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$GABA_A$ receptor antagonism in the extended amygdala decreases ethanol self-administration in rats

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Abstract

The present experiments examined the role of the extended amygdala $GABA_A$ receptors in the regulation of ethanol consumption in rats. The areas of the extended amygdala studied included the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens. The effects of bilateral microinjections of a competitive $GABA_A$ receptor antagonist, 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinium bromide (SR 95531), on ethanol consumption were assessed in Wistar rats that were trained to respond for oral ethanol (10% w/v) in a two-lever, free-choice operant task during 30-min sessions using a saccharin fading procedure. Injections of SR 95531 into the central amygdaloid nucleus decreased ethanol responding significantly at doses of 2 and 4 ng without affecting water responding. SR 95531 injections into the bed nucleus of the stria terminalis reduced ethanol responding significantly at the 8 ng and 16 ng dose, while only the 16 ng dose produced a significant effect in the shell of the nucleus accumbens. Cumulative response patterns showed that intra-amygdaloid injections did not disrupt the initiation of responding. Injections into the bed nucleus of the stria terminalis and the nucleus accumbens, however, suppressed both ethanol and water responding at the highest SR 95531 doses during the first minutes. These findings suggest that $GABA_A$ receptors in the extended amygdala may be involved in the mediation of some aspects of ethanol reward.

Keywords: Ethanol self-administration; Amygdala, extended; $GABA_A$ receptor

1. Introduction

Both biochemical and behavioral evidence indicates that ethanol interacts with the γ-aminobutyric acid (GABA)-mediated neurotransmission in the mammalian brain. Acute ethanol has been demonstrated to influence $GABA_A$/benzodiazepine receptor function by enhancing GABA-stimulated chloride flux in several preparations at pharmacologically meaningful ethanol concentrations (Ticku et al., 1986; Allan and Harris, 1986; Suzdak et al., 1986; Mehta and Ticku, 1988). Chronic ethanol treatment alters low-affinity binding of GABA (Ticku and Burch, 1980) and reduces GABA-induced chloride flux (Morrow et al., 1988). $GABA_A$ receptor antagonists bicuculline and picrotoxin and benzodiazepine inverse agonists reduce some ethanol-induced behaviors, including motor impairment, sedation, and anxiolysis (Liljequist and Engel, 1982,1984; Hellevuo et al., 1989).

There is now increasing evidence that GABAergic mechanisms are also involved in the regulation of ethanol self-administration. Benzodiazepine inverse agonists (Ro 15-4513, Ro 19-4603) decrease ethanol drinking in nondependent rats during limited ethanol access (McBride et al., 1988; Balakleevsky et al., 1990; June et al., 1991; Wegelius et al., 1994) and suppress operant responding for oral ethanol (Samson et al., 1987; Rassnick et al., 1993). $GABA_A$/benzodiazepine receptor agonists have been shown to slightly increase ethanol intake (Boyle et al., 1993; Wegelius et al., 1994) and to facilitate the acquisition of voluntary ethanol drinking (Smith et al., 1992).

The specific neuroanatomical circuits participating in the GABAergic modulation of ethanol self-adminis-

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tration are poorly understood. A particularly interesting candidate site for the GABAergic regulation is the forebrain entity known as the extended amygdala (Alheid and Heimer, 1988). This cellular continuum stretches from the bed nucleus of the stria terminalis to the centromedial amygdala and appears to include at least in part the caudomedial and ventral aspects of the shell of the nucleus accumbens (Alheid and Heimer, 1988; Heimer and Alheid, 1991). The major components of this continuum, especially the anterolateral bed nucleus of the stria terminalis and the central amygdaloid nucleus, contain large numbers of GABAergic neurons that serve mainly the intrinsic connections within the extended amygdala (Sun and Cassell, 1993). Pharmacological and anatomical evidence points that this GABAergic system may be involved in tonic inhibition of extended amygdala neurons projecting to hypothalamic and brainstem target areas. Since the extended amygdala receives extensive afferents, including those from the cortex, thalamus, basolateral amygdala, and brainstem, this GABAergic system may have an important role in integrating information from internal and external milieux and mediating associations between conditioned and unconditioned stimuli (Gaffan and Harrison, 1987; Gallagher et al., 1990; Phillips and LeDoux, 1992).

The GABAergic system in the extended amygdala, particularly in the central amygdaloid nucleus, has been implicated in expression of emotionality, including behavioral states of fear and anxiety, as well as states associated with consummatory responses (Davis, 1992; Davis et al., 1994; Minano et al., 1992). Because stress reduction has long been considered to contribute to ethanol seeking behavior in man, it was hypothesized that the amygdala and its connections might be likely sites for a GABA-like action of ethanol to mediate ethanol reinforcement. To test this hypothesis, the present studies examined the involvement of $GABA_A$ receptors of the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens in the regulation of ethanol-reinforced behavior in nondependent rats. The effects of intracerebral microinjections of a water-soluble, potent $GABA_A$ receptor antagonist, SR 95531 (Heaulme et al., 1986), on ethanol intake were assessed in rats that were trained to self-administer ethanol (10% w/v) in a two-lever, free-choice operant task (Rassnick et al., 1993).

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River Laboratory, NY, USA) weighing 160–180 g at the beginning of the experiment served as subjects. The animals were housed in groups of two in a temperature-(20 ± 2°C) and humidity-controlled vivarium on a 12-h (10:00 p.m./10:00 a.m.) light/dark cycle and had free access to food and water. All training and testing took place during the rats' dark period, 10 a.m.–10 p.m.

2.2. Behavioral testing equipment

Behavioral training and testing was conducted in operant chambers (Coulbourn Instruments) enclosed in sound-attenuation cubicles with exhaust fans. The liquid delivery system consisted of two 35-ml plastic syringes mounted on Razel infusion pumps and connected through plastic tubing to two drinking caps (volume capacity 0.15 ml) on the center front panel of each chamber. Responses on retractable levers located 3 cm to either side of the drinking cup initiated a delivery 0.1 ml of fluid into the corresponding cup. A house light in each operant chamber was turned off to indicate the beginning of the session. Recording of responses and fluid delivery were under the control of a PC microcomputer.

2.3. Oral ethanol self-administration training

Animals were trained to orally self-administer ethanol using a modification of the training protocol described by Rassnick et al. (1993), which was based on the sucrose fading technique developed by Samson (1986). In the present study, saccharin was initially added to ethanol solutions to increase the palatability of ethanol. On the first 3 days, responses at both levers were reinforced by a delivery of 0.1 ml of 0.2% (w/v) saccharin into either drinking cup on a fixed-ratio schedule (FR-1). To increase the motivation to respond in the operant chambers, rats were water deprived for 22 h prior to these training sessions. After the initial training of 3 days, the water deprivation was discontinued, and animals had always free access to food and water through the subsequent training and testing. On training days 4–9, animals were trained to alternate responding at the left and the right lever on a one-lever task to obtain 5.0% (w/v) ethanol solution with 0.2% saccharin. This method of alternating presentation of a single lever helped minimize subsequent lever biases. Then, starting on day 10, rats were introduced to a two-lever, free-choice task in which responses on one lever produced ethanol solutions (0.1 ml), while responses on the other lever resulted in water deliveries (0.1 ml), both on a FR-1 schedule. The position of the ethanol lever alternated daily. During days 10–19, the ethanol concentration was gradually increased from 5% to 8%, and then to 10%, while the presence of 0.2% saccharin varied, and was eventually eliminated. Beginning on training day 20, animals were allowed to

respond for 10% ethanol and water for approximately 5 weeks before surgery. All training and testing was conducted during 30-min daily sessions during the animals' dark period, 5 days a week.

2.4. Surgery

Rats were anesthetized with a halothane-oxygen mixture and stereotaxically implanted with bilateral 23-gauge, 10-mm stainless-steel guide cannulae aimed at 3 mm above the injection sites. Stereotaxic coordinates were based on the atlas of Paxinos and Watson (1986). With the tooth bar positioned at −3.3 mm, the coordinates for the central nucleus of the amygdala were as follows: anteroposterior (AP) = −2.5 mm, mediolateral (ML) = 4.2 mm from bregma, and dorsoventral (DV) = −5.3 mm from the skull surface. Coordinates for the bed nucleus of the stria terminalis were: AP = −0.18 mm, ML = 1.7 mm from bregma, and DV = −4.2 from the skull surface. For the shell of the nucleus accumbens, the coordinates were: AP = 1.7 mm, ML = 1.0 mm from bregma, and DV = −5.0 mm from the skull surface. Cannulae were secured on the skull by anchor screws with dental cement and sealed with a 10-mm stylet wire. Animals were allowed to recover from surgery for a week before being reintroduced to ethanol self-administration.

2.5. Drug administration

The $GABA_A$ receptor antagonist SR 95531 (2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinium bromide, Research Biochemicals, Natick, MA, USA) was dissolved in 0.9% sodium chloride (saline) and prepared immediately before administration. Injections (0.33 μl/side) were administered bilaterally via cannula guides using 30-gauge injection needles that were connected by polyethylene tubing with 10-μl Hamilton microsyringes. The injections were delivered immediately before the session over a period of 28 s with a Razel infusion pump. The injection needles (extending 3 mm beyond the tip of the guide cannula) were left in place for an additional 30 s to allow diffusion from the needle tip. On the 2 training days prior to the first drug injection, rats were habituated to the injection procedures by removing the stylets and inserting 10-mm dummy injection needles into the guide cannulae before placing the animals into the operant chambers.

2.6. Experimental design and data analysis

A between-subjects design for drug administration into the selected brain areas was used, i.e., separate animals were used for each dose. Once stable baseline responding after surgery was established (responses were ±20% of average responding for 3 consecutive days), each animal received two intracerebral injections. The first one was a vehicle (saline) injection, after which the subjects were assigned into SR 95531 treatment groups (n = 6–8 per group) matched on the basis of their ethanol responding. As the second injection, each animal received either the zero dose (saline) or one of the SR 95531 doses after at least two baseline sessions after the first injection. Responses for ethanol and water for each injection site were analyzed with separate one-way analyses of covariance using the baseline saline performance as the covariate. Analysis of covariance was used to partial out the variability within the treatment groups and the between-group differences in the baseline means. Individual means were compared with the adjusted means test (Winer, 1971). All statistical analyses were carried out using the SAS/STAT program (SAS Institute). The patterns of responding for ethanol and water over time were presented as average cumulative responses at 1-min bins over the 30-min session after different drug treatments.

2.7. Blood ethanol concentrations

Blood samples were obtained by tail bleeding immediately after a 30-min ethanol self-administration session conducted on a baseline day after the termination

Table 1
Mean (±S.E.M) number of responses for ethanol (EtOH) and water after the first baseline saline injection in groups of rats assigned to different SR 95531 treatments for each injection site

Brain region	Response	SR 95531 dose (ng)			
		0	1	2	4
Central nucleus of amygdala	EtOH	39.7 ± 4.8	37.7 ± 7.9	38.3 ± 8.1	39.8 ± 5.3
	Water	3.7 ± 4.0	12.8 ± 2.9	11.9 ± 3.8	14.7 ± 5.5
		0	4	8	16
Bed nucleus of stria terminalis	EtOH	39.0 ± 2.5	39.0 ± 7.5	39.3 ± 11.2	41.8 ± 5.5
	Water	3.8 ± 1.0 [a]	14.2 ± 3.2	15.0 ± 2.5	12.3 ± 2.8
Nucleus accumbens shell	EtOH	36.1 ± 5.5	33.0 ± 7.9	32.0 ± 4.1	33.7 ± 4.6
	Water	11.3 ± 2.6	15.4 ± 3.3	15.8 ± 1.1	16.2 ± 3.2

Significantly different from the other water groups for the bed nucleus of the stria terminalis: [a] $P < 0.05$ (Newman-Keuls test).

of the drug injections. The blood was collected in sealed Eppendorf vials containing 4 μl of heparin (1000 USP units/ml) as an anticoagulant. Samples were centrifuged at 3200 rpm for 5 min. After extraction with trichloroacetic acid, the serum was assayed for ethanol concentration using the NAD-NADH enzyme spectrophotometric method (Sigma Chemical Co.).

2.8. Histology

After completion of the behavioral tests, animals were killed by decapitation. The brains were removed, frozen immediately, and sectioned at 50 μm intervals through the cannula tract. The slides were stained with cresyl violet and examined for injection needle tip location using a low-power light microscope and the atlas of Paxinos and Watson (1986). Animals belonging to the bed nucleus of the stria terminalis groups were anesthetized lightly and injected with a minute volume (0.3–0.5 μl) of dye immediately before decapitation in order to verify that there was no leakage from the injection site into the nearby ventricle. Rats with improper cannula placements (5% of the present subjects) were excluded from the final data analysis.

3. Results

The mean number of ethanol and water responses after the baseline saline injection for rats assigned into different SR 95531 treatment groups is shown in Table 1. There were no significant differences between the treatment groups in their baseline performance, except in the mean number of water responses for rats participating in the bed nucleus of the stria terminalis injections. The one-way analysis of variance detected a significant main effect ($F(3,20) = 3.46$; $P < 0.05$). This was due to a decrease in water intake by the vehicle group.

Injections of SR 95531 into the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens decreased responding for oral ethanol in a two-lever free-choice task (Fig. 1). The most sensitive site was the central amygdaloid nucleus. One-way analysis of covariance with the baseline as the covariate revealed that SR 95531 injections into the amygdala at total doses of 0–4 ng selectively reduced responding for ethanol ($F(3,21) = 5.61$; $P < 0.01$) without affecting water responding ($F(3,21) = 0.05$; NS). Preliminary tests indicated that SR 95531 injections into the bed nucleus of the stria terminalis and the nucleus accumbens shell at doses of 1 and 2 ng had no effects on ethanol responding. Therefore, in the final experiments, the doses of SR 95531 for injections into these sites ranged from 0 to 16 ng. At these doses, SR 95531 injections into the bed nucleus of the stria terminalis attenuated responding for ethanol ($F(3,19) = 5.20$; $P < 0.01$) but did not alter water responding ($F(3,19) = 2.44$; NS). Intra-accumbens SR 95531 injections reduced both ethanol ($F(3,21) = 4.87$; $P < 0.05$) and water responding ($F(3,21) = 3.54$; $P < 0.05$).

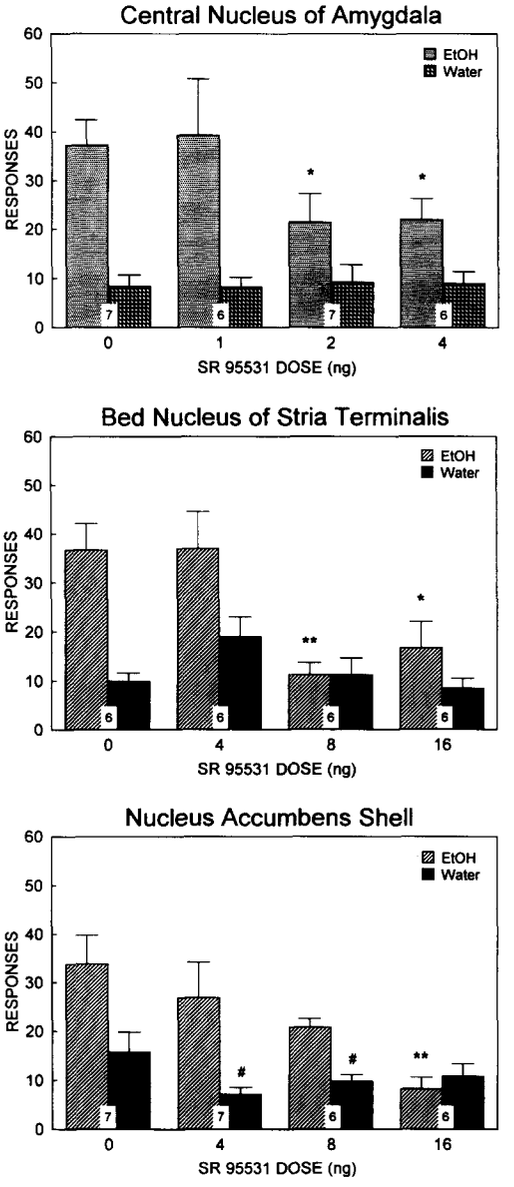

Fig. 1. The effect of SR 95531 injections into the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens on responding for ethanol (EtOH) and water. Data are expressed as the mean (±S.E.M) numbers of responses for EtOH and water during 30-min sessions for each injection site. Note the change in the abscissa scale for injections into the bed nucleus of the stria terminalis and the nucleus accumbens shell. Significance of differences from the corresponding saline control values: * $P < 0.05$, ** $P < 0.01$ for EtOH responses; # $P < 0.05$ for water responses (adjusted means test).

The cumulative ethanol responses (Fig. 2) after intracerebral saline injections showed the typical negatively accelerated pattern as rats drank most of their ethanol during the first 10 min of the session, indicating that the intracerebral injection procedures did not impair the animals' ability to respond. Intra-amygdaloid SR 95531 injections decreased ethanol responding throughout the session in a dose-dependent manner but did not alter the pattern of water responding. SR 95531 injections into the bed nucleus of the stria terminalis at 8 and 16 ng suppressed both ethanol and water responding during the first 5 min of the session. Similarly, the highest intra-accumbens dose of SR 95531 reduced the initial rates of both ethanol and water responding.

Average (±S.E.M) blood ethanol concentrations determined immediately after a 30-min baseline session were 7.8 ± 0.8 mmol (35.9 ± 3.6 mg%) for subjects participating in the intra-amygdaloid injections, 7.5 ± 0.8 mmol (34.6 ± 3.8 mg%) for subjects receiving injections into the bed nucleus of the stria terminalis, and 6.4 ± 0.6 mmol (29.3 ± 2.9 mg%) for subjects comprising the intra-accumbens group, respectively.

The locations of bilateral injection cannula tips for each injection site are presented in Fig. 3. For intra-accumbens injections, the cannula placements were veri-

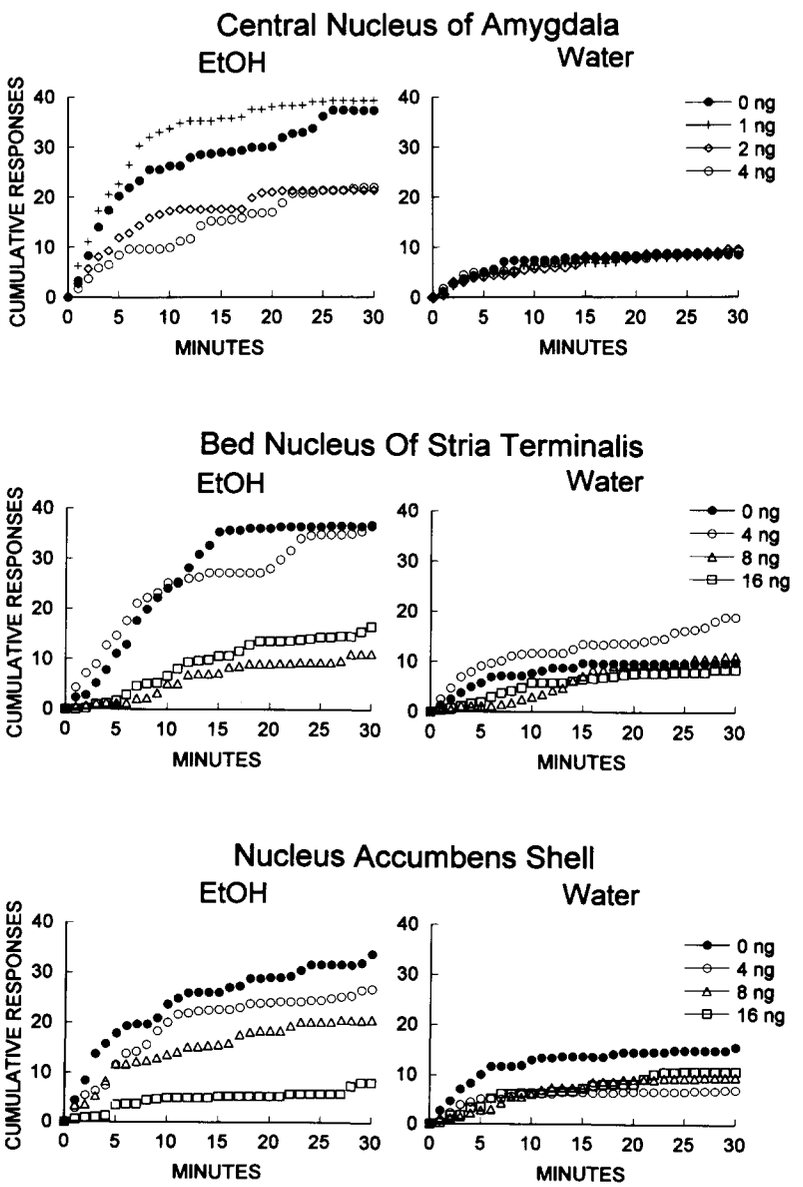

Fig. 2. Cumulative responses for ethanol (EtOH) and water after SR 95531 injections into the central amygdaloid nucleus, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens for subjects included in Fig. 1. Data are expressed as the mean number of responses during each minute of 30-min sessions for each injection site.

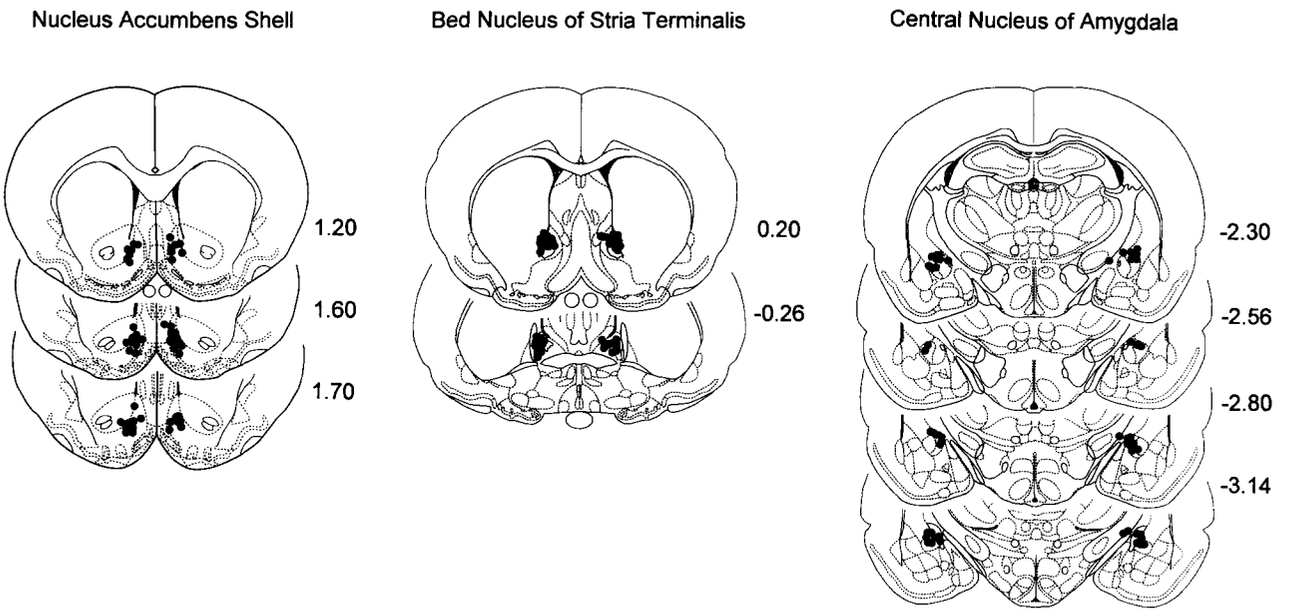

Fig. 3. Location of bilateral 30-gauge injection cannula tips for subjects included in the data analysis for each brain area. Each dot represents one rat on one side. The numbers designate the position (in mm) of coronal sections relative to bregma. Coronal sections are adapted from the rat brain atlas of Paxinos and Watson (1986).

fied within the region of from 1.20 to 1.70 mm anterior to bregma. For injections into the bed nucleus of the stria terminalis, tips were located within the region from 0.2 mm anterior to bregma to −0.26 mm posterior to bregma. Approximately half of the tips were verified between these sections. Since, however, the Paxinos and Watson atlas does not describe the section falling between these borders, the tip locations are indicated on the sections that were closest to the actual placements. For intra-amygdaloid injections, the cannula tips were verified between −2.30 to −3.14 mm posterior to bregma.

4. Discussion

The present results show that microinjections of a potent, competitive $GABA_A$ receptor antagonist, SR 95531, into the major components of the extended amygdala decrease motivated responding for oral ethanol in rats. These findings confirm and extend previous results indicating that systematically administered benzodiazepine inverse agonists (Ro 15-4513, Ro 19-4603) suppress ethanol responding in an analogous two-lever, free-choice task (Samson et al., 1987; Rassnick et al., 1993) and attenuate ethanol drinking in limited access paradigms both in fluid-deprived (McBride et al., 1988; June et al., 1991) and nondeprived animals (Balakleevsky et al., 1990; Wegelius et al., 1994). Collectively, these data suggest that compounds inhibiting $GABA_A$ receptor function have a suppressive effect on voluntary ethanol consumption. Furthermore, the present findings show that GABAergic circuits within the extended amygdala are involved in the regulation of voluntary ethanol intake in rats.

There were differences in the pattern of ethanol responding following injections of SR 95531 into distinct brain regions. Firstly, injections of the $GABA_A$ receptor antagonist into the central nucleus of the amygdala suppressed ethanol responding selectively and at lower doses than injections into the bed nucleus of the stria terminalis and the nucleus accumbens. Secondly, examination of the cumulative response patterns indicated that intra-amygdaloid SR 95531 injections did not disrupt the initiation of responding; injections into the bed nucleus of the stria terminalis and the nucleus accumbens shell, however, suppressed both ethanol and water responding at the highest SR 95531 doses during the first minutes of the session. This suggests that at least at higher doses, SR 95531 produced effects that were not specific to the regulation of ethanol intake.

The differential sensitivity of the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens in the attenuation of ethanol responding by SR 95531 could result from differences in the density of the GABAergic neurons at the site of injection. Indeed, the mediodorsal part of the nucleus accumbens contains fewer GABAergic neurons than the bed nucleus of the stria terminalis or the central amygdaloid nucleus (Sun and Cassell, 1993), and might thus be less sensitive to the

effects of GABAergic drugs. Another interpretation for the observed differences could be that these sites have functionally diverse roles in the modulation of ethanol intake.

It has been hypothesized that ethanol's tension reduction or anxiolytic properties could partially explain the positively reinforcing actions of ethanol. Ethanol injections produce anti-conflict effects in rats that can be blocked with $GABA_A$ receptor antagonists (Liljequist and Engel, 1984; Koob et al., 1986), suggesting that these effects are mediated by an enhancement of GABAergic activity. Since there are no studies on administration of GABAergic drugs directly into the central nervous system to modify ethanol's anti-conflict effects, the GABA hypothesis can be related to specific brain sites only indirectly. Intracerebral injections of benzodiazepine receptor agonists show that the amygdala, especially the anterior part of the central and basolateral nuclei, is particularly sensitive to benzodiazepines in producing an anti-conflict effect (Scheel-Krüger and Petersen, 1982; Shibata et al., 1989). The amygdaloid GABA system might mediate also ethanol's anti-conflict effects. However, it is premature to conclude that the reduction of ethanol responding in the present study produced by administration of the $GABA_A$ receptor antagonist into the central amygdaloid nucleus was caused by an attenuation of ethanol's anxiolytic properties because there is, at present, little evidence that voluntarily ingested ethanol will produce anti-conflict effects in rats.

The $GABA_A$/benzodiazepine receptor has been strongly implicated in the regulation of consummatory responses and satiety. Systemic administration of high doses of the benzodiazepine inverse agonist Ro 15-4513 abolishes preference for saccharin solution, suppresses saccharin ingestion, and reduces palatable food consumption in rats (Cooper et al., 1989). It could be argued that reductions in ethanol consumption by benzodiazepine inverse agonists are nonspecific effects mediated by satiety mechanisms. However, Ro 15-4513 decreases ethanol drinking at lower doses than intake of concomitantly available palatable food in alcohol-preferring rats (Wegelius et al., 1994). Similarly, operant responding for ethanol is reduced by lower inverse agonist doses than responding for saccharin solution (Rassnick et al., 1993). This suggests that low doses of a benzodiazepine inverse agonist might exert a selective effect on ethanol consumption.

The amygdaloid complex has previously been suggested to be involved in the mediation of consummatory responses (Nakano et al., 1986; Minano et al., 1992). The main cortical projection to the central amygdaloid nucleus comes from the insular cortex that provides this nucleus with information related to olfactory, taste, and viscerosensory modalities (Saper, 1982; Bermudez-Rattoni and McGaugh, 1991; Turner and Herkenham, 1991). It has recently been demonstrated that the afferent terminals from the rostral and middle levels of the insular cortex synapse directly to the dendritic processes of GABAergic neurons in the central nucleus of the amygdala (Sun et al., 1994). Since the rostral insular cortex contains taste-responsive neurons (Cechetto and Saper, 1987), GABAergic neurons in the central amygdaloid nucleus receive largely taste-related cortical afferents. Compared with the central amygdaloid nucleus, only a few insular cortex terminals have been seen in the bed nucleus of the stria terminalis and substantia innominata, and none of them was in contact with GABAergic neurons (Sun et al., 1994).

The taste and smell of ethanol probably provide rats with important discriminative stimuli in oral ethanol self-administration models. If the orosensory stimulus properties of ethanol are mediated by the GABA system of the central amygdaloid nucleus, intra-amygdaloid GABAergic drugs might change the processing of those stimuli and, consequently, the pattern of intake. This hypothesis is supported by the observed response patterns in the present study. Compared with the saline controls, the rate of ethanol responding after the intra-amygdaloid administration of the $GABA_A$ receptor antagonist was decreased as early as during the first 3 min of the session. Ingested ethanol reaches the brain rapidly (Nurmi et al., 1994), but because the animals earned only a small volume of ethanol during the first minutes, it is not very likely that the reduction in ethanol responding was caused by changes in the interaction of ingested ethanol with the GABAergic neurotransmission in the brain. Rather, the response patterns are suggestive of changes in ethanol's orosensory properties. Since the neuroanatomy strongly implicates the involvement of the central nucleus of the amygdala, but not the bed nucleus of the stria terminalis or the shell of the nucleus accumbens, in the mediation of information pertaining to taste, the observed difference between the brain regions in sensitivity to GABAergic manipulation might be expected. It remains to be tested whether decreases in ethanol responding by intra-amygdaloid SR 95531 at these doses are selective for ethanol solutions. In the present context, it is interesting that recent studies on the role of the amygdala in intravenous cocaine self-administration suggest that the amygdala mediates some aspect of the cocaine reinforcing stimulus (McGregor and Roberts, 1993; McGregor et al., 1994; Caine et al., 1995).

The present results suggest that the intrinsic GABAergic neurons within the extended amygdala are involved in the modulation of ethanol self-administration. Given the putative role of the extended amygdala intrinsic GABAergic neurons as the interface between the inputs and outputs of the extended amygdala (Sun

et al., 1994), changes in the GABAergic neurotransmission within this region could be forwarded through several anatomical circuits and transmitter systems. The present experimental model does not indicate directly whether the $GABA_A$ receptor antagonist modulates specifically only a certain aspect of ethanol reward or whether several ethanol-related responses are involved. Also, it would be interesting to test whether the extended amygdala GABA system participates in a general neurobiological reward circuitry. For example, the amygdala has close anatomical connections with the nucleus accumbens, and interactions between these regions have been suggested to be implicated in the modulation of motor output and conditioned reinforcement (Yim and Mogenson, 1989; Everitt et al., 1989).

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