 14 cm deep) to minimize movement.

The obturators were removed and the cannulae area was swabbed with sterile physiological saline. Injectors (33 gauge stainless steel hypodermic tubing) were then inserted to a depth of 1 mm beyond the end of the guide cannulae. Muscimol (10, 30, and 100 ng) or ACSF vehicle was then delivered bilaterally in a volume of 0.5 μl/side over a 1 min period. The injectors were removed following an additional 30-s diffusion period and new sterile obturators were inserted. Previous studies have shown that this injection protocol results in minimal gliosis or tissue perturbation beyond the injector track (33). Each rat received a total of four injections. The order of injections was determined randomly and occurred in the following sequence: ACSF, 30, 10, and 100 ng. Sham control injections were conducted each week to test for procedural effects on behavior. Sham injections were identical to actual injections except that the injectors were the same length as the guide cannulae to prevent tissue penetration, and although the pump motor was operated, the syringes were not driven. Operant sessions began 10 min after injections. During the 2 weeks prior to drug testing, the animals were handled and placed in the plastic tubs to minimize the effects of procedural changes on subsequent drug effects. The data from these sessions revealed no effect and were not used in the analysis.

Drugs

The $GABA_A$ agonist muscimol (10, 30, and 100 ng/μl) (RBI, Natick, MA) was dissolved in artificial cerebrospinal fluid (ACSF) and shaken on a mechanical shaker. New drug solutions were prepared and sterile filtered (Millipore 0.2 μm filters, Gellman) immediately prior to each injection session. Muscimol was administered bilaterally in a total volume of 1.0 μl (0.5 μl/side).

Histology

Following completion of the muscimol dose-response curves, each animal was given a lethal dose of sodium pentobarbital and transcardially perfused with a phosphate-buffered saline solution (pH 7.4) followed by 10% formalin. Their brains were removed and stored in 10% formalin 10 days. Brains were then frozen and cut into 60-μm sections and stained with cresyl violet to determine cannula placement. The data from rats with injection sites located outside the VTA (27) were not used in the analysis.

Data Analysis and Statistics

The time at which lever presses and dipper presentations occurred were recorded by the computer. The following descriptive measures were then derived: latency to the first response, total number of responses, time course of total responses (T), total response rate, time course of the first half of the responses ($T_{1/2}$), and response rate during the first half of the responses ($Rate_{1/2}$). The rationale for selecting these behavioral measures was that if GABAergic transmission is involved in the onset of responding, then response latency would be altered. Changes in response rate may indicate involvement in the maintenace of responding. Time course and $T_{1/2}$ may indicate changes in the pattern of responding. Temporal response patterns were displayed by computer-generated cumulative response records. Within each reinforcement group, the effects of muscimol were analyzed by two-way repeated measures analysis of variance with three factors for injection type (no injection, sham, and drug) and four factors for dose of muscimol (0–100 ng). Planned comparisons between drug and corresponding control conditions were conducted using the Student-Newman-Keuls procedure.

RESULTS

Histological examination showed that bilateral injections were in the VTA except for four animals in the sucrose group and one animal in the ethanol group. Data are presented only for those animals with bilateral placement: ethanol (n = 8) and sucrose (n = 5). Due to computer malfunction, data from the 100 ng dose of muscimol are not available for the sucrose group.

Ethanol Reinforcement

Muscimol produced no statistically significant effects on the total number of ethanol reinforced lever presses (Fig. 1, top). Response latency, total response time, total rate, and ethanol intake (Table 1) also showed no significant changes. However, the response measures that described responding during the first one-half of the bout revealed significant effects. Repeated measures ANOVA showed that $T_{1/2}$ was significantly increased as a function of dose, $F(3, 21) = 6.0$, $p < 0.01$, and injection type, $F(2, 14) = 13.6$, $p < 0.001$. The dose by injection type interaction on the $T_{1/2}$ measure was also significant, $F(6, 41) = 6.0$, $p < 0.001$. Post hoc analysis showed that both the 30 and 100 ng doses resulted in significant increases in $T_{1/2}$ as compared to sham control (Fig. 1, middle). Response rate during the first half of the response bout ($Rate_{1/2}$) decreased as a function of muscimol dose, $F(3, 7) = 3.6$, $p < 0.05$, but injection type failed to show a significant different for this measure due to changes in baseline values (Fig. 1, bottom). Post hoc comparisons showed that the dose-related decreases in $Rate_{1/2}$ were due to the effects at the 30 and 100 ng doses, $p < 0.05$. Thus, muscimol increased

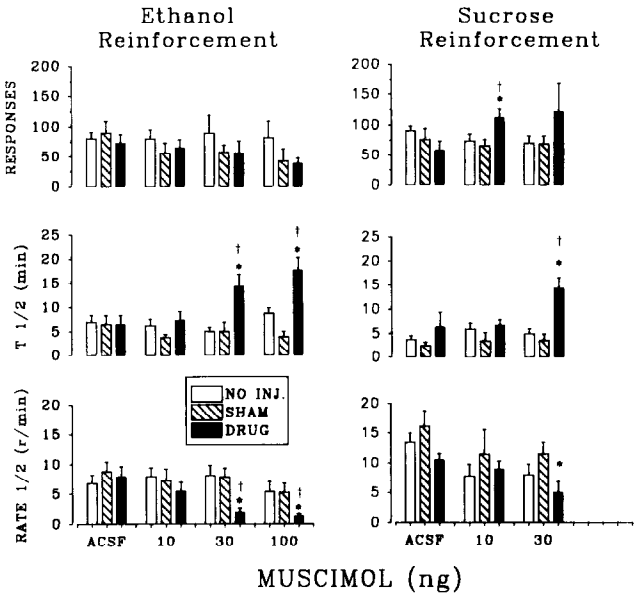

FIG. 1. Total number of responses (top), $T_{1/2}$ (middle), and $Rate_{1/2}$ (bottom) plotted as a function of muscimol dosage. Values represent mean + SEM $^{\dagger}p < 0.05$, compared to corresponding no injection (NO INJ), $^{*}p < 0.05$, compared to SHAM (Student-Newman-Keuls test).

the time required to emit the first one-half of the responses by decreasing response rate during this portion of the session.

Sucrose Reinforcement

As with ethanol reinforcement, response latency, total time, and total rate also showed no significant changes. There was no significant main effect on total number of sucrose-reinforced responses (Fig. 1, top), which was reflected in an increase in sucrose intake (Table 1). However, post hoc comparisons showed that the 10 ng dose of muscimol significantly increased the total number of sucrose-reinforced responses as compared to sham control ($p < 0.05$) (Fig. 1, middle). $T_{1/2}$ showed a statistically significant change for injection type, $F(2, 8) = 36.0$, $p < 0.001$, that was due primarily to the significant increase at the 30 ng dose of muscimol ($p < 0.05$). Repeated measures ANOVA also showed a corresponding main effect on $Rate_{1/2}$ that was related to dose, $F(2, 8) = 5.8$, $p < 0.05$, and injection type, $F(2, 8) = 7.0$, $p < 0.05$, although the interaction was not significant. Post hoc comparison showed that the 30 ng dose decreased $Rate_{1/2}$ significantly as compared to sham control ($p < 0.05$).

Cumulative Response Patterns

Cumulative response graphs were used to display local response rates and patterns during the 30-min sessions. The top four panels of Fig. 2 show representative response patterns during control conditions that are similar for both ethanol and sucrose reinforcement. In each case, responding was characterized by a high rate that began shortly after the beginning of the session and continued for approximately 8–10 min, after which response rate slowed and little or no responding occurred. This response pattern occurred regardless of the reinforcement condition. ACSF injections resulted in no changes in response pattern. The bottom four panels of Fig. 2 show the effects of microinjections of muscimol (10 and 30 ng) on the temporal distribution of responses and reinforcements. Muscimol decreased the initial high response rate at the beginning of the session and maintained this slowed rate for a longer time period with both ethanol and sucrose reinforcement. However, responding maintained by the two reinforcers was differentially sensitive. Both the 10 and 30 ng doses produced pattern changes in sucrose-reinforced responding, but this pattern change at the 10 ng dose did not continue long enough to produce a corresponding change in the $T_{1/2}$ measure (Fig. 1). Ethanol reinforced response patterns were affected by the 30 ng and 100 ng (data not shown) doses.

TABLE 1

EFFECTS OF MUSCIMOL ADMINISTRATION IN THE VTA ON ETHANOL AND SUCROSE INTAKE (g/kg)

	Intake (g/kg)	
Muscimol (ng)	Ethanol (10% v/v)	Sucrose (75% w/v)
ni	0.26 (0.03)	0.33 (0.03)
sham	0.29 (0.07)	0.29 (0.06)
0	0.24 (0.05)	0.22 (0.06)
ni	0.26 (0.05)	0.27 (0.05)
sham	0.18 (0.05)	0.27 (0.02)
10	0.21 (0.04)	0.41 (0.04)*
ni	0.30 (0.10)	0.26 (0.04)
sham	0.18 (0.04)	0.24 (0.04)
30	0.18 (0.06)	0.44 (0.16)
ni	0.31 (0.10)	
sham	0.21 (0.07)	
100	0.14 (0.03)	

Data are shown as MEAN (SEM). *Indicates significantly different from corresponding no-injection (ni) and sham injection (sham) conditions, $p < 0.05$.

DISCUSSION

The present study was designed to test the role of $GABA_A$ transmission in the VTA on ethanol and sucrose reinforced responding. Microinjections of the $GABA_A$ agonist muscimol increased the number of sucrose-reinforced lever presses and intake, but had no effect on the total number of ethanol reinforced responses or intake. Muscimol administration resulted in similar changes, however, in the temporal distribution of responses in the ethanol and sucrose reinforcement conditions. Thus, these data suggest that patterns of ethanol and sucrose reinforced responding are influenced similarly by GABAergic mechanisms in the VTA, but total ethanol reinforced responding is either not regulated by this system or exhibits less sensitivity to changes in $GABA_A$ transmission.

One plausible explanation for the differential effect of muscimol (30 ng) on ethanol and sucrose reinforced responding is that the behavioral baselines were different. Response patterns of both groups in the present study were characterized by early onset, high initial response rates, and termination of responding in less than 10-min (Fig. 2), which resulted in similar response totals between groups. This suggests that the disparate effect of muscimol on response totals between the two groups may reflect differential sensitivity of ethanol and sucrose-reinforced responding to alterations of GABAergic function within the VTA. Support for this conclusion comes from a similar study in which VTA administration of the DA D_2-like agonist quinpirole suppressed total sucrose (75%) responses per session, but required a dose 100 times greater than that needed to suppress ethanol (10%)-reinforced responding (10). These data suggest that ethanol-reinforced responding is more sensitive than sucrose-reinforced responding to changes in dopaminergic function in this system. Although the present study did not confirm whether a different dose of muscimol would have increased ethanol reinforced responding, the data suggest that responding maintained by ethanol reinforcement is less sensitive to alterations in GABAergic functions in the VTA than is responding maintained by sucrose reinforcement, or that $GABA_A$ receptor function in this brain region is not involved in the termination of ethanol reinforced responding (i.e., response totals).

The quantitative measures of response patterns during the first portion of the sessions ($T_{1/2}$ and $Rate_{1/2}$) showed that muscimol resulted in similar changes in the microstructure of responding in the two reinforcement conditions. Muscimol (30 ng, sucrose reinforcement and 30 and 100 ng, ethanol reinforcement) increased $T_{1/2}$ and decreased $Rate_{1/2}$ in a manner that corresponds with the steady prolonged response pattern shown in Fig. 2 (bottom). These changes in the response pattern were not sufficient to result in changes in response totals, but may reflect GABAergic effects on locomotor behavior in general. Conversely, the 10 ng dose of muscimol did not significantly alter any of the quantitative response pattern measures for sucrose or ethanol reinforcement. This dose increased sucrose response totals by combined, but not individu-

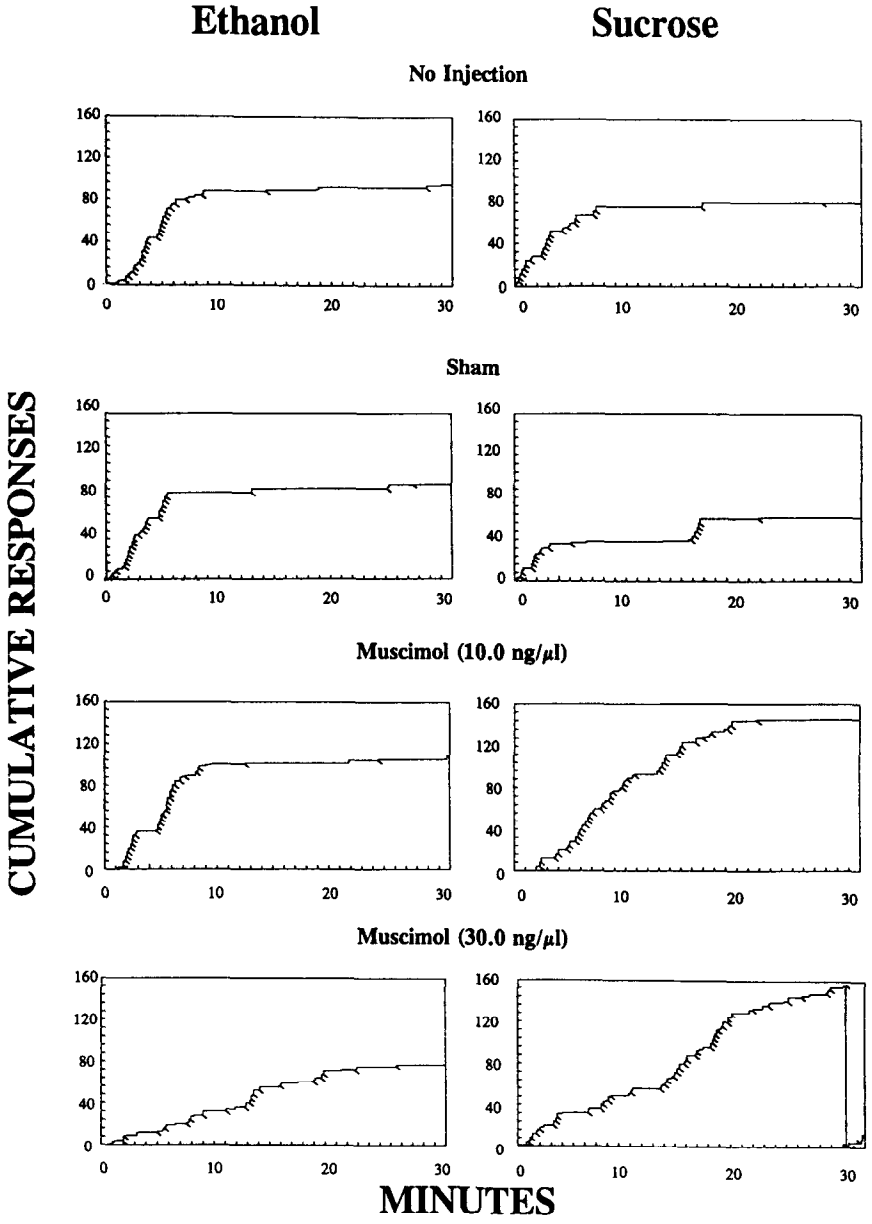

FIG. 2. Representative computer-generated cumulative response records showing temporal patterns of ethanol- (left) and sucrose-reinforced (right) responding following no injection, sham injection, and muscimol (10 and 30 mg/ml). Crosshatch marks on the graphs indicate delivery of the reinforcer. Slope of the lines indicate response rate (responses/min).

ally statistically significant, increases in response rate and total time, as shown in Fig. 2.

Muscimol (10 ng) did not produce these changes in ethanol-reinforced response patterns. In general, these data are consistent with the hypothesis that GABAergic function in the VTA influences similarly the maintenance of ethanol- and sucrose-reinforced responding. However, the failure of muscimol to increase response totals in the ethanol condition and the differential sensitivity to the response pattern altering effects at the 10 ng dose (Fig. 2) suggest that $GABA_A$ transmission in the VTA may not be involved in the termination of responding maintained by ethanol.

Increased sucrose-reinforced responding and intake observed in the present study corresponds with reports of other investigators that food and water intake are enhanced following muscimol injections in the VTA (18). It has been hypothesized (18) that muscimol-induced increases in feeding and drinking are the result of diffusion of the drug into the median raphe nucleus (MR) because injections of muscimol into the caudal VTA, which is nearest the MR, but not rostral end of the VTA, result in these effects on feeding (2). However, diffusion into the MR seems unlikely as an explanation of the present data for two reasons. First, muscimol (50-100 ng) administration in the MR has been shown to nonselectively

increase both ethanol and water drinking in a limited access paradigm (37), but the muscimol-induced increases in intake in the present study were specific to sucrose reinforcement. Second, histological examination showed that injection sites were centered in the rostral-caudal plane of the VTA (27), which would reduce the likelihood of diffusion to the MR.

One of the effects of muscimol infused into the VTA is an increase in DA levels in the nucleus accumbens (15,25). We have previously shown that increasing nucleus accumbens DA levels by direct injection of DA agonists results in increases in ethanol- and sucrose-reinforced responding by producing prolonged response patterns like those seen in the present experiment with sucrose reinforcement (10,11,33). Thus, it is possible that the increases in sucrose reinforced responding and the prolonged response patterns observed in both reinforcement conditions were due to muscimol-induced stimulation of DA release in the terminal fields of the nucleus accumbens. However, it is unclear why ethanol-reinforced responding was not increased. One plausible explanation is that ethanol may share pharmacological properties with GABAmimetic drugs (5,13,20,31,38) and the self-administered ethanol interacted additively with muscimol to produce the response pattern shown in Fig. 2. This is somewhat unlikely, however, because changes in the response pattern occurred prior to consumption of significant quantities of ethanol, as suggested by the changes in the $T_{1/2}$ and $Rate_{1/2}$ measures (Fig. 1). Another possibility is that GABAergic transmission in the mesolimbic system is involved in the subjective effects (i.e., discriminative stimulus function) of ethanol and the VTA infusion of muscimol substituted for the pharmacological effects of orally self-administered ethanol in ethanol-experienced animals, but not in the animals who had a history of sucrose reinforcement. Such a hypothesis is supported by discrimination studies that showed that the stimulus properties of peripherally administered barbiturates substitute for ethanol (16,26) and that the benzodiazepine inverse agonist Ro 15-4513 attenuates the discriminative stimulus properties of ethanol (28). Experiments that directly test the role of GABAergic systems in ethanol discrimination are needed to clarify this hypothesis.

The circuit comprising the VTA, nucleus accumbens, and pallidal areas has been implicated in the expression of locomotor behavior (2). Thus, another possible explanation of the present data is that muscimol administration in the VTA resulted in nonspecific changes in locomotor behavior that resulted in decreased response rates. However, muscimol infusions in the VTA increase locomotor activity (17). Recent evidence also indicates that GABAergic transmission in this circuit is involved in locomotor behavior in novel but not habituated environments (12). Because the operant behavior in the present study occurred under habituated conditions, it seems unlikely that the changes seen following muscimol administration were due to nonspecific locomotor effects. Direct measures of locomotor activity during operant behavior are required to confirm this possibility.

Peripheral administration studies have demonstrated GABAergic involvement in ethanol intake and reinforcement. For example, GABA agonists have been shown to decrease ethanol intake in ethanol-experienced animals (7). Alternatively, the $GABA_A$ agonist THIP increased the acquisition and maintenance of voluntary home-cage consumption of ethanol (35) by increasing the size, duration, and frequency of ethanol drinking bouts (4). Further, peripheral administration of the benzodiazepine partial inverse agonists Ro 15-4513 and FG 7142 decreased ethanol- and sucrose-reinforced responding (30). In contrast to the present study, ethanol-reinforced responding was found to be more sensitive to alteration of GABAergic transmission (30). Thus, when taken together, the differential findings from peripheral administration studies and the present findings following VTA administration of muscimol suggest that the mesolimbic system may not be the primary site of action for either increases or decreases in the reinforcement function of ethanol resultant from manipulation of GABAergic transmission. Given the finding that muscimol injections in the MR increase ethanol intake (37), the raphe nuclei may show more regionally specific involvement in ethanol reinforcement. However, studies that test the role of GABAergic transmission in the nucleus accumbens and raphe nuclei on ethanol reinforcement are required before this conclusion is warranted.

In summary, microinjections of the $GABA_A$ agonist muscimol in the VTA selectively increased the total number of sucrose- but not ethanol-reinforced responses. However, response patterns under both reinforcement conditions were similarly influenced, suggesting the possibility of locomotor effects. In each case, muscimol decreased response rates and increased the time course of response bouts in a manner similar to that previously reported following DA agonist administration in the nucleus accumbens. These data suggest that GABA transmission at $GABA_A$ receptors in the VTA is involved in maintenance of ethanol and nondrug reinforced responding. However, the VTA may not be the primary site of action whereby GABA modulates the termination of responding maintained by ethanol.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism (AA07404 and AA00142).

REFERENCES

1. Allan, A. M.; Spuhler, K. P.; Harris, R. A. Gamma-aminobutyric acid-activated chloride channels: Relationship to genetic differences in ethanol sensitivity. J. Pharmacol. Exp. Ther. 244: 866-870; 1988.
2. Arnt, J.; Scheel-Kruger, J. GABA in the ventral tegmental area: Differential regional effects in locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. Life Sci. 25:1351-1360; 1979.
3. Boismare, F.; Daoust, M.; Moore, N.; Saligaut, C.; Ljuintre, J. P.; Chretien, P.; Durlach, J. A. A homotaurine derivative reduces the voluntary intake of ethanol by rats: Are cerebral GABA receptors involved? Pharmacol. Biochem. Behav. 21:787-789; 1984.
4. Boyle, A. E.; Smith, B. R.; J.; Amit, Z. Microstructural analysis of the effects of THIP, a $GABA_A$ agonist, on voluntary ethanol intake in laboratory rats. Pharmacol. Biochem. Behav. 43:1121-1127; 1992.
5. Deitrich, R. A.; Dunwiddie, T. V.; Harris, R. A.; Erwin, V. G. Mechanisms of action of ethanol: Initial central nervous system actions. Pharmacol. Rev. 41:489-537; 1989.
6. Deutsh, J. A.; Walton, N. Y. Diazepam maintenance of alcohol preference during withdrawal. Science 198:307-309; 1977.
7. Fadda, F.; Argiolas, A.; Melis, M. R.; De Montism, G.; Gessa, G. L. Suppression of voluntary ethanol consumption in rats by gamma-butyrolactone. Life Sci. 32:1471-1477; 1983.
8. Fuchs, V.; Burbes, E.; Cooper, H. The influence of haloperidol

and amino-oxyacetic acid on etonitazene, alcohol, diazepam and harbital consumption. Drug Alcohol Depend. 14:179–186; 1984.
9. Guan, X. M.; McBride, W. J. Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. Brain Res. Bull. 23:541–547; 1989.
10. Hodge, C. W.; Haraguchi, M.; Erickson, H. L.; Samson, H. H. Microinjections of quinpirole in the ventral tegmentum decrease ethanol reinforced responding. Alcohol. Clin. Exp. Res. 17:370–375; 1993.
11. Hodge, C. W.; Haraguchi, M.; Samson, H. H. Microinjections of dopamine agonists in nucleus accumbens increase ethanol reinforced responding. Pharmacol. Biochem. Behav. 43:249–254; 1992.
12. Hooks, M. S.; Kalivas, P. W. The role of mesoaccumbens-pallidal circuitry in novelty-induced behavioral activation. Neuroscience 64:587–597; 1995.
13. Hunt, W. A. The effect of ethanol on GABAergic transmission. Neurosci. Biobehav. Rev. 7:87–95; 1982.
14. Hwang, B. H.; Lumeng, K.; Wu, J.-Y.; Li, T.-K. GABAergic neurons in nucleus accumbens: A possible role in alcohol preference. Alcohol. Clin. Exp. Res. 12:306; 1988.
15. Kalivas, P. W.; Duffy, P.; Eberhardt, H. Modulation of A_{10} dopamine neurons by γ-aminobutyric acid agonists. J. Pharmacol. Exp. Ther. 253:858–866; 1990.
16. Kline, F. S.; Young, A. M. Differential modification of pentobarbital stimulus control by *d*-amphetamine and ethanol. Pharmacol. Biochem. Behav. 24:1305–1313; 1986.
17. Klintenick, M. A.; DeWitte, P.; Kalivas, P. W. Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: An in vivo microdialysis study. Neuroscience 12:2623–2632; 1992.
18. Klitenick, M. A.; Wirtshafter, D. Comparative studies of the ingestive behaviors produced by microinjections of muscimol into the midbrain raphe nuclei of the ventral tegmental area of the rat. Life Sci. 42:775–782; 1988.
19. Koob, G. F. Drugs of abuse: Anatomy, pharmacology, and function of reward pathways. Trends Pharmacol. Sci. 13:177–184; 1992.
20. Koob, G. F.; Weiss, F. Neuropharmacology of cocaine and ethanol dependence. In: Galanter, M., ed. Recent developments in alcoholism. Vol. 10: Alcohol and cocaine: Similarities and differences. New York: Plenum Press; 1992:201–233.
21. Liljequist, S.; Engel, J. Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology (Berlin) 78:71–75; 1982.
22. Martz, A.; Deitrich, R. A.; Harris, R. A. Behavioral evidence for the involvement if gamma-aminobutyric acid in the actions of ethanol. Eur. J. Pharmacol. 89:53–62; 1983.
23. Mehta, A. K.; Ticku, M. K. Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves gamma-aminobutyric acid(A)-gated chloride channels. J. Pharmacol. Exp. Ther. 246:558–564; 1988.
24. Mudar, P. J.; LeCann, N. C.; Czirr, S. A.; Hubbell, C. L.; Reid, R. L. Methadone, pentobarbital, pimozide, and ethanol-intake. Alcohol 3:303–308; 1986.
25. Oakley, N. R.; Hayes, A. G.; Sheehan, M. J. Effect of typical and atypical neuroleptics on the behavioural consequences of activation by muscimol of the mesolimbic and nigro-striatal dopaminergic pathways. Psychopharmacology (Berlin) 105:204–208; 1991.
26. Overton, D. A. Comparison of ethanol, pentobarbital, and phenobarbital using drug vs. drug discrimination training. Psychopharmacology (Berlin) 53:195–197; 1977.
27. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1982.
28. Rees, D. C.; Balster, R. L. Attenuation of the discriminative stimulus properties of ethanol and oxazepam, but not of pentobarbital, by Ro 15-4513 in mice. J. Pharmacol. Exp. Ther. 244: 592–598; 1988.
29. Samson, H. H. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol. Clin. Exp. Res. 10:436–442; 1986.
30. Samson, H. H.; Haraguchi, M.; Tolliver, G. A.; Sadeghi, K. C. Antagonism of ethanol-reinforced behavior by the benzodiazepine inverse agonists Ro 15-4513 and FG 7142: Relation to sucrose reinforcement. Pharmacol. Biochem. Behav. 33:601–608; 1989.
31. Samson, H. H.; Harris, R. A. Neurobiology of alcohol abuse. Trends Pharmacol. Sci. 13:206–211; 1992.
32. Samson, H. H.; Hodge, C. W. Neurobehavioral regulation of ethanol intake. In: Deitrich, R. A., ed. Effects of ethanol on the central nervous system. Boca Raton, FL: CRC Press; 1996:203–226.
33. Samson, H. H.; Hodge, C. W.; Tolliver, G. T.; Haraguchi, M. Effects of dopamine agonists and antagonists on ethanol reinforced behavior: The involvement of the nucleus accumbens. Brain Res. Bull. 30:133–141; 1993.
34. Samson, H. H.; Tolliver, G. A.; Pfeffer, A. O.; Sadeghi, K. C.; Mills, F. G. Oral ethanol reinforcement in the rat: Effect of the partial inverse benzodiazepine agonist Ro 15-4513. Pharmacol. Biochem. Behav. 27:517–519; 1987.
35. Smith, B. R.; Robidoux, J.; Amit, Z. GABAergic involvement in the acquisition of voluntary ethanol intake in laboratory rats. Alcohol Alcohol. 27:227–231; 1992.
36. Suzdak, P. D.; Glowa, J. R.; Crawley, J. N.; Schwartz, R. D.; Skolnick, N.; Paul, N. A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243–1247; 1986.
37. Tomkins, D. M.; Sellers, E. M.; Fletcher, P. J. Median and dorsal raphe injections of the 5-HT_{1A} agonist, 8-OH-DPAT, and the $GABA_A$ agonist, muscimol, increase voluntary ethanol intake in Wistar rats. Neuropharmacology 33:349–359; 1993.
38. Ticku, M. K. Alcohol and GABA-Benzodiazepine receptor function. Ann. Med. 22:241–246; 1990.