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Effects of Acute Ethanol on GABA Release and $GABA_A$ Receptor Density in the Rat Mesolimbic System

MICHAEL COWEN, FENG CHEN, BEVYN JARROTT AND ANDREW J. LAWRENCE

Department of Pharmacology, Monash University, Clayton, Victoria, Australia

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COWEN, M., F. CHEN, B. JARROTT AND A. J. LAWRENCE. *Effects of acute ethanol on GABA release and $GABA_A$ receptor density in the rat mesolimbic system.* PHARMACOL BIOCHEM BEHAV **59**(1) 51–57, 1998.—The present study has addressed whether acute ethanol administration mediates changes in GABA release and $GABA_A$ receptor density in the rat mesolimbic system. In vivo microdialysis was performed in the ventral pallidum and between the ventral tegmental area and substantia nigra of conscious ethanol-naive rats. Extracellular levels of endogenous GABA were stable in both brain regions and not significantly affected following administration of 5 ml of 20% ethanol by gavage, despite clear overt behavioral signs of intoxication. Two hours following ethanol administration, animals were decapitated and the brains processed for autoradiography. Adjacent tissue sections were incubated with [^{3}H]SR95531 or [^{3}H]muscimol and the resulting autoradiograms quantified. Binding of both radioligands was significantly reduced in the striatum of rats treated with ethanol compared to vehicle (15 ± 2% for [^{3}H]SR95531 and 33 ± 6% for [^{3}H]muscimol). In contrast, ethanol had no effect on [^{3}H]SR95531 binding in hippocampus, while that of [^{3}H]muscimol was increased by 100 ± 30%. Ethanol had no effect on the binding of either radioligand in all other areas examined. Therefore, while acute ethanol had no effect on the release of GABA in either the ventral pallidum or ventral tegmental area, changes in the binding density of $GABA_A$ ligands were observed in selected brain regions. © 1998 Elsevier Science Inc.

Ethanol GABA Microdialysis Receptors Autoradiography

A common feature of drugs that have a tendency to be abused is an ability to act on, or interact with, components of the so-called mesolimbic reward system [for a recent review see (34)]. While the afferent component of this mesolimbic reward pathway is a well-described dopaminergic projection from the ventral tegmental area (VTA) to the cortex, striatum, and importantly, the nucleus accumbens, the efferent projections from the nucleus accumbens to the ventral pallidum (VP) and VTA/ substantia nigra are less understood. A favored candidate transmitter of striatopallidal and striatonigral neurons is the inhibitory amino acid, γ-aminobutyric acid (GABA). Thus, both the globus pallidus/ventral pallidum and the VTA/substantia nigra receive a dense and topographically organised GABAergic innervation from the striatum (10,32). In addition, GABAergic neurons originating in the nucleus accumbens and the VP directly innervate the VTA (14). Furthermore, stimulation of the nucleus accumbens evokes complex changes in firing patterns in the VP that are susceptible to modulation by the $GABA_A$ antagonist, bicuculline (3). Therefore, both anatomical and electrophysiological data indicate the likely involvement of GABA and $GABA_A$ receptors in mesolimbic reward efferent neurons.

The ability of ethanol to interact with GABA has long been known (30), since early behavioral and neurochemical observations (1,29). A direct interaction between ethanol and $GABA_A$ receptors was demonstrated by the observation that ethanol enhanced chloride transport through the $GABA_A$ receptor ion channel (28). In addition, more recent studies have shown that the binding properties of various radioligands that recognise the $GABA_A$ receptor complex are modulated by ethanol. For example, the binding of the benzodiazepine partial inverse agonist, [^{3}H]Ro15-4513, increases in rat cortex and cerebellum following chronic ethanol administration (18), while that of [^{3}H]flunitrazepam is unaltered (27).

Therefore, while the ability of ethanol to interact with GABAergic pathways and $GABA_A$ receptors has been well

Requests for reprints should be addressed to Dr. A. J. Lawrence, Department of Pharmacology, Monash University, Wellington Road, Clayton, Victoria 3168, Australia.

established, relatively little is known about the ability of ethanol to modulate the endogenous release of GABA in vivo. Systemic injection of ethanol has been shown to have no effect on the release of GABA in the rat nucleus accumbens (12), the site of "reward" afferent terminals. The present study has therefore employed in vivo microdialysis, in conscious freely moving rats, to measure endogenous GABA in the VP and SN/VTA region, and to determine the effect of oral ingestion of ethanol on GABA release in these two brain regions that receive projections from the nucleus accumbens. Furthermore, after the termination of release experiments, $GABA_A$ receptor density was studied in the rat brains by way of autoradiography utilising agonist, [^{3}H]muscimol, and antagonist, [^{3}H]SR95531 radioligands on adjacent brain sections.

METHOD

All experiments described herein were performed in accordance with the Prevention of Cruelty to Animals Act 1986 under the guidelines of the Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

Materials

[^{3}H]muscimol (14.9 Ci/mmol) and [^{3}H]SR95531 (49.5 Ci/mmol) were obtained from NEN Products, Du Pont. γ-Aminobutyric acid (GABA) was obtained from Research Biochemicals Inc. Hyperfilm and tritium microscales were obtained from Amersham International. All other reagents were of either analytical or laboratory grade from various suppliers.

In Vivo Microdialysis

Male Wistar–Kyoto (WKY) rats (250–320 g) were anesthetized with sodium methohexitone (60 mg/kg, IP) and placed in a stereotaxic frame (Stoelting, USA) set up for use with the atlas of Paxinos and Watson (24). A burr hole was drilled in the skull above the ventral pallidum (AP −0.3 mm, ML −2.6 mm, measured from bregma) or between the substantia nigra pars compacta and the VTA (AP −4.8 mm, ML −1.6 mm, measured from bregma) and the dura pierced. A stainless steel guide cannula (20 G) was inserted to a depth of 6.8 or 7.5 mm, respectively, from the skull surface, then secured with a skull screw and dental cement. The skin incision was sutured and the animals allowed to recover for 24 h.

The following day a microdialysis probe was inserted into the guide cannula as follows. The microdialysis probe, prepared as previously described (16), of length such that the whole membrane would protrude beyond the guide cannula into the region of interest (2 mm, ventral pallidum; 1.5 mm ventral tegmental area/substantia nigra), was perfused with artificial cerebrospinal fluid (aCSF, pH 7.4; of the following composition in mM: glucose, 5; NaCl, 125; $NaHCO_3$, 27; KCl, 2.5; NaH_2PO_4, 0.5, Na_2HPO_4, 1.2; Na_2SO_4, 0.5; $MgCl_2$, 1; $CaCl_2$, 1) at a rate of 1 μl/min. The rats were restrained briefly with halothane (2%), and the probe inserted and secured in place. Following a 90-min equilibration and recovery period, samples were taken every 20 min for GABA analysis. At the end of the third 20-min sample period, the rat was gavaged with 5 ml of either 20% (v/v) ethanol or distilled water. A further five 20-min sample periods elapsed before the rats were killed by decapitation, their brains removed, and then frozen over liquid nitrogen. The tissue was then stored at −80°C until processed further. Probe locations were verified histologically following staining of cryostat-cut sections with 0.1% thionin (Fig. 1). In cases where the membrane insertion fell outside the region of interest, the microdialysis data was excluded from analysis.

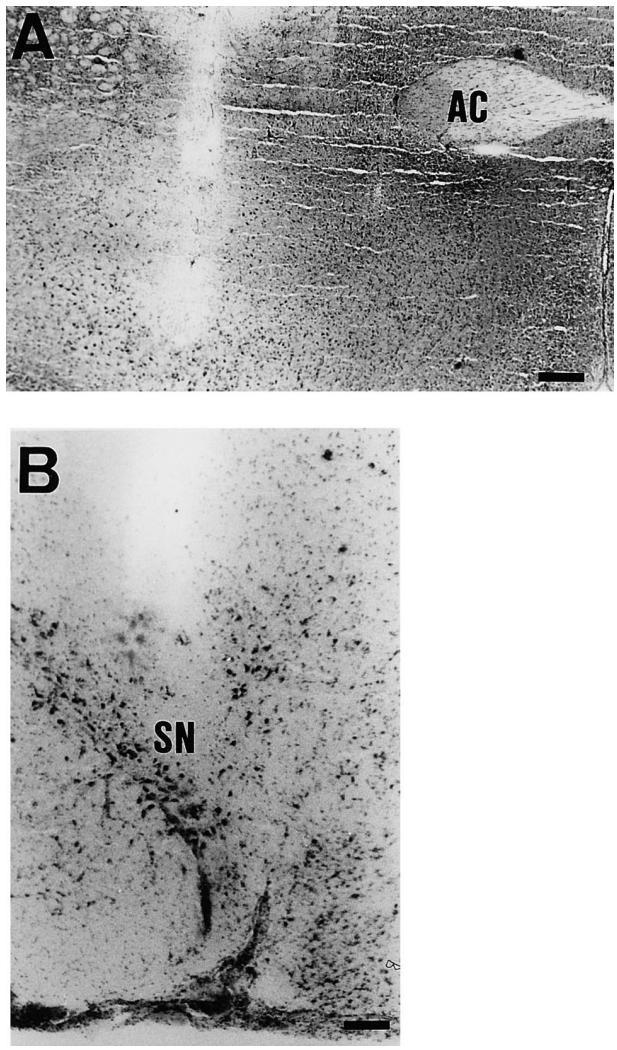

FIG. 1. Photomicrographs of dialysis probe placements in WKY rats: (A) the ventral pallidum, membrane (arrow) placed lateral and ventral of the anterior commissure (ac), bregma −0.3 mm. Scale bar = 454 μm. (B) lateral to the ventral tegmental area, membrane (arrow) placed immediately dorsal of the substantia nigra pars compacta (sn), bregma −4.8 mm. Scale as for A.

GABA content of dialysates was determined by reverse-phase high-pressure liquid chromatography (HPLC) with electrochemical detection. Precolumn derivatization of samples with o-phthaldialdehyde/2-mercaptoethanol was performed as described (15). The dual glassy carbon working electrode was held at +0.7 V vs. an Ag/AgCl reference electrode. Separation was achieved at room temperature using an octadecylsilane (ODS II) reverse phase column (100 × 3.2 mm, 3 μm particle size) with an isocratic mobile phase (Sodium acetate 0.15 M/EDTA 1 mM, pH 6.0 with 20% acetonitrile) flowing at 0.4 ml/min. Samples were quantified by comparison of peak heights with those of standards determined on the same day.

Autoradiography

In addition to verification of microdialysis probe placement, cryostat sections of brain (14 μm) were taken between bregma 0.0 to −1.0 mm and between bregma −4.5 to −6.0 mm (24) and thaw-mounted onto gelatin/chrome alum coated microscope slides. The slide-mounted sections were stored at −80°C until used in autoradiographic studies.

Autoradiography was performed using two different ligands, the $GABA_A$ agonist, [^{3}H]muscimol, and the $GABA_A$ antagonist, [^{3}H]SR95531 (2,11). In the case of [^{3}H]SR95531, the sections were allowed to warm to room temperature before preincubation (30 min, room temperature) in Tris/citrate buffer (50 mM, pH 7.4) containing 100 mM $MgCl_2$. Sections were then cooled in ice-cold buffer for 5 min before incubation with [^{3}H]SR95531 (6.5 nM) at 4°C for 30 min in the same buffer as used for the preincubation. Nonspecific binding was determined in the presence of 10 mM GABA. The slide mounted sections were then washed in ice-cold buffer (3 × 5 s) and distilled water (2 × 10 s), and allowed to dry under a gentle stream of cool air. [^{3}H]Muscimol autoradiography followed a similar protocol except phosphate buffer (5 mM K_2HPO_4/5 mM KH_2PO_4, pH 7.4) containing 100 mM KCl was used throughout the experiment, the slide-mounted sections were incubated with [^{3}H]muscimol (10 nM) for 40 min, and only one wash with distilled water was used. Nonspecific binding was again determined in the presence of 10 mM GABA.

Dried sections were apposed to tritium-sensitive film (Hyperfilm) in the presence of tritium microscales for 1 week, in the case of sections incubated with [^{3}H]SR95531, and for 7 weeks in the case of sections incubated with [^{3}H]muscimol. Developed films were subsequently quantified, using an MCID M4 image analysis system (Imaging Research), by comparison of optical density, under constant illumination, of the autoradiograms compared to the standard microscales.

Statistics

The statistical software program SigmaStat (Jandel) was employed throughout. Due to a lack of normal distribution for much of the data sets, Mann–Whitney *U*-tests were routinely employed to determine differences between control and ethanol groups. In the case of multiple tests, a Bonferroni correction was utilized. A Mann–Whitney *U*-test was also used to determine whether significant differences in binding were observed with the two tritiated ligands. A significance level of $p < 0.05$ was employed throughout.

RESULTS

Microdialysis

Stable levels of extracellular GABA could be measured over the experimental time course, with basal values (defined as the mean of the first three samples) of 1.51 ± 0.2 pmol/20 μl ($n = 11$) in the region between the VTA/SN and 1.65 ± 0.4 pmol/20 μl ($n = 10$) in the VP (Fig. 2). Levels of extracellular GABA in both the VTA/SN and VP were unchanged following administration of 5 ml of water by gavage (Fig. 2). In a similar manner, the release of GABA in either the VP or VTA/SN was not significantly affected by oral administration of 5 ml of 20% ethanol, although there was a tendency for a small increase in both regions (Fig. 2). Animals showed clear signs of intoxication, such as a marked increase in locomotor activity, within 20 mins of ethanol administration, tending toward exaggerated movement, within 30 min. This culminated in the majority of animals sleeping within 40 min of ethanol administration, generally remaining asleep throughout the remainder of the experiment.

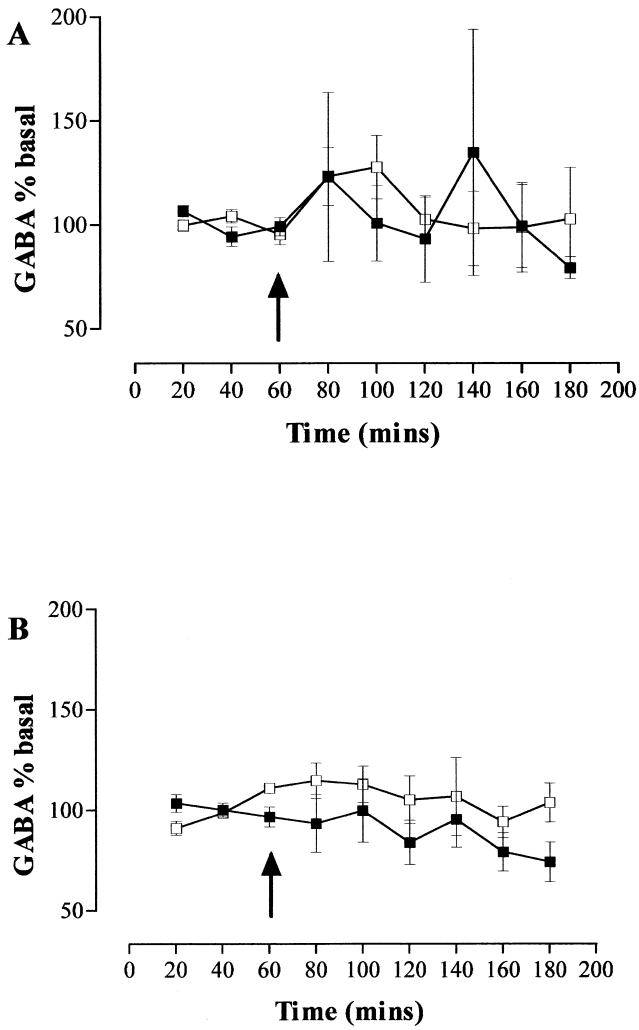

FIG. 2. Time courses of microdialysis experiments studying the effect of acute ethanol on levels of extracellular GABA in the ventral pallidum (A) and ventral tegmental area/substantia nigra (B) of conscious WKY rats. Administration of either vehicle (5 ml of water, closed symbols) or ethanol (5 ml of 20% ethanol, open symbols) by gavage was peformed at the end of the third sample point, as indicated by the arrow. Data represent the mean ± SEM ($n = 5$ per group).

Autoradiography

Both [^{3}H]muscimol and [^{3}H]SR95531 bound avidly to rat brain sections (Fig. 3), with nonspecific binding representing <5% of total binding. [^{3}H]SR95531 binding in sections from control rats was observed in the cortex, the bed nucleus of the stria terminalis, the striatum including the fundus, the hippocampus, the lateral septal nucleus, the mammillary body, the medial preoptic area, the periaqueductal gray, the superior colliculus, superficial and intermediate grays, the substantia nigra, the ventral pallidum and several thalamic nuclei, including the paraventricular nucleus, precommissural nucleus, parafascicular nucleus, lateral posterior nucleus, and the dor-

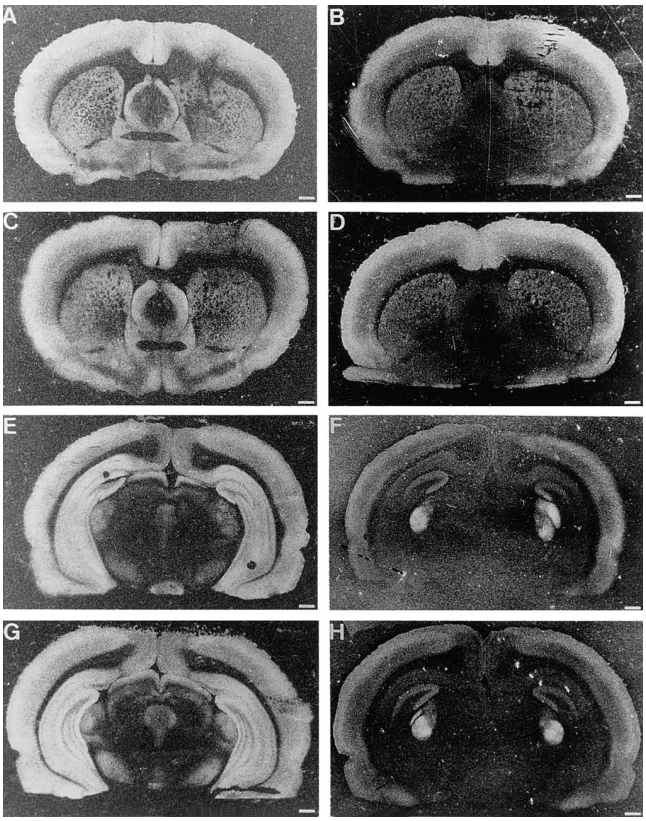

FIG. 3. Autoradiograms of [^{3}H]SR95531 (left hand panel, A, C, E, G) and [^{3}H]muscimol (right hand panel, B, D, F, H) binding to sections of rat brain following acute ethanol administration. In all cases scale bar = 770 μm. (A, B) Control, vehicle-treated animals (5 ml of water by gavage) at the level of the ventral pallidum (bregma ~−0.3 mm). Note the differential distribution profiles for the two radioligands such as the very low density of $GABA_A$ sites labeled by [^{3}H]muscimol (B) in the lateral septum (asterix) when compared to [^{3}H]SR95531 (A). (C,D) Acute ethanol-treated animals (5 ml of 20% ethanol by gavage) at the same level of the ventral pallidum as shown in A and B. (E,F) Control, vehicle-treated animals (5 ml of water by gavage) at the level of the ventral tegmental area (bregma ~−5.6 mm). Note the very dense labeling in the medial geniculate nucleus (arrow) with [^{3}H]muscimol (F), whereas dense labeling with [^{3}H]SR95531 (E) is apparent in the cortex, superior colliculus, periaqueductal gray matter, and throughout the hippocampal formation. (G,H) Acute ethanol-treated animals (5 ml of 20% ethanol by gavage) at the same level of the ventral tegmental area as shown in E and F.

sal and medial geniculate nuclei (MGN) (Table 1). In areas of common binding, [^{3}H]muscimol binding was significantly lower overall than [^{3}H]SR95531 (fmol/mm^2; Mann–Whitney *U*-test, $p < 0.05$), and regions were less easily delineated with [^{3}H]muscimol than with [^{3}H]SR95531 (see Fig. 3). [^{3}H]Muscimol binding in sections from control rats was observed only in the cortex, the striatum, the hippocampus, and several thalamic nuclei including the lateral posterior thalamic nucleus and the dorsal and medial geniculate nuclei (Table 2).

Relative differences in the binding of the two ligands in sections from control rats was noted. Binding of [^{3}H]SR95531 in the dorsal posterior thalamic nuclei (DPTN; including the lateral posterior thalamic nucleus and the dorsal geniculate nucleus) and the medial geniculate nucleus was 57 and 43% of binding in the cortex, respectively (Table 1), while binding of

TABLE 1

EFFECT OF ETHANOL ON [^{3}H]SR95531 BINDING IN SECTIONS OF THE RAT

	Control		Ethanol	
Region	fmol/mm^2 (Mean ± SEM)	% Cortex	fmol/mm^2 (Mean ± SEM)	% Cortex
Cortex	4.5 ± .01	100	4.3 ± 0.1	100
BNST†	3.5 ± 0.2	79	3.2 ± 0.1	75
DPTN†	2.6 ± 0.1	57	2.6 ± 0.2	62
Fundus striatum	3.5 ± 0.1	79	3.1 ± 0.1	72
Hippocampus	4.6 ± 0.1	102	4.6 ± 0.1	108
Lateral septal nucleus	3.8 ± 0.2	86	3.6 ± 0.1	84
Mammillary body	1.9 ± 0.1	42	1.9 ± 0.2	45
MGN	1.9 ± 0.1	43	2.2 ± 0.2	51
Medial preoptic area	2.8 ± 0.3	63	2.9 ± 0.2	68
MPTN†	1.9 ± 0.2	43	2.3 ± 0.3	53
Periaqueductal gray	1.7 ± 0.1	38	1.7 ± 0.2	40
SCIG†	1.6 ± 0.1	36	1.6 ± 0.2	38
SCSG†	3.5 ± 0.1	78	3.5 ± 0.2	81
Striatum	2.8 ± 0.1	63	2.4 ± 0.1*	57
Substantia nigra	1.4 ± 0.1	32	1.5 ± 0.1	36
Ventral pallidum	3.5 ± 0.2	78	3.1 ± 0.1	73

*Significant difference to control, Mann–Whitney *U*-test with Bonferroni correction, $p < 0.05$. The data are from four to six animals.

†Abbreviations are as follows: BNST, bed nucleus, stria terminalis; DPTN, dorsal posterior thalamic nuclei, including the lateral posterior thalamic nucleus and the dorsal genicultate nucleus; MGN, medial geniculate nucleus; MPTN, medial posterior thalamic nuclei, including the paraventricular thalamic nucleus, the precommissural nucleas and the parafascicular thalamic nucleus; SCIG, superior colliculus, intermediate gray; SCSG, superior colliculus, superficial gray.

[^{3}H]muscimol in the same regions was much higher at 126 and 109% of binding in the cortex, respectively (Table 2). In contrast, binding of [^{3}H]SR95531 in the hippocampus was 102% of binding in the cortex (Table 1), while binding of [^{3}H]muscimol in the same region was much lower at 17% of binding in the cortex (Table 2). The pattern of binding in the hippocampus of the two ligands was different, as [^{3}H]SR95531 covered the entire extent of the hippocampal formation (Fig. 3), whereas [^{3}H]muscimol bound predominantly to the dentate gyrus and CA1 field (Fig. 3).

Ethanol caused a significant decrease in binding in the striatum with both [^{3}H]SR95531 (15 ± 2%) and [^{3}H]muscimol (33 ± 6%). In contrast, ethanol caused a significant increase in binding of [^{3}H]muscimol in the hippocampus (100 ± 30%), but this change was not observed with [^{3}H]SR95531 (Mann–Whitney *U*-tests with Bonferroni corrections, $p < 0.05$). No significant differences were observed in any other regions.

Relative differences in the binding of the two ligands was preserved in sections from ethanol-treated rats. Binding of [^{3}H]SR95531 in the DPTN and the medial geniculate nucleus was 62 and 51% of binding in the cortex, respectively (Table 1), while binding of [^{3}H]muscimol in the same regions was again much higher at 136 and 117% of binding in the cortex, respectively (Table 2). Similarly as for sections from control rats,

TABLE 2

EFFECT OF ETHANOL ON [^{3}H]MUSCIMOL BINDING IN SECTIONS OF THE RAT CNS

Region	Control		Ethanol	
	fmol/mm^2 (Mean ± SEM)	% Cortex	fmol/mm^2 (Mean ± SEM)	% Cortex
Cortex	2.1 ± 0.1	100	2.0 ± 0.1	100
DPTN†	2.7 ± 0.2	126	2.7 ± 0.2	136
Hippocampus	0.4 ± 0.1	17	0.7 ± 0.1*	37
MGN†	2.3 ± 0.2	109	2.3 ± 0.4	117
Striatum	1.2 ± 0.1	58	0.8 ± 0.1*	41

*Significant difference to control, Mann–Whitney *U*-test with Bonferroni correction, $p < 0.05$. The data are from four to six animals.

†Abbreviations are as follows: DPTN, dorsal posterior thalamic nuclei, including the lateral posterior thalamic nucleus and the dorsal geniculate nucleus; MGN, medial geniculate nucleus.

binding of [^{3}H]SR95531 in the hippocampus was 108% of binding in the cortex (Table 1), while binding of [^{3}H]muscimol in the same region was much lower at 37% of binding in the cortex (Table 2).

DISCUSSION

The present data demonstrate differential effects of acute ethanol administration on the release of endogenous GABA and the density of $GABA_A$ receptors in the rat mesolimbic system. Specifically, while GABA release did not change in either the VP or VTA/SN following a high dose of ethanol given acutely, the density of $GABA_A$ receptor binding decreased in striatum and increased in hippocampus 2 h after ethanol intake. Furthermore, hippocampal binding was only altered for the agonist [^{3}H]muscimol, and not when measured with [^{3}H]SR 95531, suggesting the presence of receptor populations with different sensitivities. This hypothesis is further strengthened by the marked variations in the binding profiles of [^{3}H]muscimol and [^{3}H]SR 95531 observed in rat brain. The concentrations of the two radioligands employed (6.5 nM for [^{3}H]SR 95531 and 10 nM for [^{3}H]muscimol) were chosen as they equate to the respective K_D values for the radioligands in rat brain (11,23), and therefore should be optimal for labelling $GABA_A$ receptors on slide-mounted sections. The differential binding profiles of the two radioligands does not, therefore, reflect inappropriate experimental conditions. A more likely explanation is the possibility that the two radioligands bind with differing affinity to $GABA_A$ receptors with different subunit composition. For example, [^{3}H]muscimol binds predominantly to α and subunits (35) and it is therefore possible that receptors with different subunit composition predominate in regions where [^{3}H]SR 95531 binding is high compared to [^{3}H]muscimol. Alternatively, the agonist [^{3}H]muscimol may only bind to certain affinity states of the receptor that are not discriminated by the antagonist [^{3}H]SR 95531.

The anatomical delineation of reciprocal GABAergic projections between the nucleus accumbens, VP and VTA (4,14) provided a clear substrate for GABA to be considered a major neurotransmitter of reward efferents, a hypothesis with functional correlates (3). It was somewhat surprising, therefore, when administration of ethanol had no significant effect on the release of GABA from either the VP or the VTA/SN region; however, studies employing intraperitoneal injection of ethanol have also failed to detect changes in GABA release measured in the nucleus accumbens (7,12). In vitro studies of [^{3}H]GABA release in rat hippocampus have demonstrated that chronic ethanol consumption did not affect basal release but did enhance stimulated release (31). Neuroanatomical studies have demonstrated a loss of GABAergic synapses in the dentate fascia of ethanol-sensitive mice during withdrawal from chronic ethanol exposure (8). Interestingly, biphasic effects of ethanol on presynaptic GABA uptake have been documented, with stimulation at low concentrations and inhibition at high concentrations (9). Thus, it is distinctly possible that the actual concentration of ethanol achieved in the rat brain in the present study was in the range that stimulates GABA uptake, therefore resulting in no net observed effect on release. Alternatively, much longer exposure to ethanol may be necessary to cause measurable changes in GABA release. It must be remembered also that the technique of in vivo microdialysis requires long sampling times due to the low flow rates employed, and as such can only readily quantify substantial changes in release that are maintained for a period of time. Clearly, it is possible that acute ethanol administration evoked a transient change in neurotransmitter release that was masked in a 20-min sample. Whatever the explanation, an inability to measure changes in the release of endogenous GABA in either the VP or VTA/SN following acute ethanol does not necessarily mean that GABA is not a transmitter at mesolimbic reward efferent neurons. The present study employed a relatively high acute dose of ethanol that caused sedation, as has been previously observed (13). This dose was chosen with the particular route of administration (gavage) to mimic the situation of human ethanol consumption, rather than using an intraperitoneal injection. It is clearly possible that lower doses of ethanol, devoid of sedative properties, may indeed mediate changes in GABA release in the VP or VTA.

The distribution of $GABA_A$ receptors has been mapped throughout the rat brain, both by traditional autoradiographic techniques (2,22) and also by molecular biological studies of the expression of the various $GABA_A$ receptor subunit genes (25,33). The differential distribution patterns of individual receptor subunits and mature receptors, dependent upon the radioligand used, clearly suggests that $GABA_A$ receptors are likely to exist in different isoforms throughout the brain. In relation to $GABA_A$ receptors that show sensitivity to ethanol, evidence indicates that [^{3}H]zolpidem binds to ethanol-sensitive $GABA_A$ receptors that are likely to be comprised of α_1, β_2, and γ_2-subunits (5). These researchers have further shown that in various limbic regions, both zolpidem and ethanol can enhance GABAergic transmission in areas where the mRNA encoding the α_1, α_2, α_3, β_2, β_3, and γ_2 receptor subunits are localized (6).

In the present study, acute ethanol resulted in a reduction of striatal $GABA_A$ receptor binding as measured by both [^{3}H]muscimol and also [^{3}H]SR 95531, whereas the hippocampal binding of [^{3}H]muscimol, but not that of [^{3}H]SR 95531, was increased by ethanol. In mice, [^{3}H]muscimol binding has been shown to increase in both the molecular and granule cell layers of the cerebellum following acute ethanol administration, along with a similar increase in expression of the mRNA encoding the α_1 and β_3 subunits (35). Indeed, sensitivity of $GABA_A$ receptors to ethanol has been linked to α subunits (17). A number of studies have also examined the effects of chronic ethanol consumption on $GABA_A$ receptor density. In the rat visual cortex and hippocampus, chronic ethanol treatment had no effect on the density of [^{3}H]flunitrazepam binding sites, whereas GABA-mediated enhancement of [^{3}H]flunitraz-

epam binding was increased in the cortex but not hippocampus (19,20). On the other hand, [^{3}H]flunitrazepam binding is increased in the cerebellum and red nucleus following both chronic ethanol and withdrawal from chronic ethanol (21); however, the ability of GABA to modulate [^{3}H]flunitrazepam binding is not altered during withdrawal from chronic ethanol (26). Other limbic structures, such as the frontal cortex and nucleus accumbens were not included in the study due to a concentration on the efferent loop of the reward pathway (VP, VTA/SN) rather than the afferent component.

In summary, the present data indicate that $GABA_A$ receptors in the striatum and hippocampus are sensitive to acute ethanol administration; however, the ability to measure a change in receptor density depends upon the radioligand employed. This observation clearly lends weight to the hypothesis that $GABA_A$ receptors exist in multiple isoforms, only a proportion of which are ethanol sensitive. Further studies are required to ascertain the subunit stoichiometry of such receptors. In contrast to the effects of ethanol on $GABA_A$ receptors, a high dose of acute ethanol had no discernible effect on the release of endogenous GABA from either the VP or VTA/SN as measured by in vivo microdialysis. This may reflect no net change in the extracellular concentration of GABA, or may be due to shortfalls in the technique employed. Changes in receptor density may be due to a direct action of ethanol on a subpopulation of $GABA_A$ receptors, or possibly may reflect an ability of ethanol to directly act upon the chloride channel without affecting the release of GABA per se.

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