

The Irreversible γ -Aminobutyric Acid (GABA) Transaminase Inhibitor γ -Vinyl-GABA Blocks Cocaine Self-Administration in Rats¹

STEPHANIE A. KUSHNER,² STEPHEN L. DEWEY,³ and CONAN KORNETSKY

Laboratory of Behavioral Pharmacology, Department of Pharmacology, Boston University School of Medicine, Boston, Massachusetts

Accepted for publication April 7, 1999 This paper is available online at <http://www.jpvet.org>

ABSTRACT

γ -Vinyl γ -aminobutyric acid (GABA) (GVG) is an irreversible inhibitor of GABA transaminase, the primary enzyme involved in GABA metabolism. Acute administration of GVG increases brain GABA levels and blocks cocaine-induced locomotor activity, cocaine-induced lowering of brain stimulation reward thresholds, and cocaine-induced conditioned place preference. To further evaluate the effects of GVG on cocaine-induced reward, we examined its effects on cocaine self-administration in male Wistar rats on fixed ratio 5 and progressive ratio schedules of reinforcement. Additionally, the effects of GVG on operant responding for a food reward were examined on the same two schedules to determine whether the effects of GVG were specific to cocaine reward or generalized to other

types of reward. GVG dose dependently decreased responding for cocaine on both schedules of reinforcement, suggesting that GVG attenuated the reward value of the cocaine. Responding for food was also decreased by GVG, suggesting that the effects of increased GABA levels induced by GVG may have a general effect on central reward systems. Data from this and other studies indicate that GVG does not induce motor impairment, decrease spontaneous locomotor activity, or induce catalepsy. Taken together, these data suggest that increases in GABAergic activity induced by GVG have an attenuating effect on centrally mediated reward systems and that the GABA system may be a useful target in the development of new therapeutic strategies for cocaine addiction.

The results of numerous studies implicate the central dopaminergic systems, primarily the mesolimbic system, as the major anatomical and neurochemical substrates that mediate the rewarding effects of various abused compounds, including the psychomotor stimulants and the opiates (for review, see Koob, 1992). There is a significant body of evidence indicating that the mesolimbic dopamine (DA) system is regulated by the inhibitory neurotransmitter γ -aminobutyric acid (GABA). The ventral tegmental area (VTA) receives GABAergic input from three different sources: the nucleus accumbens (NA), the ventral pallidum, and the GABAergic interneurons located within the VTA itself (Heimer et al., 1991; Kalivas et al., 1993). Additionally, both GABA_A and

GABA_B receptors are located in the VTA, on the interneurons and the dopaminergic cell bodies, respectively (Kalivas et al., 1990; Churchill et al., 1992).

Neurochemical evidence also indicates that GABA modulates dopaminergic activity. Infusion of the GABA_A receptor agonist muscimol into the VTA of rats results in an increase in locomotor activity that can be antagonized with either the GABA_A receptor antagonist bicuculline or the DA receptor antagonist haloperidol. This suggests that the increase in locomotor activity is mediated via GABA_A receptors and DA receptors, respectively. However, whereas intra-VTA administration of the GABA_B receptor agonist baclofen has no effect of its own on locomotor activity, it is able to block the ability of both intra-VTA muscimol and peripherally administered amphetamine and cocaine to increase locomotor activity (Kalivas et al., 1990). These data suggest that GABA_A and GABA_B receptors play different, and perhaps opposing, modulatory roles in the VTA. However, to determine the effect of an overall increase in GABAergic activity, a compound that is not specific for either receptor subtype needs to be utilized.

γ -Vinyl-GABA (GVG) is an irreversible inhibitor of GABA transaminase (GABA-T), which is the primary enzyme in-

Received for publication January 8, 1999.

¹This work was supported by National Institute on Drug Abuse Grants DA-02326 and KO5-DA-00099 to C.K. Portions of this work previously appeared in a doctoral dissertation by S.A.K. titled "The Irreversible GABA Transaminase Inhibitor γ -Vinyl GABA Attenuates Dopamine-Mediated Reward Systems in the Rat" (1998) and in *Society for Neuroscience Abstracts*, 1997.

²Corresponding author: Stephanie A. Kushner, Ph.D., New York University Medical Center, Department of Psychiatry, Room HN512, 550 First Ave., New York, NY 10016. E-mail: sk53@is7.nyu.edu.

³Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973.

ABBREVIATIONS: DA, dopamine; BSR, brain-stimulation reward; FR1, fixed ratio 1; FR5, fixed ratio 5; GABA, γ -aminobutyric acid; GABA-T, γ -aminobutyric acid transaminase; GVG, γ -vinyl-GABA; NA, nucleus accumbens; PR, progressive ratio; VTA, ventral tegmental area.

volved in GABA metabolism. In vitro studies have demonstrated that GVG significantly reduces GABA-T activity without affecting the activity of glutamic acid decarboxylase, the primary enzyme involved in GABA synthesis (Lippert et al., 1977). Acute administration of GVG to mice results in a significant increase in brain GABA levels (Jung et al., 1977), and it has been demonstrated that the "extra" GABA is stored within the nerve terminal and is only released during synaptic transmission (Mattson et al., 1995). Therefore, GVG is a compound that can be used to determine effects of an overall increase in GABAergic activity.

It has been demonstrated that GVG-induced increases in GABAergic activity result in a decrease in extracellular DA in the caudate-putamen of rats (Morgan and Dewey, 1998), inhibit cocaine-induced increases in extracellular DA in the caudate-putamen and NA as measured by in vivo microdialysis, and inhibit cocaine-induced increases in locomotor activity (Dewey et al., 1997). Positron emission tomography studies in primates have provided additional evidence demonstrating that GVG inhibits cocaine-induced increases in extracellular striatal DA (Dewey et al., 1998). Behavioral studies have shown that GVG dose dependently increases brain-stimulation reward (BSR) thresholds and blocks cocaine-induced lowering of BSR thresholds without any effect on locomotor activity as measured by response latencies (Kushner et al., 1997). Additionally, it has been demonstrated that GVG abolishes both the acquisition and expression of cocaine-induced conditioned place preference without affecting food-induced conditioned place preference, locomotor activity, or inducing catalepsy (Dewey et al., 1998). Collectively, these data suggest that GVG-induced increases in GABAergic activity attenuate the rewarding properties of cocaine, possibly by inhibiting cocaine-induced increases in extracellular DA.

To further evaluate the effects of increased GABAergic activity on the rewarding properties of cocaine, the effects of acute administration of GVG were determined in rats trained to self-administer cocaine on a fixed ratio 5 (FR5) and a progressive ratio (PR) schedule. The specificity of GVG on the rewarding properties of cocaine were examined by acute administration of GVG to rats responding for a food reward on each reinforcement schedule. The food experiments were also used to determine any possible motor effects of GVG in the operant paradigms.

Materials and Methods

Subjects. In the self-administration experiments, 14 male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) were used, weighing 325 to 350 g at the time of surgery and housed individually in stainless steel hanging cages with water available ad libitum (except during experimental sessions) and food available to maintain animals at 85% body weight throughout the course of the experiment. The colony room was maintained at a constant temperature with a 12-h light/dark cycle (lights on at 7:00 AM). All testing took place during the light phase of the cycle. An additional six male Wistar rats (Charles River), the same weight and housed under the same conditions, were used in the food experiments.

Apparatus. The apparatus (MED Associates, St. Albans, VT) used for both the food and self-administration experiments consisted of an operant chamber (28 × 22 × 21 cm) made of acrylic and stainless steel housed inside a sound attenuating chamber (63 × 44 × 58 cm). Animals could be viewed through an acrylic window

(18 × 19 cm) located in the center of the door of the sound attenuating chamber, which also contained a fan (for white noise) and a house light for illumination. One of the stainless steel walls of the operant chamber contained two retractable levers located 7.5 cm from the grid floor of the chamber. A stimulus light was located 5.5 cm above each lever, and an opening between the two levers, located 2 cm above the grid floor, contained a receptacle for sweetened food pellets (Noyes, Lancaster, NH) that were dispensed by a motorized feeder (MED Associates). The opening containing the receptacle was covered by a stainless steel plate during all self-administration experiments. The ceiling of the operant chamber had a keyhole-shaped opening that allowed free movement of a catheter/spring connector (Plastics One, Roanoke, VA). The catheter/spring connector attached to a liquid swivel, which was connected with Tygon tubing to a syringe located in an infusion pump housed on a shelf attached to the back wall of the sound attenuating chamber above the operant chamber. The operant chamber was connected to an interface (MED Associates), which was in turn connected to an IBM-compatible 386 computer that ran the software for the operant procedures (see below) and recorded the data. All equipment was powered by a 28-V power supply (MED Associates).

Surgery. The surgical procedure was adapted from the laboratory of Emmett-Oglesby (see Lane et al., 1992, for details). All animals were anesthetized with a combination of pentobarbital sodium (65 mg/kg i.p.) and chloral hydrate (80 mg/kg i.p.). Atropine sulfate (0.54 mg/kg s.c.) was given as a preanesthetic adjuvant to prevent pulmonary edema. A catheter made of Silastic tubing (0.012 inch i.d. and 0.025 inch o.d.) was inserted approximately 4.5 cm into the right external jugular vein. The other end of the silastic tubing was connected to an exit cannula made of stainless steel and plastic (Plastics One). After checking for blood flow with 1 U of heparin in sterile saline, the exit cannula was threaded s.c. from the point at which it was secured to the jugular vein through an incision made on the top of the skull. The cannula was secured to the skull with a small amount of Krazy Glue and a platform made of stainless steel screws and dental cement. Gentamicin sulfate (6.5 mg/kg) was administered i.m., and the animal was allowed to recover on a warmed heating pad before being returned to the colony room. The animal was allowed to recover for 1 full week before any experimental testing was conducted. If at any time during the course of the experiment it became clear that the animal's catheter was no longer patent, the left jugular vein was catheterized as described above, and the exit cannula was secured to the existing skull platform.

Training. Before surgery, animals were put into the operant chamber for 15 to 20 min to acclimate to the environment. They were then shaped to press the lever located to the right of the food receptacle to obtain a sweetened food pellet. Animals learned the lever-pressing task in one or two 30-min training sessions. Once reliable responding was established, an indwelling catheter was implanted in the right jugular vein (see above for surgical procedure). The animals to be used in the food experiments were trained in the same manner but did not undergo surgery. After a 1-week recovery period from surgery, the rats were trained to self-administer either 0.3 or 0.6 mg · kg⁻¹ · infusion⁻¹ of cocaine HCl dissolved in sterile saline (the opening for the food receptacle in the operant chamber was covered by a stainless steel plate). These doses of cocaine were chosen based on previous work carried out in our laboratory (Duvauchelle et al., 1998). Before each session, the pump was turned on manually to check for drug flow and to make sure that there were no air bubbles in the tubing. Once this was done, the catheter/spring connector was secured to the exit cannula, the animal was placed in the chamber, and the pump was turned on manually for 3 s to displace the heparin from the catheter. Animals were initially trained on a fixed ratio 1 (FR1) schedule of reinforcement. Depression of the right lever resulted in a 6-s illumination of the stimulus light above the lever, and a simultaneous infusion of cocaine for 6 s at a rate of 0.0178 ml/s. A 20-s time-out period, signaled by turning the house light off, followed each infusion to prevent overdose. Re-

sponses made on the lever during this time were counted but had no scheduled consequences. The end of the time-out period was signaled by illumination of the house light. Responses made on the left (inactive) lever were always counted but never had any scheduled consequences. Training sessions ended after 3 h or after a maximum of 28 reinforcements had been obtained. Once animals received 28 reinforcements for 5 consecutive days, they were advanced to either an FR5 or a PR schedule (see below). Animals in the food experiments were advanced to either an FR5 or PR schedule after lever-pressing behavior had been established.

Self-Administration Experiments. Animals on the FR5 ($n = 10$) schedule received an infusion of 0.3 or 0.6 mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ cocaine after every fifth response on the lever. Each reinforcement was followed by a 20-s time-out period signaled by turning off the house light, which was then turned on to signal the end of the period. Sessions lasted for 3 h or until a maximum of 160 reinforcements were obtained (note that animals never reached this maximum). The criterion for a stable baseline was met when the number of reinforcements per session varied by less than 20% for 3 consecutive days. After three of the animals had been challenged with all five doses of GVG (0, 100, 200, 300, and 400 mg/kg), they were advanced to a PR schedule. Additional naive animals ($n = 4$) went directly from the FR1 training schedule to the PR schedule. Under the PR schedule, the number of responses required to obtain a reinforcement increased such that one response was required to receive the first reinforcement, two responses were required to receive the next reinforcement, four to receive the next, and so on according to the following series: 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 178, 219, 268, 328, 402, 492, 603, 737, and 901 (Roberts et al., 1989). The levers in the operant chamber retracted after either 3 h or when a period of 1 h elapsed during which there was no response. The criterion for a stable baseline was met when the number of reinforcements obtained per session varied by less than 20% for 3 consecutive days.

Food Experiments. Animals ($n = 6$) on the FR5 schedule received a sweetened food pellet after every fifth response, followed by a 20-s time-out signaled as described above for the self-administration experiments. Sessions lasted 1 h. Once the rats had received all doses of GVG tested, four were advanced to a PR schedule, identical to that described above except that sessions lasted 1 h. The criterion for a stable baseline on each of the schedules was identical to that described above for the self-administration experiments.

Drugs and Treatment Regimen. The drug and treatment regimen was identical for all animals in both the self-administration and food experiments, except as noted. GVG (supplied by Stephen L. Dewey) was dissolved in physiological saline and injected i.p. at a volume of 1 ml/kg. Doses of 0 (saline vehicle), 100, 200, 300, and 400 mg/kg were administered to each animal in randomized order, and each animal received each dose twice unless the catheters of the self-administering animals lost viability before the study was completed. Animals self-administering the 0.3-mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ dose of cocaine on the FR5 schedule did not receive the 400-mg/kg dose of GVG, because preliminary data indicated that responding for this dose of cocaine was almost completely suppressed at a dose of 300 mg/kg GVG. Each animal in both the self-administration and food experiments had a single daily session as described above for 5 consecutive days, followed by 2 days off, and then another 5 consecutive daily sessions, etc. If baseline criterion were met (see above), on the 4th day, the animal received an injection of GVG or saline 3 h before the experimental session. On the 5th day, animals were tested as on days 1 through 3 (no challenge injections).

Statistical Analysis. The effects of GVG on responding on each schedule in both the self-administration and food experiments were determined by dividing the number of reinforcements obtained after GVG administration by the mean number of reinforcements obtained on the previous three baseline days. The results indicate percentage of baseline responding. Dunnett's analysis for multiple comparisons was carried out to determine whether there was a significant effect

of dose of GVG on cocaine or food intake. To evaluate any potential effects of GVG that may have remained 24 h after administration, the number of reinforcements obtained on the 5th day was divided by the mean number of reinforcements obtained on the 3 baseline days (percentage of baseline responding). Dunnett's analysis was carried out as described above to determine whether there was a significant effect of dose of GVG on cocaine or food intake 24 h after administration.

Results

As illustrated in Fig. 1, Dunnett's analysis revealed a statistically significant effect ($p < .05$) of 300 mg/kg GVG on responding for 0.3 mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ of cocaine on an FR5 schedule of reinforcement 3 h after GVG administration. Doses of 100 and 200 mg/kg did not have a statistically significant effect on intake. No statistically significant effect of any dose of GVG was seen on responding for the same dose of cocaine 24 h after GVG administration, although there was a trend for a decrease in responding as GVG dose increased.

Figure 2 illustrates the effects of GVG on responding for 0.6 mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ of cocaine on an FR5 schedule of reinforcement 3 and 24 h after GVG administration. Dunnett's analysis indicated a statistically significant effect ($p < .05$) on responding at doses of 200, 300, and 400 mg/kg GVG compared with saline vehicle 3 h after administration. Twenty-four hours after GVG administration, a statistically significant effect ($p < .05$) on responding was still observed after treatment with the 400-mg/kg dose of GVG compared with saline vehicle.

The effects of GVG on responding for 0.3 mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ of cocaine on a PR schedule 3 and 24 h after administration are indicated in Fig. 3. Dunnett's analysis indicated a statistically significant effect ($p < .05$) of 300 and 400 mg/kg GVG on cocaine intake compared with saline vehicle 3 h after GVG administration. There were no statis-

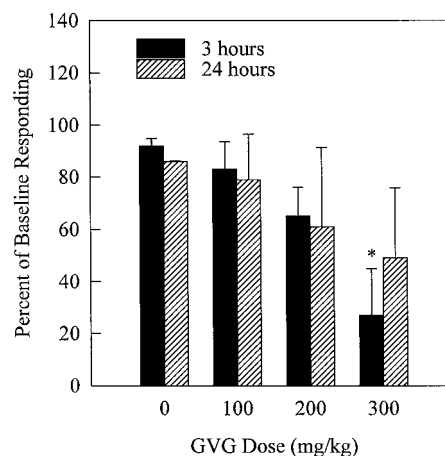


Fig. 1. Mean \pm S.E. percent change in baseline responding for 0.3 mg \cdot kg $^{-1}$ \cdot infusion $^{-1}$ cocaine on an FR5 schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration. Three hours post-GVG administration, $n = 5$ at 0, 200, and 300 mg/kg GVG, and $n = 4$ at 100 mg/kg GVG. Twenty-four hours post-GVG administration, $n = 3$ at 0 mg/kg GVG, $n = 4$ at 100 and 200 mg/kg GVG, and $n = 5$ at 300 mg/kg GVG. * $p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) as determined by Dunnett's analysis. The group baseline value (mean \pm S.D. of the number of reinforcements received in the absence of GVG or saline administration) was 52 ± 29 reinforcements.

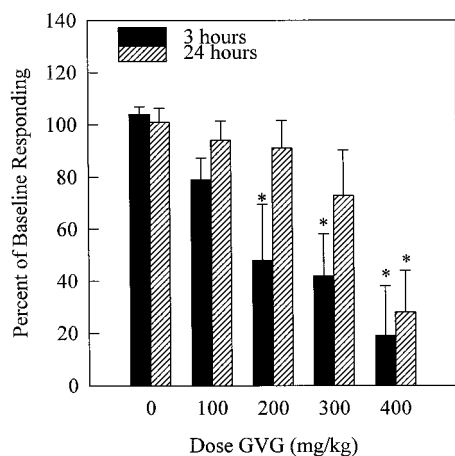


Fig. 2. Mean \pm S.E. percent change in baseline responding for 0.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{infusion}^{-1}$ cocaine on an FR5 schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration ($n = 5$ at all doses both 3 and 24 h post-GVG administration). $*p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) by Dunnett's analysis. The group baseline value (mean \pm S.D.) was 38 ± 7 reinforcements.

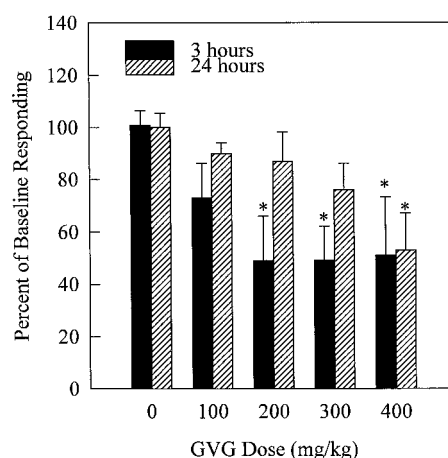


Fig. 4. Mean \pm S.E. percent change in baseline responding for 0.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{infusion}^{-1}$ cocaine on a PR schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration ($n = 5$ at all doses both 3 and 24 h post-GVG administration). $*p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) by Dunnett's analysis. The group baseline value (mean \pm S.D.) was 14 ± 4 reinforcements.

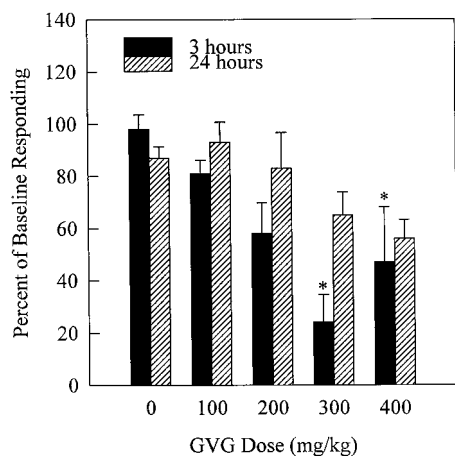


Fig. 3. Mean \pm S.E. percent change in baseline responding for 0.3 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{infusion}^{-1}$ cocaine on a PR schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration ($n = 5$ at all doses both 3 and 24 h post-GVG administration). $*p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) by Dunnett's analysis. The group baseline value (mean \pm S.D.) was 12 ± 4 reinforcements.

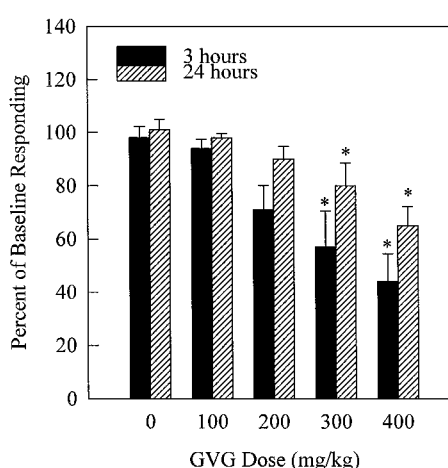


Fig. 5. Mean \pm S.E. percent change in baseline responding for food pellets on an FR5 schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration ($n = 6$ at all doses both 3 and 24 h post-GVG administration). $*p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) by Dunnett's analysis. The group baseline value (mean \pm S.D.) was 148 ± 5 reinforcements.

tically significant effects on responding for cocaine 24 h after administration of GVG at any dose tested.

Figure 4 illustrates the effects of GVG on responding for 0.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{infusion}^{-1}$ of cocaine on a PR schedule 3 and 24 h after administration of GVG. Dunnett's analysis indicated a statistically significant effect ($p < .05$) of GVG at doses of 200, 300, and 400 mg/kg. There was a statistically significant effect ($p < .05$) of 400 mg/kg GVG on responding for this dose of cocaine 24 h after administration.

The effects of GVG on responding for sweetened food pellets on an FR5 schedule are depicted in Fig. 5. Dunnett's analysis revealed a statistically significant effect ($p < .05$) of 300 and 400 mg/kg GVG on lever pressing for food compared with saline vehicle 3 h after GVG administration. Twenty-four hours after GVG administration, there was still a statistically significant effect ($p < .05$) at these two doses.

Finally, Fig. 6 shows the effects of GVG on responding for

sweetened food pellets on a PR schedule 3 and 24 h after GVG administration. There was a statistically significant effect ($p < .05$) of GVG on responding compared with saline vehicle at a dose of 300 mg/kg 3 h after administration, as determined by Dunnett's analysis. No statistically significant effect of GVG on responding for food was found 24 h after administration at any dose tested.

Discussion

Previous data (Dewey et al., 1997, 1998; Kushner et al., 1997; Morgan and Dewey, 1998) have suggested that GVG will attenuate the rewarding properties of cocaine. Therefore, the finding that GVG decreased responding for two doses of cocaine on an FR5 schedule of reinforcement was an unexpected one. According to the standard interpretation of self-administration data on fixed-ratio schedules outlined by Yonel and Wise (1975, 1976), these data suggest that GVG is

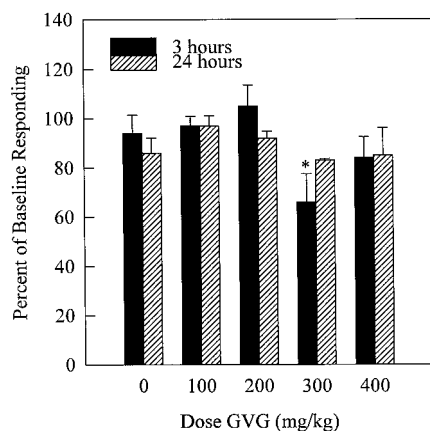


Fig. 6. Mean \pm S.E. percent change in baseline responding for food pellets on a PR schedule as a function of GVG dose 3 h (filled columns) and 24 h (hatched columns) after GVG administration ($n = 4$ at all doses both 3 and 24 h post-GVG administration). * $p < .05$ between dose of GVG and saline vehicle (0 mg/kg GVG) by Dunnett's analysis. The group baseline value (mean \pm S.D.) was 16 ± 2 reinforcements.

potentiating the rewarding effects of cocaine. However, the results of a study by Roberts and Koob (1982) employing 6-hydroxydopamine lesions of the ventral tegmental area in the rat suggest that the Yokel and Wise model may not be applicable in all cases and that a decrease in responding on a fixed-ratio schedule could indicate a decrease in the rewarding properties of the self-administered compound. The difficulty in interpreting fixed-ratio data in self-administration studies is discussed in greater detail in a review by Arnold and Roberts (1997), who suggest that a PR schedule provides data that are less ambiguous in their interpretation.

To help clarify the results obtained in the FR5 experiments, rats were trained to self-administer the same doses of cocaine on a PR schedule, and the same doses of GVG were used as a drug challenge. GVG dose dependently lowered breakpoints on the PR schedule at both doses of cocaine tested. As outlined by Arnold and Roberts (1997), this indicates that GVG had an attenuating effect on the rewarding properties of the cocaine. Additionally, these results support the interpretation of the FR5 data as indicating a decrease (rather than an increase, as the "standard" interpretation of such data would suggest) in the reward value of the cocaine.

The decrease in cocaine self-administration after GVG administration is consistent with data from other studies. GVG is able to block both cocaine-induced lowering of BSR thresholds (Kushner et al., 1997) and cocaine-induced conditioned place preference (Dewey et al., 1998), suggesting that acute administration of GVG decreases the reward value of cocaine. Like GVG, the specific GABA_B receptor agonist baclofen has been shown to lower breakpoints for cocaine self-administration on a PR schedule (Roberts et al., 1996). Similarly, baclofen administered directly into the VTA of rats results in a rightward shift of the rate-frequency curve for intracranial self-stimulation to the medial forebrain bundle, indicating an attenuating effect of GABA_B receptor activation on central DA reward systems (Willick and Kokkinidis, 1995). GVG may also decrease the reward value of other abused compounds, because it has been shown to decrease oral self-administration of morphine (Buckett, 1981) and voluntary consumption of alcohol in a strain of alcohol-prefering rats (Wegelius et al., 1993).

In our set of experiments, GVG decreased responding for a food reward on both the FR5 and PR schedules. These results suggest that the effects of GVG may not be specific to the rewarding properties of cocaine per se but that GVG may have a more general effect on centrally mediated reward systems. Note that, as discussed above in regard to the self-administration data, the decrease in breakpoint on the PR schedule supports the interpretation that the decrease in rate of responding for food on the FR5 schedule is due to a decrease in the reward value of the food.

It is possible that GVG has an effect on feeding or satiety that is independent of reward systems. However, previous studies examining the effects of GVG on food intake have yielded differing results. Huot and Palfreyman (1982) found that peripheral administration of GVG decreased whole-night food intake in non-food-deprived rats. Similarly, it was found that i.c.v. administration of the GABA-T inhibitor ethanolamine-*O*-sulfate decreased the food intake of freely fed and food-deprived rats (Olgiati et al., 1980). In contrast, Stratford and Kelley (1997) found that injections of GVG directly into the shell of the NA-stimulated feeding behavior in non-food-deprived rats. It is possible that the differing effects of GVG in these studies is the result of the different routes of administration employed.

Unlike the studies outlined above, the effects of GVG were examined not on measured intake of food presented to the animal in a dish but on operant responding for a food reward. It has been demonstrated that release of DA in the NA of rats increases during lever pressing for food on an FR5 schedule of reinforcement but does not change during free food consumption (Salamone et al., 1994). These results suggest that dopaminergic activity in the NA may be more related to the potential reward value of the food rather than to mechanisms of hunger and/or satiety and that increases in extracellular DA may be necessary to maintain the behavior. Because GVG has been shown to decrease extracellular levels of DA in the NA of awake, freely moving rats (Morgan and Dewey, 1998), it is possible that GVG administration results in a sufficient decrease in DA in the NA such that the lever-pressing behavior will not be maintained to the same degree as when GVG is not present. Therefore, it can be argued that GVG-induced decreases in the rate of food intake on the FR5 schedule and the decrease in breakpoint on the PR schedule are at least partially due to a direct effect on DA that is independent of hunger/satiety mechanisms.

The results of our food experiments also indicate that it is unlikely that the decrease in rate of response in the self-administration studies was caused by GVG-induced motor impairments. On average, rats responded at a rate of 20 to 30 reinforcements per hour when self-administering a dose of $0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{infusion}^{-1}$ of cocaine on an FR5 schedule and at an average rate of 145 reinforcements per hour when responding for food on an FR5 schedule (data not shown). At the highest dose of GVG tested (400 mg/kg), rats lever-pressed for food at a rate of 50 reinforcements h (data not shown). Although this represents a significant decrease in overall responding, it is still a greater rate than that seen during cocaine self-administration in the absence of GVG. Note that no attempt is being made to compare reinforcing efficacies of the cocaine and the food but simply to illustrate that animals are still capable of performing the operant task in the presence of GVG.

Other studies also suggest that the decreases in rate of responding in our experiments are not caused by GVG-induced motor impairments. Response latencies in a BSR procedure are unaffected by treatment with doses of GVG in the same range as used in the present study (Kushner et al., 1997). Additionally, it has been demonstrated that GVG does not affect locomotor activity or induce catalepsy in rats (Dewey et al., 1998).

Note that an overall increase in brain GABA levels will affect many brain areas in addition to the mesolimbic DA systems mentioned in the introduction. Several of these, including the prefrontal cortex and the amygdala, have been implicated in contributing to aspects of conditioning, learning, and reward. However, a full discussion of these systems would be beyond the scope of this article (for review, see Bardo, 1998).

The data outlined in our studies indicate that acute administration of GVG decreases responding for cocaine on two different schedules of reinforcement, suggesting that GVG-induced increases in GABAergic activity have an attenuating effect on the reinforcing properties of cocaine. Additionally, GVG decreases operant responding for food reinforcement, suggesting that the effects of GVG on central reward systems may generalize to other types of reinforcement. However, rates of response for food remained higher in the presence of GVG than rates of response for cocaine in the absence of GVG, suggesting that the decrease in rate of responding for cocaine was not caused by GVG-induced motor impairment but by a decrease in the reward value of the cocaine. Collectively, these results suggest that GABA has an inhibitory effect on DA-mediated reward systems and that the GABA system may be useful to target in the development of new therapeutic strategies for cocaine addiction.

References

- Arnold JM and Roberts DCS (1997) A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav* **57**:441–447.
- Bardo MT (1998) Neuropharmacological mechanisms of drug reward: Beyond dopamine in the nucleus accumbens. *Crit Rev Neurobiol* **12**:37–67.
- Buckett WR (1981) The influence of a GABA transaminase inhibitor, γ -vinyl GABA, on voluntary morphine consumption in the rat. *Psychopharmacology* **75**:214–216.
- Churchill L, Dilts RP and Kalivas PW (1992) Autoradiographic localization of gamma-aminobutyric acidA receptors within the ventral tegmental area. *Neurochem Res* **17**(1):101–106.
- Dewey SL, Chaurasia CS, Chen C, Volkow ND, Clarkson FA, Porter SP, Straughter-Moore RM, Alexoff DL, Tedeschi D, Russo NB, Fowler JS and Brodie JD (1997) GABAergic attenuation of cocaine-induced dopamine release and locomotor activity. *Synapse* **25**:393–398.
- Dewey SL, Morgan AE, Ashby CR, Horan B, Kushner SA, Logan J, Volkow ND, Fowler JS, Gardner EL and Brodie JB (1998) A novel strategy for the treatment of cocaine addiction. *Synapse* **30**:119–129.
- Duvauchelle CL, Sapoznik T and Kornetsky C (1998) The synergistic effects of combining cocaine and heroin ("speedball") using a progressive-ratio schedule of reinforcement. *Pharmacol Biochem Behav* **61**:297–302.
- Heimer L, Zahm DS, Churchill L, Kalivas PW and Wohltmann C (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience* **41**:89–125.
- Huot S and Palfreyman MG (1982) Effects of gamma-vinyl GABA on food intake of rats. *Pharmacol Biochem Behav* **17**:99–106.
- Jung MJ, Lippert B, Metcalf BW, Bohlen P and Schechter PJ (1977) γ -Vinyl GABA (4-amino-hex-5-enoic acid), a new selective inhibitor of GABA-T: Effects on brain GABA metabolism in mice. *J Neurochem* **29**:797–802.
- Kalivas PW, Churchill L and Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* **57**:1047–1060.
- Kalivas PW, Duffy P and Eberhardt H (1990) Modulation of A10 dopamine neurons by γ -aminobutyric acid agonists. *J Pharmacol Exp Ther* **253**:858–866.
- Koob GF (1992) Drugs of abuse: Anatomy, pharmacology, and function of reward pathways. *Trends Pharmacol Sci* **13**:177–184.
- Kushner SA, Dewey SL and Kornetsky C (1997) Gamma-vinyl GABA attenuates cocaine-induced lowering of brain stimulation reward thresholds. *Psychopharmacology* **133**:383–388.
- Lane JD, Pickering CL, Hooper ML, Fagan K, Tyers MB and Emmett-Oglesby MW (1992) Failure of odansetron to block the discriminative or reinforcing stimulus effects of cocaine in the rat. *Drug Alcohol Depend* **30**:151–162.
- Lippert B, Metcalf BW, Jung MJ and Casara P (1977) 4-Amino-hex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric aminotransferase in mammalian brain. *Eur J Biochem* **74**:441–445.
- Mattson RH, Petroff OA, Rothman D and Behar K (1995) Vigabatrin: Effect on brain GABA levels measured by nuclear magnetic resonance spectroscopy. *Acta Neurol Scand Suppl* **162**:27–30.
- Morgan AE and Dewey SL (1998) Effects of pharmacologic increases in brain GABA levels on cocaine-induced changes in extracellular dopamine. *Synapse* **28**:60–65.
- Olgiati VR, Netti C, Guidobono F and Pecile A (1980) The central GABAergic system and control of food intake under different experimental conditions. *Psychopharmacology* **68**:163–167.
- Roberts DCS, Andrews MM and Vickers GJ (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. *Neuropsychopharmacology* **15**:417–423.
- Roberts DCS and Koob GF (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* **17**:901–904.
- Roberts DCS, Loh EA and Vickers G (1989) Self-administration of cocaine on a progressive-ratio schedule in rats: Dose-response relationship and effect of haloperidol pretreatment. *Psychopharmacology* **97**:535–538.
- Salamone JD, Cousins MS, McCullough LD, Carriero DL and Berkowitz RJ (1994) Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. *Pharmacol Biochem Behav* **49**:25–31.
- Stratford TR and Kelley AE (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. *J Neurosci* **17**:4434–4440.
- Wegelius K, Halonen T and Korpi ER (1993) Gamma-vinyl GABA decreases voluntary alcohol consumption in alcohol-preferring AA rats. *Pharmacol Toxicol* **73**:150–152.
- Willick ML and Kokkinidis L (1995) The effects of ventral tegmental administration of GABA_A, GABA_B, and NMDA receptor agonist on medial forebrain bundle self-stimulation. *Behav Brain Res* **70**:31–36.
- Yokel RA and Wise RA (1975) Increased lever pressing for amphetamine after pimozone in rats: Implication for a dopamine theory of reward. *Science* **187**:547–549.
- Yokel RA and Wise RA (1976) Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology* **48**:311–318.

Send reprint requests to: Dr. Conan Kornetsky, Boston University School of Medicine, Department of Pharmacology, 715 Albany St., L-602, Boston, MA 02118.
