Gamma-Aminobutyric Acid Binding to Receptor Sites in the Rat Central Nervous System
(neurotransmitter/bicuculline/synaptic membranes/glycine/strychnine)

STEPHEN R. ZUKIN, ANNE B. YOUNG, AND SOLOMON H. SNYDER*

Departments of Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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ABSTRACT [H]Gamma-aminobutyric acid (GABA) binds to synaptic membrane fractions of rat brain in a selective fashion representing an interaction with postsynaptic GABA receptors. Inhibition of [H]GABA binding by a variety of amino acids closely parallels their ability to mimic the synaptic inhibitory actions of GABA and does not correlate with their relative affinity for the presynaptic synaptosomal GABA uptake system. [H]GABA binding is saturable with an affinity constant of about 0.1 εM. The GABA antagonist bicuculline inhibits [H]GABA binding with half maximal effects at 5 εM, whereas it requires a concentration of 0.5 mM to reduce synaptosomal GABA uptake by 50%. In subcellular fractionation experiments [H]GABA binding is most enriched in crude synaptic membranes. [H]GABA binding is greatest in the cerebellum, least in the spinal cord and medulla oblongata, with intermediate values in the thalamus, hippocampus, hypothalamus, cerebral cortex, midbrain, and corpus striatum.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (1–4). Its inhibitory actions are selectively antagonized by the alkaloid bicuculline, in contrast to the inhibitory actions of glycine, which are selectively antagonized by strychnine (5–8). The synaptic activities of GABA may be terminated by a high-affinity, sodium-requiring uptake system into GABA nerve terminals (9, 10) and possibly glia (11–13). The sodium-dependent binding of GABA to membrane preparations of mammalian brain largely represents binding of GABA to uptake sites, since the relative affinity of the variety of amino acids and drugs in preventing this binding parallels their affinity for the GABA uptake system but differs markedly from their relative abilities to mimic the synaptic inhibitory actions of GABA (6, 14, 15,†).

Here we report binding of [H]GABA to crude synaptic membrane fractions of the rat central nervous system which appears to represent a specific interaction with the postsynaptic GABA receptor.

MATERIALS AND METHODS

Tissue Preparation. Subcellular fractionation utilized a modification of previously described techniques (16–20). Male Sprague-Dawley rats (100–200 g) were decapitated and the brains were rapidly removed and homogenized in 15 volumes of ice-cold 0.32 M sucrose in a Potter-Elvehjem glass homogenizer fitted with a Teflon® pestle. The homogenate was centrifuged at 1000 × g for 10 min, the pellet was discarded, and the supernatant fluid was centrifuged at 20,000 × g for 20 min. The crude mitochondrial pellet was resuspended in distilled water and dispersed with a Brinkmann Polytron PT-10 sonicator (setting 6) for 30 sec. The suspension was centrifuged at 8000 × g for 20 min. The supernatant was collected and the pellet, a bilayer with a soft, buffy uppercoat, was rinsed carefully with the supernatant fluid to collect the upper layer. The combined supernatant fraction was then centrifuged at 48,000 × g for 20 min. The final crude synaptic membrane pellets were resuspended in H2O and centrifuged at 48,000 × g for 20 min and then stored at −30° for at least 18 hr. The frozen pellets were resuspended in H2O, maintained at 25° for 20 min, centrifuged at 48,000 × g for 10 min and the pellets were then suspended in the buffer for GABA binding assay. [H]GABA binding capacity remained intact for at least 30 days under these conditions. Storing frozen tissue enhances GABA receptor binding and markedly lowers sodium-dependent GABA binding unrelated to receptor sites†.

Binding Assay. Aliquots of crude synaptic membranes (0.3–1.2 mg of protein) were incubated in triplicate at 4° for 5 min in 2 ml of 0.05 M Tris-citric acid buffer (pH 7.1 at 4°) containing approximately 32 nM [H]-G-aminobutyric acid (GABA) (500,000 cpm) alone or in the presence of 1 mM GABA, 0.1 mM bicuculline, or other indicated drugs. Time course experiments indicated that specific [H]GABA binding is at equilibrium within 5 min under these conditions. After incubation, the reaction was terminated by centrifugation for 10 min at 48,000 × g. The supernatant fluid was decanted, and the pellet was rinsed rapidly and superficially with 5 ml, then 10 ml of ice-cold distilled H2O. Bound radioactivity was extracted into 1 ml of Protosol (New England Nuclear Corp.), 10 ml of toluene phosphor was added, and radioactivity was assayed by liquid scintillation spectrometry (Packard Tricarb model 3385 or 3375), at a counting efficiency of 34%. Analysis of the membrane-bound radioactivity by thin layer chromatography in three solvent systems indicated that all of the bound material was authentic GABA.

Specific [H]GABA binding was obtained by subtracting from the total bound radioactivity the amount not displaced by high concentrations of bicuculline (0.1 mM) or GABA (1 mM). Except when otherwise indicated, values for nonspecific binding were the same whether bicuculline or GABA was used.
TABLE 1. Subcellular distribution of specific [\textsuperscript{3}H]GABA binding in the rat brain

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific [\textsuperscript{3}H]GABA binding</th>
<th>Specific: non specific binding ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding (cpm/mg of protein)</td>
<td>Total binding per fraction (cpm)</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>282</td>
<td>12,708</td>
</tr>
<tr>
<td>Crude nuclear pellet (P1)</td>
<td>209</td>
<td>2100</td>
</tr>
<tr>
<td>Crude mitochondrial pellet (P2)</td>
<td>1068</td>
<td>14,585</td>
</tr>
<tr>
<td>Crude microsomal pellet (P3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Osmotically shocked P1 subfractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria–myelin pellet</td>
<td>210</td>
<td>1829</td>
</tr>
<tr>
<td>Crude synaptic membrane pellet</td>
<td>1517</td>
<td>18,177</td>
</tr>
</tbody>
</table>

Tissues were prepared and subjected to differential centrifugation as described in the text. The crude microsomal pellet was obtained by centrifugation of the supernatant fluid remaining after centrifugation of the crude mitochondrial pellet at 100,000 \( \times g \) for 1 hr. The various pellets were resuspended in 50 mM Tris–citric acid buffer (pH 7.1) (0.7–1.2 mg of membrane protein per 2 ml) and assayed as described. The experiment has been replicated twice.

as a displacer. Protein was measured by the method of Lowry et al. (21).

Uptake Studies. Uptake of radioactive GABA was assayed as previously described (22–25).

Materials. [\textsuperscript{3}H]GABA (specific activity 10 Ci/mmol) was obtained from New England Nuclear Corp. Picrotoxin, purchased from Sigma Chemical Corp., and bicuculline, from Pierce Chemical Co., were dissolved immediately prior to use because of their chemical lability.

RESULTS

Subcellular localization of [\textsuperscript{3}H]GABA binding in the rat brain

The distribution of specific [\textsuperscript{3}H]GABA binding in subcellular fractions of whole rat brain was measured in fractions prepared by a modification of previously described techniques (16–20) (Table 1). Among the primary fractions obtained by differential centrifugation, the largest amount of binding is recovered in the crude mitochondrial pellet (P2) which contains both pinched-off nerve endings ("synaptosomes") and free mitochondria. Total specific binding in the P2 pellet is as great or greater than that of the whole homogenate, while binding in the crude nuclear pellet is only about \( \frac{1}{4} \)th that of the whole homogenate, and no specific binding can be detected in the crude microsomal pellet. The specific activity of binding in the P2 pellet is more than four times that of the whole homogenate. Substantial GABA binding in the P2 pellet might conceivably occur but dissociate during the prolonged centrifugation required to recover this pellet. However, since all the binding of the whole homogenate can be accounted for in terms of binding recovered in the P1 and P2 fractions, the crude microsomal pellet probably does not contain large amounts of GABA binding activity.

When the P2 pellet is subjected to hypotonic shock, nerve endings are lysed and subsequent differential centrifugation can resolve a low-speed pellet containing mitochondria and myelin from a high-speed fraction which is enriched in synaptic membranes (16). The crude synaptic membrane fraction contains about 10 times as much GABA binding as the mitochondria–myelin pellet and has a specific activity more than five times that of the original homogenate. The crude synaptic membrane fraction has been used for routine GABA binding assays. Specific binding of [\textsuperscript{3}H]GABA to synaptic membranes is linear between 0.5 and 2 mg of whole brain membrane protein (Fig. 1). Binding studies are routinely performed within this linear range.

During preparation of the crude synaptic membrane fraction from brain homogenates, the ratio of specific to nonspecific GABA binding increases progressively. Thus in the whole homogenate specific GABA binding is only 10% of the total. The ratio of specific to nonspecific binding in the crude mitochondrial pellet is four times that of the whole homogenate, while the crude synaptic membrane fraction displays a ratio six times that of the whole homogenate. Thus in routine assays with the synaptic membrane fraction, about 40–50% of [\textsuperscript{3}H]GABA binding is displaced by 0.1 mM bicuculline or 1 mM GABA.

Saturability of [\textsuperscript{3}H]GABA binding to synaptic membranes of whole rat brain

Specific [\textsuperscript{3}H]GABA binding is saturable with increasing concentration, with half-maximal binding occurring at approximately 0.1 \( \mu \text{M} \). By contrast, nonspecific binding, indicated by the binding of [\textsuperscript{3}H]GABA in the presence of 1 mM GABA or 0.1 mM bicuculline, is not saturable and increases linearly with increasing [\textsuperscript{3}H]GABA. Displacement of [\textsuperscript{3}H]GABA by nonradioactive GABA is half-maximal at 0.1 \( \mu \text{M} \) nonradioactive GABA, correlating closely with the data obtained in saturation analysis using [\textsuperscript{3}H]GABA alone. Maximal displace-
ment with nonradioactive GABA occurs at about 10 \( \mu \)M (Fig. 2). The extent of maximal displacement of \([\text{H}]\)GABA binding by bicuculline is similar to maximal displacement obtained with GABA. Half-maximal displacement occurs at 5 \( \mu \)M bicuculline with maximal displacement at about 100 \( \mu \)M bicuculline. Thus, bicuculline has about \( 1/\omega \) with the affinity of GABA for the receptor binding sites.

**Amino acid and drug competition for \([\text{H}]\)GABA binding**

Several authors have evaluated the relative potency of amino acids mimicking the synaptic inhibitory properties of GABA (26–29). To discriminate between inhibitory effects produced by amino acids that structurally resemble both glycine and GABA, investigators have evaluated the degree to which the inhibition can be antagonized selectively by strychnine or bicuculline (30, 31). \([\text{H}]\)GABA can bind to membranes and associate with the presynaptic neuronal uptake of GABA (14, f). To differentiate between the binding of the presynaptic GABA receptor and of presynaptic GABA uptake sites, we have compared the abilities of a variety of amino acids to inhibit \([\text{H}]\)GABA binding to synaptic membranes and \([\text{H}]\)GABA accumulation by synaptosomal fractions of rat brain (Table 2).

The potencies of various amino acids in inhibiting \([\text{H}]\)GABA binding to synaptic membranes closely parallel their neurophysiologic inhibitory potency and differ markedly from their relative affinities for the synaptosomal GABA uptake system. Thus 3-aminopropane sulfonic acid, which neurophysiologically is somewhat more potent than GABA, in repeated experiments has a slightly greater affinity for GABA binding sites than GABA itself. By contrast, 3-amino propane sulfonic acid has very little affinity for synaptosomal uptake sites for GABA, producing only 50% inhibition at 1 mM concentration, whereas GABA itself has an affinity for the presynaptic uptake system of about 20 \( \mu \)M. Similarly, imidazoleacetic acid, which neurophysiologically is 50–75% as potent as GABA, is almost as potent as GABA itself in lowering \([\text{H}]\)GABA binding. However, imidazoleacetic acid has negligible affinity for synaptosomal GABA uptake, producing only 13% inhibition at 1 mM concentration. 3-Hydroxy GABA is slightly weaker than imidazoleacetic acid both neurophysiologically and in its effects on specific GABA binding to synaptic membranes. It lowers GABA binding by 50% at 1 \( \mu \)M concentration, while requiring 100 \( \mu \)M concentration to reduce synaptosomal uptake of GABA by 50%.

While imidazoleacetic acid potently mimics the synaptic inhibitory effects of GABA, addition of a methyl group to form 1-methylimidazole acetic acid produces a compound devoid of neurophysiologic activity and essentially devoid of ability to compete for \([\text{H}]\)GABA binding sites in synaptic membrane preparations. Yet both imidazoleacetic acid and 1-methylimidazoleacetic acid have the same low affinity for the synaptosomal GABA uptake system. Similarly imidazole lactic acid has only 20% neurophysiologic activity of imidazoleacetic acid in mimicking GABA and is substantially less potent than imidazoleacetic acid in displacing \([\text{H}]\)GABA binding to synaptic membranes.

While numerous amino acids with potent GABA-like neurophysiologic actions lack affinity for the synaptosomal GABA uptake system, 2,4-diaminobutyric acid is a potent inhibitor of synaptosomal GABA uptake (10) but is devoid of neurophysiologic inhibitory action in the mammalian central nervous system. In our experiments, 2,4-diaminobutyric acid is a very weak inhibitor of \([\text{H}]\)GABA binding to synaptic membranes, reducing binding only 24% at 1 mM concentration, while it lowers synaptosomal GABA uptake 65% at 0.1 mM concentration.

\( \alpha \)-Alanine, \( \beta \)-alanine, taurine, and \( \beta \)-aminoisobutyric acid structurally resemble both glycine and GABA. However, in most systems examined, their synaptic inhibitory effects are antagonized predominantly by strychnine and much less by bicuculline, so that they appear to have major affinities for the glycine receptor and much less affinity for the synaptic GABA receptor (30, 31). Glycine, \( \beta \)-aminoisobutyric acid, \( \beta \)-alanine, and taurine have very little affinity for \([\text{H}]\)GABA binding sites in synaptic membrane fractions, none of them producing more than 6% inhibition of specific \([\text{H}]\)GABA binding even at 10 \( \mu \)M concentration. Strychnine, whose affinity constant for the glycine receptor of rat central nervous system is 2 nM (17, 18, 20), requires 10,000 times higher concentrations to reduce \([\text{H}]\)GABA binding to synaptic membranes by 50%.

To further assess the specificity of amino acids that displace GABA binding to synaptic membranes, we evaluated a wide range of putative neurotransmitters and psychotropic agents, none of which inhibits \([\text{H}]\)GABA binding to synaptic membranes at 0.1 mM concentration (Table 2). Of special importance is the lack of effect of aminoxyacetic acid, a compound that inhibits GABA transaminase, suggesting that no relevant binding to this enzyme occurs in the present system.

**Regional distribution of \([\text{H}]\)GABA binding to synaptic membrane fractions in the rat central nervous system**

There are marked regional variations in \([\text{H}]\)GABA binding to synaptic membrane fractions of rat brain (Table 3). Highest binding occurs in the cerebellum. The thalamus, hippocampus, cerebral cortex and midbrain display intermediate values, about 50–60% of binding in the cerebellum. \([\text{H}]\)GABA binding in the corpus striatum is only about two-thirds of binding in the midbrain. Lowest levels of GABA binding are obtained in the medulla oblongata–pons and the spinal cord, which have only half the binding of the corpus striatum and one-
TABLE 2. Competition by amino acids and other compounds for specific [\(^3\)H]GABA binding and synaptosomal [\(^3\)H]GABA uptake

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent inhibition of specific [(^3)H]GABA binding at a concentration of:</th>
<th>Percent inhibition of [(^3)H]GABA uptake at a concentration of:</th>
<th>Percent of GABA-like neurophysiological activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>5 × 10^{-5} M       10^{-5} M       10^{-4} M       10^{-3} M       10^{-2} M</td>
<td>10^{-4} M       10^{-3} M       10^{-2} M</td>
<td></td>
</tr>
<tr>
<td>GABA</td>
<td>30                  50                  96                  96                  100</td>
<td>40                  85                  100                  100</td>
<td></td>
</tr>
<tr>
<td>3-Aminopropanoic acid</td>
<td>41                  67                  93                  100                 100</td>
<td>55                  130–150                50–70</td>
<td></td>
</tr>
<tr>
<td>Sulfonic acid</td>
<td>10                  45                  79                  100                 —</td>
<td>13                  50–70                 50–70</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxy GABA</td>
<td>48                  96                  96                  —                   —</td>
<td>10                  52                  100                  100</td>
<td></td>
</tr>
<tr>
<td>(\beta)-Alanine</td>
<td>0                   6                   6                   —                   —</td>
<td>35                  0                    0                    0</td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>0                   5                   5                   —                   —</td>
<td>8                   —                    —                    —</td>
<td></td>
</tr>
<tr>
<td>2,4-Diaminobutyric acid</td>
<td>0                   3                   0                   0                   0</td>
<td>11                  65                  100                  100</td>
<td></td>
</tr>
<tr>
<td>1-Methylimidazol- (\alpha)-acetic acid</td>
<td>0                   —                   —                   —                   —</td>
<td>—                   —                    —                    —</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>0                   —                   —                   42                  —</td>
<td>—                   —                    —                    —</td>
<td></td>
</tr>
<tr>
<td>Imidazole lactic acid</td>
<td>0                   0                   0                   0                   10</td>
<td>0                   10                   0                    0</td>
<td></td>
</tr>
<tr>
<td>L-Histidine</td>
<td>0                   0                   0                   —                   —</td>
<td>13                  0                    0                    0</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Alanine</td>
<td>0                   0                   0                   —                   —</td>
<td>0                   0                    13                   —</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0                   0                   0                   —                   —</td>
<td>0                   0                    0                    14</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0                   0                   0                   —                   —</td>
<td>0                   0                    0                    13</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0                   0                   0                   —                   —</td>
<td>0                   0                    0                    0</td>
<td></td>
</tr>
<tr>
<td>Imidazole</td>
<td>0                   0                   0                   —                   —</td>
<td>0                   0                    0                    0</td>
<td></td>
</tr>
<tr>
<td>Aminoxyacetic acid</td>
<td>0                   0                   0                   —                   —</td>
<td>23                  0                    0                    0</td>
<td></td>
</tr>
<tr>
<td>(\beta)-aminobutyric acid</td>
<td>0                   0                   0                   —                   —</td>
<td>—                   —                    —                    —</td>
<td></td>
</tr>
</tbody>
</table>

**Other Compounds**

| Bicuculline          | 34                  67                  100                 5                   —                    |                                                  |
| Strychnine           | 38                  71                  52                  52                  52                  |                                                  |

Values are means of data from two separate experiments, each performed in triplicate, whose values varied less than 15%. Values presented as zero inhibition represent less than 5% inhibition, the minimum that can be detected reliably.

Compounds that have no effect on [\(^3\)H]GABA binding to crude synaptic membrane fractions at 0.1 mM concentration include: 3-p-aminoalicyclic acid, isoniazid, pyridoxine, nicotinamide, pyridoxal chloride, urea, ergocristine, tõlbuthamide, acetazolamide, \(\alpha\)-methyl-dopa, methylergide, hippuric acid, alloxan, antiobiotic acid, melatonin, oxotremorine, meprobamate, pentobarbital, diphenylhydantoin, ketamine, lithium carbamate, ethanol, acetophenetidine, reserpine, benzoylsody, haloperidol, chlor Diazepoxide, cyclandelate, diethylbarbazine, dichlorphenamides, lidocaine, ethosuximide, chloro phenesin, phenacemide, carbamazepine, aspartic acid, \(\alpha\)-alanine, morpine, aminoxyacetic acid.

* Data for imidazole analogues are from Godfraind et al. (43) and data for other compounds are from Curtis et al. (30), and Curtis and Watkins (27, 29).

sixth the binding of the cerebellum. In general, these regional differences do not closely resemble regional variations in endogenous GABA, whose levels are generally highest in the corpus striatum and hypothalamus and relatively low in the cerebellum (32–34). However, in monkey brain the regional distribution of GABA receptor binding does correlate closely with endogenous GABA. Since the receptor assay was performed on the disrupted P\(_1\) fraction it was possible that the regional differences observed were related to a loss of receptor binding in the P\(_2\) fraction, which may vary from one brain area to another. This is unlikely, because in other experiments† regional variations in specific [\(^3\)H]GABA binding using osmotically lyzed whole homogenates are identical to those seen using a disrupted P\(_2\).

**DISCUSSION**

The specific binding of [\(^3\)H]GABA to synaptic membrane fractions appears to represent an interaction with the postsynaptic receptor for the neurotransmitter actions of GABA. There is an impressive correlation between the ability of amino acids to mimic the neurophysiologic actions of GABA and their ability to inhibit [\(^3\)H]GABA binding to synaptic membranes. Bicuculline, a neurophysiologic GABA antagonist, has about 1/4th the affinity of GABA for the GABA binding sites in synaptic membranes. By contrast, strychnine, the synaptic GABA antagonist, has about 10,000 times the affinity of GABA for the GABA receptor. These data are consistent with neurophysiologic evidence that bicuculline is a much weaker agonist of GABA than strychnine of GABA. These data are consistent with neurophysiologic evidence that bicuculline is a much weaker antagonist of GABA than strychnine of GABA. However, this inhibition of [\(^3\)H]GABA binding to synaptic membranes. Neurophysiologically, bicuculline is thought to impair chloride conductance changes associated with GABA in the crayfish and not to compete for the GABA recognition site (35). This conceivably may account for its inability to inhibit the [\(^3\)H]GABA binding sites.

Several other investigators have measured [\(^3\)H]GABA binding to membrane fractions of mammalian brain (36, 37) and crayfish (38). Peck et al. (36) employed chlorpromazine in their incubation mixtures in an attempt to inhibit the nerve terminal GABA uptake system. However, since their incubations were conducted in the presence of sodium, it is likely that a majority of binding occurred to presynaptic uptake sites.
and not to the receptor, as evidenced by the very weak effects of bicuculline on GABA binding and the failure of variations in sodium concentration to alter the bicuculline sensitivity. Moreover, the affinity constants they obtained for GABA (21 μM) and bicuculline (80 μM) are the same as the affinity constants of these compounds for the presynaptic GABA uptake system (3, 4) and 100 times greater than values we have observed for the specific receptor binding of GABA to synaptic membranes. Omission of sodium from the incubation medium in our studies appears to facilitate identification of postsynaptic GABA receptors. The inability of other workers (36, 37) to detect the GABA receptor even in the absence of sodium is probably explained by their use of fresh tissue in which sodium-dependent binding unrelated to the postsynaptic GABA receptor predominates. Though we can measure GABA receptor binding in fresh tissue in the absence of sodium, storing frozen synaptic membranes greatly lowers the sodium-dependent binding and enhances GABA receptor binding.

In the presence of sodium GABA binding to synaptic membranes appears to involve uptake sites. Sodium-dependent binding is about 10 times greater than sodium-independent receptor binding. Sodium-dependent binding is potently inhibited by diamoobutyric acid, which has little receptor binding affinity, but is hardly affected by bicuculline, imidazoleacetic acid and 3-aminopropanesulfonic acid, which have considerable receptor affinity.

GABA binding to membrane fractions of crayfish muscle measured in the presence of sodium has an affinity which is greater than that of the GABA uptake process, but is inhibited by a variety of amino acids and bicuculline in proportion to their affinity for the GABA presynaptic uptake, correlating poorly with neurophysiologic effects of GABA in crayfish muscle (38). The affinity constant for [H]GABA binding to synaptic membranes in our study is about 1/100th that of GABA for the presynaptic uptake sites. [H]GABA binding is inhibited 50% by bicuculline at concentrations less than 1/100th of those required to inhibit synaptosomal GABA uptake and is affected by amino acids in proportion to their neurophysiologic receptor actions, which show no correlation with amino-acid affinity for presynaptic GABA uptake.

The regional distribution of [H]GABA binding to synaptic membranes obtained from rat brain does not correlate closely with regional variations in endogenous GABA. However, in 30 regions of monkey brain† we have observed a close correlation between postsynaptic GABA receptor binding and endogenous GABA. Moreover, it should be borne in mind that receptor density need not correlate with the content of endogenous transmitter in presynaptic boutons. The relative surface area of postsynaptic membrane may vary throughout the brain independently of the volume or transmitter content of associated nerve terminals. Thus, the relative density of muscarinic cholinergic receptors in various regions of the brain only shows a limited correlation with levels of endogenous acetylcholine (39, 40). Similarly, [H]lysergic acid diethyl amide binding to apparent postsynaptic serotonin receptors shows very little correlation with endogenous levels of serotonin, being higher in the cerebral cortex than in the hypothalamus, while levels of endogenous serotonin are markedly greater in the hypothalamus than in the cerebral cortex (41; J. P. Bennett and S. H. Snyder, in preparation). Moreover, norepinephrine-sensitive cyclic AMP accumulating systems, presumably associated with norepinephrine receptor sites in the brain, are most enriched in the cerebellum, which contains the lowest levels of endogenous norepinephrine in the brain (42).

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<table>
<thead>
<tr>
<th>Region</th>
<th>Specific (bicuculline-displaceable) [H]GABA binding (cpm/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>2946 ± 407</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1824 ± 43</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1752 ± 280</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1610 ± 78</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>1593 ± 145</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1553 ± 122</td>
</tr>
<tr>
<td>Corpus striatum</td>
<td>1080 ± 95</td>
</tr>
<tr>
<td>Medulla oblongata-pons</td>
<td>523 ± 46</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>465 ± 38</td>
</tr>
</tbody>
</table>

Brains and spinal cords from 24 rats were pooled, rapidly dissected by the method of Gliwinski and Iversen (44), and placed in ice-cold 0.32 M sucrose. The tissues were prepared as described for the whole brains in Materials and Methods, and samples were assayed by the standard technique. The values given are the means of triplicate determinations from two separate experiments ± the standard error of the mean.