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Distribution of $GABA_A$ and $GABA_B$ Receptors in Mammalian Brain: Potential Targets for Drug Development

Anne B. Young and Dorothy Chu

Department of Neurology, University of Michigan, Ann Arbor, Michigan

ABSTRACT

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GABA is the major inhibitory neurotransmitter in mammalian brain. GABA receptors and the metabolism of GABA are significant targets for new centrally acting drugs to treat neurological and behavioral disorders. The simple neutral amino acid is likely to subserve a neurotransmitter role at 25–50% of all synapses in the central nervous system. GABA's actions are mediated by two different receptors, $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors are ligand-gated chloride channels that are sensitive to the convulsant alkaloid bicuculline and modulated by benzodiazepines and barbiturates. $GABA_B$ receptors affect calcium and potassium conductance through GTP binding proteins and are insensitive to bicuculline and sensitive to the agonist baclofen. Both receptors are widely distributed in cerebral cortex, hippocampus, basal ganglia, thalamus, cerebellum, and brainstem.

Key words: autoradiography, receptor localization, human, rat

INTRODUCTION

GABA (gamma-aminobutyric acid) is a neutral amino acid that subserves neurotransmitter functions at an estimated 25–50% of synapses in the mammalian central nervous system. GABA is present in brain in concentrations of 1–10 mM. The amino acid is synthesized from glutamate by the neuronal cytoplasmic enzyme glutamate decarboxylase and it is metabolized by mitochondrial enzymes to alpha-ketoglutarate that enters the Kreb's cycle. The synthetic and metabolic enzymes have been purified and antibodies raised against them. The enzymes have been localized in rat and human brain by using immunocytochemistry and the

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Address reprint requests to Anne B. Young, M.D., Ph.D., Neuroscience Building, 1103 East Huron St., Ann Arbor, MI 48104-1687.

GABAergic pathways have been mapped by using this approach [Fagg and Foster, 1983; Ribak et al., 1979].

In the nerve terminal, GABA is stored in vesicles by a unique sodium-independent, ATP-dependent transport system that is selective for GABAergic neurons [Fykse and Fonnum, 1988; Hell et al., 1988]. This uptake system is biochemically and pharmacologically distinct from the neuronal and glial membrane high-affinity transport system and is driven by an electrochemical proton gradient [Wood and Sidhu, 1986; Debler and Lajtha, 1987; Krogsgaard-Larsen et al., 1987; Larsson et al., 1988]. GABA in vesicles and, perhaps, in the cytoplasm is released into the synaptic cleft upon depolarization of the terminal by a calcium-dependent mechanism. After release, GABA diffuses across the synaptic cleft to interact with postsynaptic GABA receptors. GABA is inactivated by diffusion and by a high-affinity, sodium-dependent transport system into synaptic terminals and glial cells. The biochemical and pharmacological properties of this transport system have been studied in detail and will be reviewed in other articles in this volume.

GABA interacts with two receptor types, so-called $GABA_A$ and $GABA_B$ receptors [Stephenson, 1988; Bowery et al., 1988; Bormann, 1988]. $GABA_A$ receptors are ligand-gated chloride channels that are modulated by benzodiazepines and barbiturates and inhibited by the convulsant alkaloid, bicuculline. $GABA_B$ receptors are insensitive to bicuculline and are sensitive to the agonist baclofen. In this chapter, the distribution of $GABA_A$ and $GABA_B$ receptors in normal rat and human brain will be reviewed.

MATERIALS AND METHODS

Male Sprague-Dawley rat brains and coronal sections of human brain from persons who died without neurological disease were stored in sealed containers until assay. Tissues were mounted onto cryotome pedestals with embedding matrix (Lipshaw) and were allowed to equilibrate in the cryostat at −20°C [Chu et al., 1990, 1987b, 1987a]. Serial brain sections (20 μm thick, in horizontal or coronal plane) were cut on a Lipshaw cryotome at −20°C to −15°C and thaw mounted onto chrome-alum gelatin-coated slides. Prior to assay, sections were prewashed for 15 min in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ (pH 7.40) at 4°C. Slides were then removed from the buffer and dried under a stream of cool air.

[^{3}H]GABA was used to examine both $GABA_A$ and $GABA_B$ receptors. $GABA_B$ receptors were assayed by incubating sections for 45 min at 4°C with [^{3}H]GABA (20–25 nM) in 50 mM Tris-HCl buffer (pH 7.4), 2.5 mM $CaCl_2$, and 10 μM isoguvacine (a $GABA_A$ agonist which specifically blocks [^{3}H]GABA binding to $GABA_A$ receptors). Nonspecific binding was determined in the presence of 100 μM (±)baclofen. $GABA_A$ receptors were assayed by incubating sections for 45 min at 4°C with [^{3}H]GABA (20–25 nM) in 50 mM Tris-HCl buffer (pH 7.4), 2.5 mM $CaCl_2$, and 100 μM (±)baclofen (which blocks specifically binding to $GABA_B$ receptors). Nonspecific binding was determined in the presence of 100 μM isoguvacine.

After incubation, slides were individually removed, subjected to three rapid squirts with buffer followed by one quick rinse with 2.5% glutaraldehyde in acetone and immediately blown dry with warm air. This rinse and dry procedure was completed within 15 sec. The slides were mounted in x-ray cassettes and apposed to sheets of tritium-sensitive Ultrafilm ^{3}H (LKB) for 3 weeks at 4°C. The films were developed in Kodak D19 for 3 min at 25°C, fixed, and dried. Autoradiograms were analyzed by computer-assisted densitometry. Twenty to 25 readings from each region of interest on each section were averaged. Optical densities were quantified in pmol [^{3}H]GABA bound per mg protein by comparing film densities of brain with those generated by ^{14}C-embedded plastic standards previously calibrated against brain paste standards containing known amounts of tritium and protein.

TABLE 1. Comparison of B_{max} and K_D Values for $GABA_A$ and $GABA_B$ Receptors in Various Regions of Rat and Human Brain*

Rat brain area	B_{max} (pmol GABA bound/mg protein) $GABA_A$	$GABA_B$
Cerebral cortex		
Layers I–III	4.02 ± 0.26	3.53 ± 0.26
Layer IV	4.69 ± 0.22	1.87 ± 0.24
Neostriatum	1.36 ± 0.11	0.91 ± 0.12
Dentate gyrus		
Molecular layer	3.97 ± 0.13	2.97 ± 0.51
Cerebellum		
Molecular layer	2.64 ± 0.18	3.58 ± 0.49
Granular layer	9.30 ± 0.38	1.66 ± 1.65
	K_D (nM)	
Cerebral cortex		
Layers I–III	92 ± 10	341 ± 41
Layer IV	100 ± 19	222 ± 46
Neostriatum	70 ± 14	313 ± 58
Dentate gyrus		
Molecular layer	139 ± 15	462 ± 54
Cerebellum		
Molecular layer	164 ± 26	481 ± 35
Granular layer	119 ± 17	458 ± 193
	B_{max} (pmol GABA bound/mg protein)	
Human brain area	$GABA_A$	$GABA_B$
Cerebral cortex		
Layers II–III	3.17 ± 0.33	1.26 ± 0.08
Layer V	2.36 ± 0.31	1.08 ± 0.12
Hippocampus		
Stratum moleculare dentate gyrus	3.51 ± 0.69	1.09 ± 0.15
Stratum lacunosum-moleculare of CA1	2.33 ± 0.21	0.85 ± 0.10
Stratum pyramidale of CA	3.50 ± 0.42	1.04 ± 0.14
Subiculum	1.99 ± 0.23	0.55 ± 0.17
	K_D (nM)	
Cerebral cortex		
Layers II–III	121 ± 30	443 ± 139
Layer V	136 ± 33	401 ± 79
Hippocampus		
Stratum moleculare dentate gyrus	234 ± 84	414 ± 113
Stratum lacunosum-moleculare of CA1	167 ± 30	318 ± 74
Stratum pyramidale of CA	192 ± 45	355 ± 99
Subiculum	191 ± 46	261 ± 85

*Values represent mean ± S.E.M. of four brains.

RESULTS

The affinity of GABA for rat and human $GABA_A$ receptors varied between 70 and 170 nM between regions whereas its affinity for $GABA_B$ receptors was 220–480 nM and Hill-numbers for the two binding sites were very close to unity in all regions [Chu et al., 1990, 1987b, 1987a] (Table 1). Both receptor subtypes were widely distributed in mammalian brain but the relative densities of the two varied from region to region (Figs. 1,2). In olfactory bulb, $GABA_B$ binding was highest in the glomerular layers, whereas $GABA_A$ binding was most

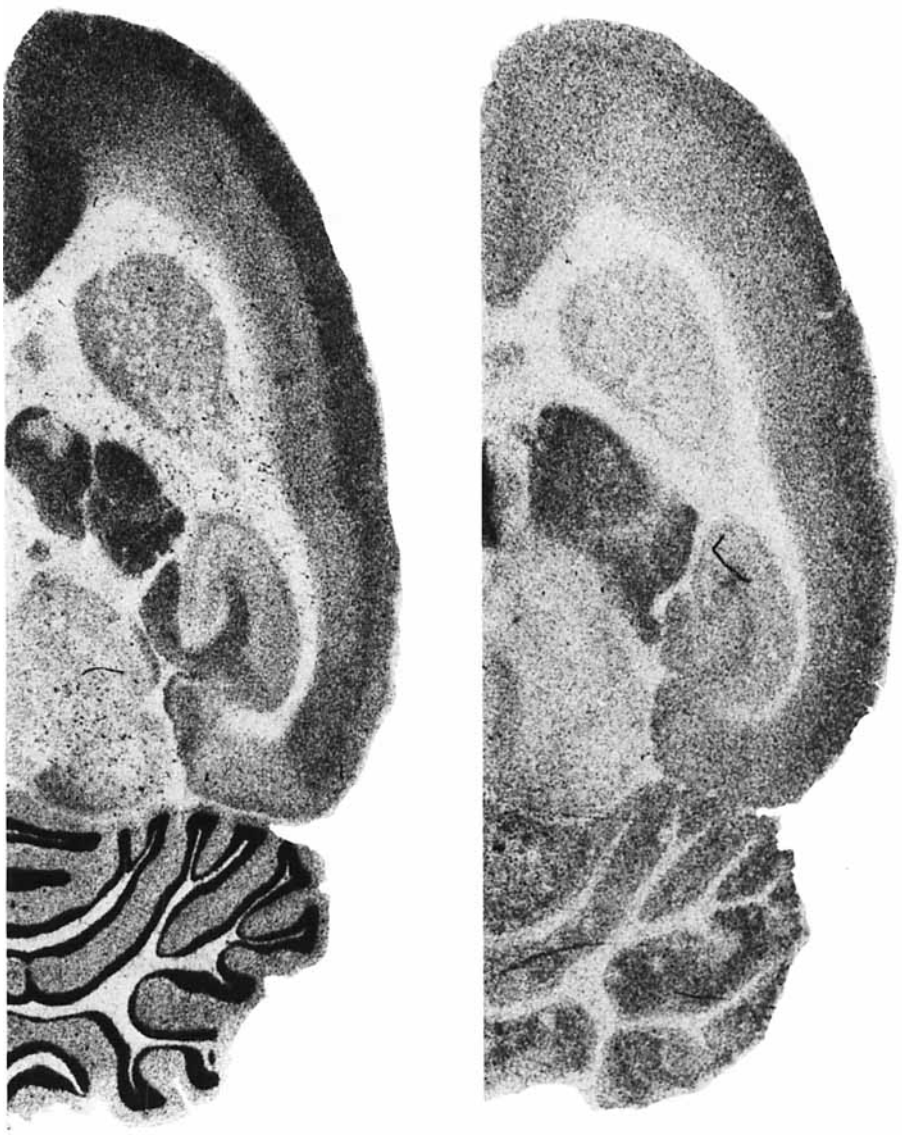

Fig. 1. $GABA_A$ (left) and $GABA_B$ (right) receptors in horizontal hemisections of rat brain. [^{3}H]GABA concentration was 24 nM (magnification: ×7.25).

dense in the external plexiform and inner granular layers. GABA receptor binding was dense for both subtypes of receptor in cerebral cortex although $GABA_A$ receptors were localized in distinct cortical layers (dense in layer I–IV) whereas $GABA_B$ binding was more diffusely distributed through cortex.

In most areas, $GABA_A$ receptors outnumbered $GABA_B$ receptors [Chu et al., 1990]. The exceptions were the medial habenula, the glomerular layer of the olfactory bulb, the superficial gray of the superior colliculus, the interpeduncular nucleus, the pontine nuclei, and the molecular layer of cerebellum where $GABA_B$ receptors were high and $GABA_A$ receptors were relatively low.

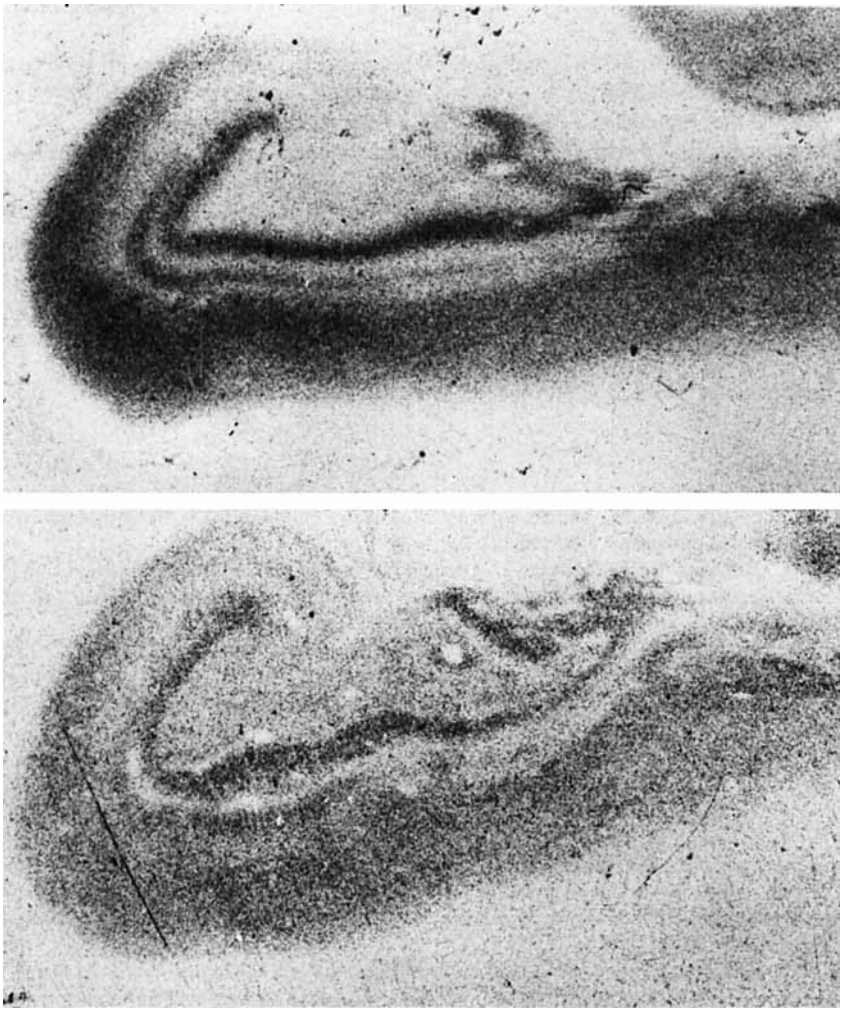

Fig. 2. $GABA_A$ **(above)** and $GABA_B$ **(below)** receptors in human hippocampus. [^{3}H]GABA concentration was 20 nM. The person died of nonneurological disease and was not on centrally active medications at the time of death (magnification: $\times 10$).

In human hippocampus, $GABA_A$ receptors outnumber $GABA_B$ receptors approximately three or four to one [Chu et al., 1987a]. The ratio of the two are fairly constant throughout the various hippocampal regions. The two receptor subtypes were most dense in stratum moleculare of dentate gyrus and stratum pyramidale of CA1 and less dense in stratum lacunosum-moleculare of CA1 and subiculum.

DISCUSSION

$GABA_A$ and $GABA_B$ receptors were widely distributed in mammalian brain and are in high concentration in cortical, hippocampal, thalamic, basal ganglia, and cerebellar structures [Chu et al., 1990, 1987b,a; Wamsley et al., 1986; McCabe and Wamsley, 1986; Zezula et al.,

1988]. Drugs mediating actions at GABAergic synapses are likely to affect GABAergic function in multiple brain areas.

In recent years, the $GABA_A$ receptor has been purified and studied at the molecular level [Stephenson, 1988; Schofield et al., 1987; Barnard et al., 1987; Blair et al., 1988; Levitan et al., 1988]. Several subunits exist for the $GABA_A$ receptor and different isoforms of the various subunits have also been identified. The different subunits have unique pharmacological and electrophysiological properties. Antibodies against the receptor subunits have been used to map receptor localization [Houser et al., 1988; De Blas et al., 1988]. The various subunits are differentially distributed in brain [Wisden et al., 1988]. In the future, it may prove possible to design drugs that act on specific subpopulations of $GABA_A$ receptors.

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