Neuroscience Vol. 34, No. 2, pp. 341–357, 1990
Printed in Great Britain

0306-4522/90 $3.00 + 0.00
Pergamon Press plc

DISTRIBUTION AND KINETICS OF $GABA_B$ BINDING SITES IN RAT CENTRAL NERVOUS SYSTEM: A QUANTITATIVE AUTORADIOGRAPHIC STUDY

D. C. M. CHU, R. L. ALBIN, A. B. YOUNG and J. B. PENNEY*
Department of Neurology and the Neuroscience Program, University of Michigan, Ann Arbor, MI 48104, U.S.A.

Abstract—[^{3}H]GABA quantitative autoradiography was used to examine the binding kinetics and regional distribution of $GABA_B$ receptors in rat brain. The regional distribution was compared to that of $GABA_A$ receptors. At 4°C, [^{3}H]GABA binding to $GABA_B$ receptors reached equilibrium within 45 min. The association and dissociation rate constants for $GABA_B$ binding to outer neocortical layers were $2.87 \pm 0.17 \times 10^5$ min^{-1} M^{-1} and 0.0966 ± 0.0118 min^{-1}, respectively, indicating a dissociation constant of 336 ± 40 nM. Saturation binding studies in the same region yielded a dissociation constant for $GABA_B$ receptors of 341 ± 41 nM while that of $GABA_A$ receptors was 92 ± 10 nM. While the affinities of each type of GABA receptor were uniform across brain regions, the maximal number of binding sites for both types of GABA receptor varied across regions. The distributions of the two receptors in rat brain were different in the olfactory bulb, cerebellum, thalamus, neocortex, medial habenula and interpeduncular nucleus. Areas high in $GABA_B$ binding included the medial and lateral geniculates, the superior colliculus and certain amygdaloid nuclei. Binding to white matter tracts and ventricles was negligible.

The distribution of $GABA_B$ receptors was in agreement with previously postulated sites of action of baclofen.

Much evidence supports the existence of at least two classes of receptors for the inhibitory amino acid neurotransmitter GABA.[11,25,27] These pharmacologically and physiologically distinct classes of GABA receptors have been termed the $GABA_A$ and $GABA_B$ receptors. Several agonists selectively activate $GABA_A$ receptors, including muscimol,[67] isoguvacine[42] and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP).[28] To date, baclofen (Lioresal, *p*-chlorophenyl GABA) is the only known agonist selective for $GABA_B$ receptors.[12–14] $GABA_A$ receptors have traditionally been characterized by their sensitivity to blockade by the GABA antagonist, bicuculline,[46] while $GABA_B$ receptors are by definition bicuculline-insensitive.[11] Both types of GABA receptors cause inhibition, but activate different ionic mechanisms.[26] $GABA_A$ receptors are coupled to the benzodiazepine/barbiturate receptor-linked chloride channel.[35,55,79] $GABA_B$ receptors have been associated with an inhibitory GTP-binding protein[2] and adenylate cyclase activity.[3,41,83] In hippocampal pyramidal neurons, $GABA_B$ receptor activation is associated with an increased outward potassium conductance[2,32,43] or a calcium-dependent potassium conductance.[9] The effect of baclofen on dorsal root ganglion neurons, however, is a direct decrease in calcium conductance.[23,26]

GABA receptor autoradiography also indicates that these two receptors have distinct anatomical distributions[13,15,35] within certain brain and spinal cord regions. In this report, we describe the kinetic properties of $GABA_B$ receptors and their regional distribution in rat brain as measured by quantitative autoradiography using [^{3}H]GABA. A comparison of the relative affinities and number of $GABA_A$ and $GABA_B$ binding sites in various brain regions is presented. The characterization of these receptors in normal rat brain in the present study has enabled us to examine their regulation after various experimental lesions[19] and to investigate their status in normal and pathologic human brains.[17,18]

EXPERIMENTAL PROCEDURES

Materials

[^{3}H]GABA (57 or 71.5 Ci/mmol) was obtained from Amersham Inc. (Arlington Heights, IL). Isoguvacine was purchased from Cambridge Research Biochemicals Cambridgeshire, U.K.). The racemic mixture of baclofen was donated by Ciba-Geigy Pharmaceuticals (Suffern, NY). Non-radioactive GABA was purchased from Sigma Chemicals (St. Louis, MO).

Tissue preparation

Male Sprague–Dawley rats (Spartan Sprague–Dawley) weighing 200–250 g were decapitated. Their brains were rapidly dissected and frozen over powdered dry ice. Brains were mounted with embedding matrix onto cryotome chucks and were allowed to thermoequilibrate in the cryostat at −20°C. Serial brain sections (20-μm-thick, in the horizontal or coronal plane) were cut on a Lipshaw cryotome at −20 to −15°C. The section thickness of 20 μm affords preservation of optimal tissue integrity through the various rinse procedures. The latent images generated on tritium-sensitive film represent emissions arising 5 μm or less from the tissue surface[75] and thus, at

*To whom correspondence should be addressed.
Abbreviation: THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol.

20 μm, variations in the thickness of the sections do not affect the density of the autoradiographic image.[39]

The tissue sections were placed on microscope slides previously coated with chrome–alum gelatin. These sections were then thaw-mounted onto the slides over a warming plate. Tissue sections thus obtained were subjected to a 15 min prewash 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 quantitative autoradiography

[^{3}H]GABA was used to study both $GABA_B$ and $GABA_A$ receptors. $GABA_B$ receptors were examined with [^{3}H]GABA in the presence of 10 μM isoguvacine, an agonist which specifically occupies $GABA_A$ binding sites. Conversely, $GABA_A$ receptors were assayed with [^{3}H]GABA in the presence of 100 μM (±)baclofen. The concentrations of baclofen and isoguvacine needed to block binding to either $GABA_B$ or $GABA_A$ receptors, respectively, were determined in separate inhibition experiments. Non-specific [^{3}H]GABA binding was determined by the inclusion of 100 μM isoguvacine and 100 μM (±)baclofen. Unless otherwise indicated, assay conditions for both $GABA_B$ and $GABA_A$ binding sites involved a 45 min incubation at 4°C with [^{3}H]GABA in 50 mM Tris–HCl + 2.5 mM $CaCl_2$ (pH 7.40). Slides were placed in vials containing the radioligand–buffer mixture. 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 s. The slides were mounted in an X-ray cassette and apposed to a sheet of tritium-sensitive Ultrofilm-^{3}H (LKB) for 3 weeks at 4°C. After that time, the films were developed in Kodak D19 for 3 min at 25°C, fixed and dried. The films were placed in a photographic enlarger and optical densities in various regions of the film were quantified using computer-assisted microdensitometry with a spot densitometer.[22] Twenty to twenty-five readings from each region of interest on each section were averaged. Optical densities in various regions of interest were quantified in units of pmol of [^{3}H]GABA bound/mg protein. These values were obtained by comparing film densities of the brain regions with those generated by ^{14}C-embedded plastic standards which had previously been calibrated against brain paste standards containing known amounts of tritium and protein.[65] The relationship between the amount of radioactivity and the optical density was described by a computer-generated fourth order polynomial function.

Determination of kinetic constants

Separate experiments to study the association and dissociation of [^{3}H]GABA to $GABA_B$ receptors were conducted using four animals for each study. To investigate the association of [^{3}H]GABA (19.48 nM, 57 Ci/mmol) to $GABA_B$ sites, tissues sections were incubated with [^{3}H]GABA for increasing periods of time ranging from 30 s to 120 min. Non-specific binding, assessed by the inclusion of 100 μM (±)baclofen, was determined in adjacent sections for each time point examined. The assay medium also contained 10 μM isoguvacine to prevent binding of [^{3}H]GABA to $GABA_A$ sites. For the determination of dissociation rate constants, binding of [^{3}H]GABA (24 nM, 71.5 Ci/mmol) under $GABA_B$-preferring conditions was carried out for 45 min at 4°C. At this point, the dissociation of [^{3}H]GABA from $GABA_B$ sites was assessed by placing slides into large volumes of buffer (method of infinite dilution) for varying periods of time ranging from 0 to 90 min. For each time point, non-specific binding was determined in an adjacent section. The slides were then rapidly rinsed once with 2.5% glutaraldehyde in acetone and dried as previously described. Bound values of [^{3}H]GABA for both experiments were analysed densitometrically in layers I–III of cerebral cortex. Association of [^{3}H]GABA to $GABA_B$ receptors was assumed to obey pseudo-first order kinetics. The observed rate constant of association (k_{obs}) was obtained from the slope of a plot of $\ln(B_e/B_e - B_t)$ vs time, where B_e was the amount of [^{3}H]GABA bound at equilibrium (45 min) and B_t was that bound at time t. Similarly, the dissociation rate constant (k_{-1}) was determined by the slope of $\ln(B_0/B_t)$ vs time, where B_0 was the amount of [^{3}H]GABA bound at 45 min and B_t was that which bound at various times after infinite dilution of label. The association rate constant (k_{+1}) was calculated from the following relationship between the observed rate constant (k_{obs}) and the dissociation rate constant (k_{-1}): $k_{+1} = (k_{obs} - k_{-1})/[\text{free GABA}]$. All slopes were calculated by computer-assisted linear regression. An equilibrium dissociation rate constant (K_D) was determined for these data and compared to K_D values obtained by saturation studies.

Saturation analyses

In order to determine affinities (K_D values) and densities (B_{max} values) of both $GABA_B$ and $GABA_A$ receptors, saturation studies were conducted using the method of isotopic dilution of [^{3}H]GABA (5–28 nM, 57 Ci/mmol) with non-radioactive GABA. For $GABA_B$ receptors, 10 μM isoguvacine was present in all the vials, while 100 μM (±)baclofen was added to the $GABA_A$ assay medium. The range of free [^{3}H]GABA concentrations spanned from 5 nM to 1 μM. Four animals each were used for $GABA_B$ and $GABA_A$ saturation experiments. Non-specific binding was assessed in serially adjacent sections for each concentration of free radioactive [^{3}H]GABA examined. Values of bound [^{3}H]GABA were quantified in several regions of interest: sensorimotor cortex, neostriatum, hippocampal dentate molecular layer, and cerebellar granule and molecular layers. These were used to construct Scatchard plots which were analysed by the iterative computer curve-fitting program LIGAND (SCAFIT).[59] B_{max} and K_D values and corresponding Hill coefficients were determined for both GABA receptor subtypes.

Inhibition experiments

Competition of [^{3}H]GABA binding by isoguvacine or baclofen was investigated in separate series of experiments (each $n = 4$). Isoguvacine competition assays were conducted in the absence of any baclofen; however, baclofen competition experiments were performed in the presence of 40 μM isoguvacine. Hill plots were constructed to determine IC_{50} values for binding to discrete layers of the cerebellum. K_I values were calculated according to the equation: $K_I = IC_{50}/(1 + [\text{GABA}]/K_D)$.

Regional distribution of GABA receptor subtypes

Total GABA, $GABA_A$ and $GABA_B$ binding sites were mapped in serial sections at nine coronal levels of the rat brain using [^{3}H]GABA (23 nM, 71.5 Ci/mmol). Values of [^{3}H]GABA bound under $GABA_A$- or $GABA_B$-preferring conditions were quantified in several regions of interest in four animals. As before, non-specific binding was that which remained on adjacent sections in the presence of 100 μM isoguvacine and 100 μM (±)baclofen. The corresponding tissue sections were stained for Nissl substance with Cresyl Violet to identify the structures in the autoradiograms.

RESULTS

Kinetics of [^{3}H]GABA binding to baclofen-sensitive $GABA_B$ sites in rat brain

Binding of [^{3}H]GABA to $GABA_B$ sites reached equilibrium within 45 min at 4°C (Fig. 1). Preliminary studies revealed that $GABA_B$ binding at 4°C for 45 min was identical in all respects to $GABA_B$ binding

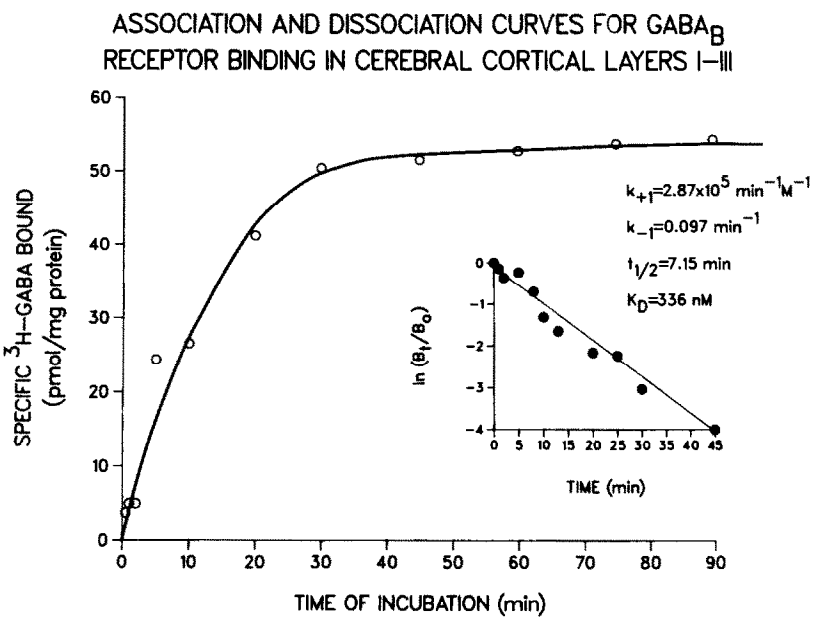

Fig. 1. Association and dissociation curves for [^{3}H]GABA binding of $GABA_B$ receptors in rat cerebral cortical layers I–III from a representative animal. Association of [^{3}H]GABA (19 nM, 57 Ci/mmol) to $GABA_B$ receptors for increasing periods of time was studied in rat brain horizontal sections in the presence of 10 μM isoguvacine to block binding to $GABA_A$ sites. Individual serial sections were used for total $GABA_B$ binding and non-specific binding at each time point examined. Non-specific $GABA_B$ binding was defined as the amount of [^{3}H]GABA bound in the presence of 100 μM (±)baclofen and 100 μM isoguvacine. Inset: dissociation of [^{3}H]GABA binding (24 nM, 71.5 Ci/mmol) under $GABA_B$-preferring conditions was obtained after attaining equilibrium (after 45 min). Dissociation was initiated by the method of infinite dilution of label for varying periods of time (see Experimental Procedures section). All bound values were determined by computer-assisted spot densitometry. The kinetic values given in the figure represent the mean of four animals. B_o, the amount of [^{3}H]GABA bound to $GABA_B$ receptors at equilibrium (45 min); B_t, the amount bound at time t.

Table 1. Comparison of B_{max}, K_D and n_H values for $GABA_A$ and $GABA_B$ receptors in various regions of rat brain

Area	$GABA_A$	$GABA_B$
	B_{max} (pmol GABA bound/mg protein)	
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
	Hill number (n_H)	
Cerebral cortex		
Layers I–III	0.98 ± 0.02	0.98 ± 0.05
Layer IV	1.08 ± 0.09	1.02 ± 0.05
Neostriatum	0.99 ± 0.10	0.99 ± 0.05
Dentate gyrus		
Molecular layer	1.00 ± 0.05	1.11 ± 0.03
Cerebellum		
Molecular layer	1.03 ± 0.05	1.09 ± 0.04
Granular layer	1.01 ± 0.07	0.95 ± 0.10

Values represent mean ± S.E.M. of four animals.

at 20°C for 20 min, with the advantage that non-specific binding was greatly reduced at the lower temperature. Binding to baclofen-sensitive $GABA_B$ sites was reversible and displayed association and dissociation rate constants within layer I–III of cerebral cortex of $2.87 \pm 0.17 \times 10^5$/min per M and 0.097 ± 0.012/min, respectively. These values yielded an equilibrium dissociation constant (K_D) of 336 ± 40 nM. This estimate of $GABA_B$ affinity was in close agreement with the value of 341 ± 41 nM for cerebral cortex as disclosed by saturation experiments (Table 1). Figure 2 shows a typical saturation isotherm and the associated Scatchard plot for layers I–III of cerebral cortex.

The binding affinity (K_D) of [^{3}H]GABA for $GABA_B$ receptors was roughly three- to four-fold less than its affinity for $GABA_A$ receptors (Table 1). The K_D values of $GABA_B$ binding sites in various regions of the rat CNS generally ranged from 300 to 500 nM, whereas the K_D values of $GABA_A$ binding sites were within the range of 100–200 nM. While the affinities of $GABA_B$ and $GABA_A$ receptors were fairly constant across different brain regions, their densities (B_{max}) varied from region to region. For example, within cortical layers I–III there were roughly equal numbers of $GABA_B$ and $GABA_A$ receptors, while more $GABA_A$ than $GABA_B$ receptors were present in layer IV. Similarly, the molecular layer of the

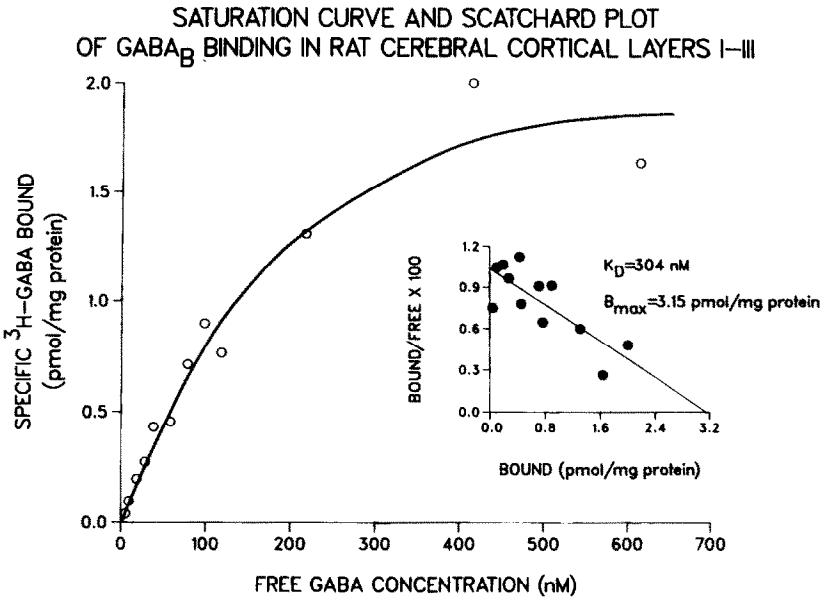

Fig. 2. Saturation of $GABA_B$ binding sites by increasing concentrations of [^{3}H]GABA in the presence of 10 μM isoguvacine. The curve shows $GABA_B$ binding in cerebral cortical layers I–III from a representative normal rat brain. Saturation was achieved by isotopic dilution of [^{3}H]GABA with known concentrations of non-radioactive GABA. Bound values were calculated with appropriate adjustments of the resultant specific activity. Inset: Scatchard (Rosenthal) plot of the same data to yield B_{max} (x-intercept) and K_D (negative reciprocal slope) values.

cerebellum was enriched in $GABA_B$ receptors while $GABA_A$ receptors were more numerous in the cerebellar granule cell layer. In striatum and dentate gyrus, where both types of receptors were found, $GABA_B$ binding sites accounted for roughly 40% of all GABA receptors. The Hill coefficients for both $GABA_B$ and $GABA_A$ binding sites in several regions were close to unity (Table 1).

Displacement studies in the cerebellum (Fig. 3) demonstrated that a combination of 40 μM isoguvacine plus 100 μM (±)baclofen was able to displace all specific [^{3}H]GABA binding. These data suggest that under these conditions only single populations of $GABA_A$ and $GABA_B$ receptors can be distinguished.

Regional distribution of $GABA_B$ and $GABA_A$ binding sites in rat brain

Specific [^{3}H]GABA binding of $GABA_B$ and $GABA_A$ receptors in a number of brain regions is shown in Table 2. The distribution of $GABA_B$ and $GABA_A$ binding sites displayed marked heterogeneity in some regions, and striking similarity in others, as demonstrated in the autoradiograms and corresponding Nissl-stained sections in Fig. 4–9. For example, in the olfactory bulb, $GABA_B$ binding was highest in the glomerular layers, whereas $GABA_A$ binding occurred predominantly in the external plexiform and inner granular layers. In several other regions, as exemplified by the geniculate bodies and superior colliculus, the distribution of $GABA_B$ receptors paralleled that of $GABA_A$ receptors.

[^{3}H]GABA exhibited differential binding to $GABA_B$ and $GABA_A$ sites in various cortical layers and lobes. Binding to both types of GABA receptors was highest in parietal cortex, followed in order by striate, temporal, frontal, retrosplenial, piriform and entorhinal cortices. The anterior cingulate cortex exhibited greater [^{3}H]GABA binding than did the posterior cingulate cortex. In general, binding to $GABA_A$ receptors occurred in a distinct laminar pattern to cortical layers I–IV and VIa, while $GABA_B$ binding was primarily restricted to cortical layers I–III.

With notable exceptions, a consistent finding in virtually all brain regions examined was that $GABA_A$ binding accounted for roughly 70–80% of total [^{3}H]GABA binding, while $GABA_B$ binding represented 20–30% (Table 2). Exceptions to this generalization were seen in 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 the cerebellum. These regions exhibited the highest levels of $GABA_B$ binding, where these receptors accounted for as much as 90% of total [^{3}H]GABA binding. Other regions exhibiting high absolute values of $GABA_B$ binding sites were found in various cortical and thalamic structures and certain amygdaloid subnuclei (see Table 2).

Brain regions which exhibited intermediate levels of $GABA_B$ binding included the external plexiform layer of the olfactory bulb, accessory olfactory nucleus, lateral septal nuclei, posterior cingulate cortex, ventrolateral and ventromedial thalamus, anterior cortical and medial amygdala, the molecular layer of the dentate gyrus, and the granular layer of the cerebellum.

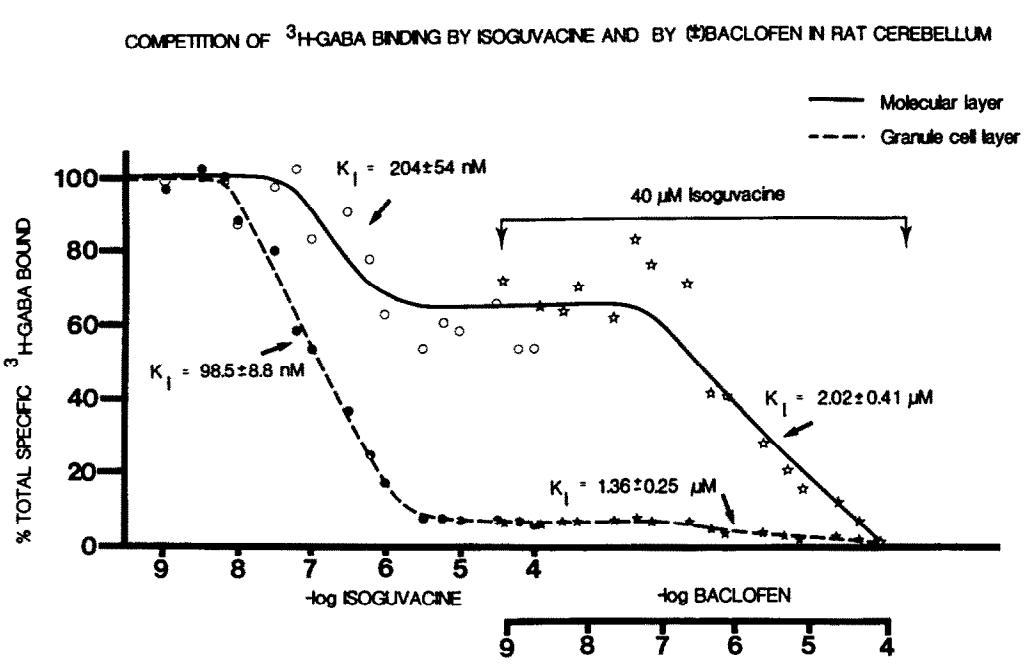

Fig. 3. Competition of [^{3}H]GABA (16 nM, 57 Ci/mmol) binding by isoguvacine and (±)baclofen in normal rat cerebellum. The solid line represents values obtained from the molecular layer while the dashed line represents data obtained from the granule cell layer. Increasing concentrations of isoguvacine compete for roughly 40% of total specific [^{3}H]GABA binding sites in the molecular layer and for 90% of total [^{3}H]GABA sites in the granule cell layer. In the presence of 40 μM isoguvacine, increasing concentrations of (±)baclofen effectively compete for the remaining 60% of total specific [^{3}H]GABA binding sites in the molecular layer and for 10% of [^{3}H]GABA binding sites in the granule cell layer. Inhibition constants (K_i for isoguvacine and baclofen were calculated by the equation: $K_i = IC_{50}/(1 + [GABA]/K_D)$ where the dissociation constants (K_D) were obtained by separate saturation studies. The points represent the mean of four animals. Standard errors of the mean were not plotted for the sake of clarity.

Relatively low amounts of binding to $GABA_B$ receptors (<0.1 pmol/mg protein) were observed in the neostriatum, globus pallidus, hippocampal formation, substantia nigra and other brainstem regions. $GABA_B$ binding to white matter tracts or ventricles was indistinguishable from non-specific background levels.

$GABA_A$ receptors generally predominated in cortical, septal and hippocampal regions as well as in certain thalamic relay nuclei (VPM and the geniculate bodies). Binding to $GABA_A$ receptors was also high in the external plexiform layer of the olfactory bulb and the granular layer of the cerebellum. Intermediate levels of binding to $GABA_A$ receptors occurred in basal ganglia, basal forebrain, amygdala, thalamus, superficial gray of the superior colliculus, central nucleus of the inferior colliculus, pre- and parasubiculum and the molecular layer of the cerebellum. Low amounts of $GABA_A$ binding were found in the hypothalamus, habenula, mammillary bodies, subthalamus, substantia nigra, and other midbrain and pontine regions.

DISCUSSION

Kinetics of [^{3}H]*GABA binding*

The distribution of $GABA_B$ receptors as revealed by [^{3}H]GABA autoradiography confirms and extends the findings of previous autoradiographic and homogenate binding studies which have used either [^{3}H](−)baclofen or [^{3}H]GABA as ligands for $GABA_B$ receptors.[12,13,15,24,34,42,81] The technique described here provides a sensitive assay for determining the affinity and number of both $GABA_B$ and $GABA_A$ receptors in anatomically discrete brain regions.

One concern is whether a component of the observed [^{3}H]GABA binding sites could represent uptake or sequestration of the ligand into membrane-bound saccules. However, the specific [^{3}H]GABA binding assay used here is unlikely to reflect binding to the GABA uptake site for the following reasons. Sodium ions, which have been shown to be required for [^{3}H]GABA uptake binding in tissue homogenates,[47] have been excluded from the assay medium. Furthermore, a specific antagonist of the GABA uptake site, nipecotic acid, did not block any component of [^{3}H]GABA binding (unpublished observations). The autoradiographic method involves freezing and thawing of brain sections—a step which has been shown to disrupt membrane-bound saccules.[47] The equilibrium dissociation constant (K_D) derived by kinetic experiments agreed well with the K_D determined by equilibrium saturation experiments—a feature of reversible equilibrium binding conditions that is inconsistent with sequestration of label into membrane saccules. In addition, all steps in the present assay

Table 2. Comparative regional distribution of $GABA_A$ and $GABA_B$ binding sites in rat brain

Area	$GABA_A$	$GABA_B$
Olfactory bulb		
External plexiform layer (EPL)	1627 ± 96	128 ± 9
Glomerular layer (Glom)	410 ± 39	521 ± 63
Inner granular layer (IGL)	212 ± 32	79 ± 12
Anterior olfactory nuclei (AON)	276 ± 61	140 ± 9
Cortex		
Frontal (Fr)		
layer I	626 ± 76	350 ± 40
layer II	721 ± 90	384 ± 31
Parietal (Par)		
layer I	891 ± 98	488 ± 58
layer II	981 ± 84	521 ± 74
layer III	936 ± 102	422 ± 35
layer IV	957 ± 102	360 ± 35
layer V	610 ± 80	260 ± 23
layer VIa	708 ± 93	230 ± 26
layer VIb	304 ± 50	111 ± 11
Anterior cingulate (ACg)	765 ± 142	335 ± 18
Posterior cingulate (PCg)	529 ± 56	160 ± 14
Entorhinal (Ento)	411 ± 38	208 ± 9
Primary olfactory (PO)	508 ± 83	208 ± 21
Retrosplenial (Rspl)	582 ± 64	169 ± 23
Temporal (Tem)	741 ± 51	335 ± 24
Visual (Str 17)	822 ± 58	339 ± 23
Septal area		
Lateral septal, dorsal (LSD)	343 ± 62	172 ± 24
Lateral septal, ventral (LSV)	312 ± 76	100 ± 22
Lateral septal, intermed. (LSI)	230 ± 41	92 ± 14
Horizontal diagonal band (HDB)	242 ± 29	53 ± 10
Medial septal (MS)	240 ± 50	63 ± 15
Septofimbrial nucleus (SFO)	48 ± 12	25 ± 12
Basal forebrain		
Ventral pallidum (VP)	298 ± 41	85 ± 22
Olfactory tubercle (Tu)	308 ± 53	55 ± 14
Bed nucleus stria terminalis		
medial (BSTM)	168 ± 35	108 ± 37
lateral (BSTL)	233 ± 47	92 ± 33
Nucleus basalis of Meynert (NBM)	194 ± 21	52 ± 15
Deep telencephalic nuclei		
Neostriatum (CPu)	336 ± 62	75 ± 8
Nucleus accumbens (NA)	351 ± 85	87 ± 17
Globus pallidus (GP)	123 ± 14	51 ± 13
Major island of Calleja (MCj)	523 ± 94	93 ± 12
Claustrum (Claus)	686 ± 92	205 ± 28
Endopiriform nucleus (Endo)	584 ± 151	183 ± 26
Amygdala (Amyg)		
basolateral nucleus (BLa)	299 ± 19	110 ± 32
lateral nucleus (Lat)	385 ± 34	170 ± 32
central nucleus (Ce)	231 ± 37	73 ± 11
anterior cortical (ACo)	338 ± 74	122 ± 21
basomedial nucleus (BM)	315 ± 25	186 ± 19
medial nucleus (Med)	341 ± 43	124 ± 20
posteromedial cortical (PMCo)	479 ± 17	217 ± 21
Hippocampus		
Dentate gyrus		
molecular layer (SMDG)	627 ± 80	170 ± 13
granular layer (SGDG)	164 ± 41	75 ± 15
CA3 region		
stratum oriens (Or)	275 ± 38	90 ± 22
stratum lucidum (Luc)	178 ± 39	77 ± 23
stratum radiatum (Rad)	297 ± 36	113 ± 25
lacunosum/moleculare (LM)	278 ± 41	112 ± 24
CA1 region		
stratum oriens	392 ± 69	84 ± 20
stratum pyramidale	422 ± 55	88 ± 17
stratum radiatum	442 ± 61	92 ± 21
lacunosum/moleculare	389 ± 58	102 ± 21
Subiculum (Sub)	285 ± 25	85 ± 12
Presubiculum (PreS)	638 ± 84	328 ± 54
Parasubiculum (ParaS)	416 ± 51	186 ± 36
Thalamic nuclei		
Paraventricular (Pv)	368 ± 67	216 ± 19
Dorsomedial (DM)	567 ± 101	286 ± 39
Lateral dorsal (LD)	611 ± 70	315 ± 47
Reticular (Ret)	84 ± 12	33 ± 12
Ventrolateral (VL)	486 ± 46	162 ± 17
Ventroposterolateral (VPL)	445 ± 68	137 ± 36
Ventroposteromedial (VPM)	702 ± 63	207 ± 14
Ventromedial (VM)	315 ± 73	156 ± 27
Globosus (Glb)	521 ± 108	201 ± 21
Reuniens (Re)	313 ± 69	210 ± 32
Centromedian (CM)	295 ± 24	176 ± 20
Parafascicular (Pf)	229 ± 37	96 ± 31
Lateral posterior (LP)	674 ± 123	291 ± 33
Posterior (Po)	560 ± 63	255 ± 29
Dorsolateral geniculate (DLG)	853 ± 93	317 ± 40
Ventrolateral geniculate (VLG)	150 ± 13	70 ± 16
Dorsomedial geniculate (MGD)	610 ± 84	317 ± 49
Ventromedial geniculate (MGV)	918 ± 100	367 ± 49
Stria medullaris thalami (Sm)	178 ± 68	87 ± 36
Lateral habenula (LHb)	88 ± 31	173 ± 19
Medial habenula (MHb)	64 ± 30	632 ± 58
Hypothalamus, other		
Medial preoptic area (MPOA)	207 ± 53	95 ± 25
Lateral preoptic area (LPOA)	162 ± 28	62 ± 14
Septohypothalamic nucleus (SHy)	145 ± 28	78 ± 26
Anterior hypothalamus (AH)	192 ± 28	60 ± 16
Lateral hypothalamus (LH)	102 ± 16	41 ± 12
Ventromedial hypothalamus (VMH)	234 ± 52	94 ± 18
Posterior hypothalamus (PH)	240 ± 26	105 ± 29
Arcuate nucleus (Arc)	182 ± 32	90 ± 22
Medial mammillary body (MM)	220 ± 83	119 ± 60
Lateral mammillary body (LM)	243 ± 81	111 ± 16
Subthalamus (ST)	234 ± 34	60 ± 10
Zona incerta (ZI)	108 ± 11	31 ± 10
Midbrain		
Superior colliculus		
superficial grey (SuGr)	611 ± 80	497 ± 74
optic nerve layer (ONL)	241 ± 16	114 ± 7
intermediate grey (IntGr)	278 ± 26	96 ± 14
intermediate white (IntWh)	144 ± 15	40 ± 6
deep grey (DpGr)	252 ± 10	76 ± 7
Anterior pretectal nucleus (APT)	176 ± 23	43 ± 5
Pretectal nucleus (Pt)	346 ± 61	93 ± 22
Dorsal central grey (CGD)	283 ± 17	147 ± 30
Central grey (CG)	210 ± 21	110 ± 25
Rostrolinear raphe (RLi)	194 ± 28	92 ± 20
Red nucleus (Red)	71 ± 15	11 ± 7
Substantia nigra		
pars compacta (SNC)	127 ± 29	66 + 11
pars reticulata (SNR)	217 ± 13	84 ± 5
Ventral tegmental area (VTA)	114 + 13	72 + 12
Other brainstem nuclei		
Inferior colliculus		
central nucleus (CIC)	498 ± 55	144 ± 28
Interpeduncular nucleus (IPN)	111 ± 21	362 ± 227
Dorsal raphe (DR)	275 ± 61	105 ± 15
Parabigeminal nucleus (PBG)	511 ± 92	57 ± 10
Cuneiform nucleus (Cn)	125 ± 8	58 ± 14
Pontine reticular formation (RF)	59 ± 3	22 ± 7
Pontine nuclei (PnN)	52 ± 18	56 ± 17
Pontine central grey (PnCG)	273 ± 9	144 ± 8
Dorsal parabrachial (DPN)	146 ± 14	106 ± 19
Mesencephalic nucleus of V (m5)	477 ± 188	145 ± 43
Ventral cochlear nucleus (VCN)	70 ± 15	42 ± 9
Cerebellum		
Molecular layer (Mol)	411 ± 27	453 ± 60
Granule cell layer (Gran)	2410 ± 155	158 ± 17

Values of [^{3}H]GABA bound (fmol/mg protein) represent mean ± S.E.M. of four animals. [^{3}H]GABA concentration was 23 nM. Autoradiograms were generated and quantified as described in the text.

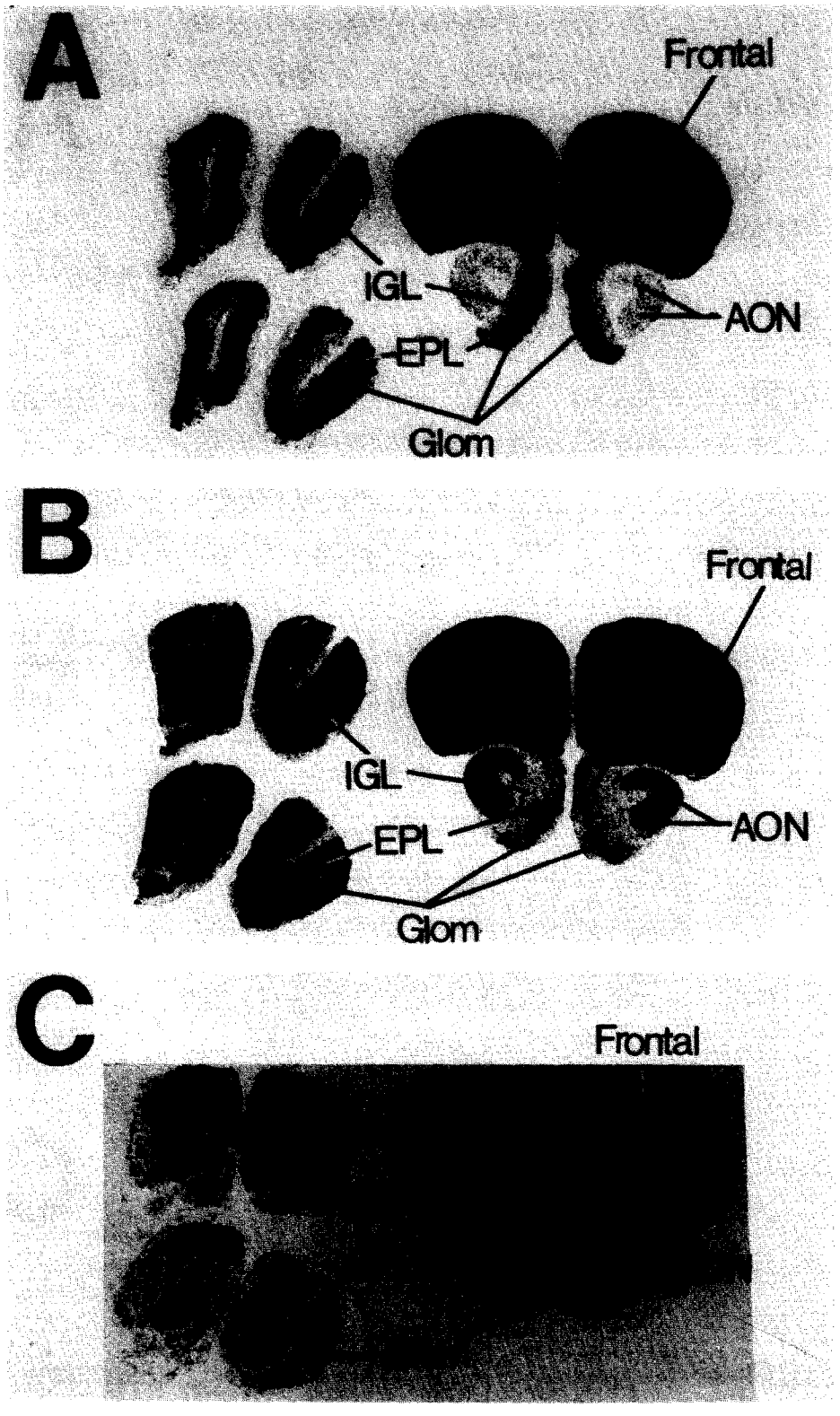

Fig. 4. Binding at the level of the olfactory bulb.

Figs 4–9. These figures depict the regional distribution of GABA binding sites in rat brain sections as generated by quantitative autoradiography using [^{3}H]GABA (23 nM, 71.5 Ci/mmol). Autoradiograms were generated as described in the Experimental Procedures section. All abbreviations are given in Table 2. Each figure shows [^{3}H]GABA binding to $GABA_A$ and $GABA_B$ sites in adjacent coronal sections as well as Cresyl Violet stained micrograph, in the following manner. (A) $GABA_A$ sites as shown by binding of [^{3}H]GABA in the presence of 100 μM (±)baclofen. (B) $GABA_B$ sites as shown by the binding of [^{3}H]GABA in the presence of 10 μM isoguvacine. (C) Nissl-stained micrographs of the corresponding brain sections.

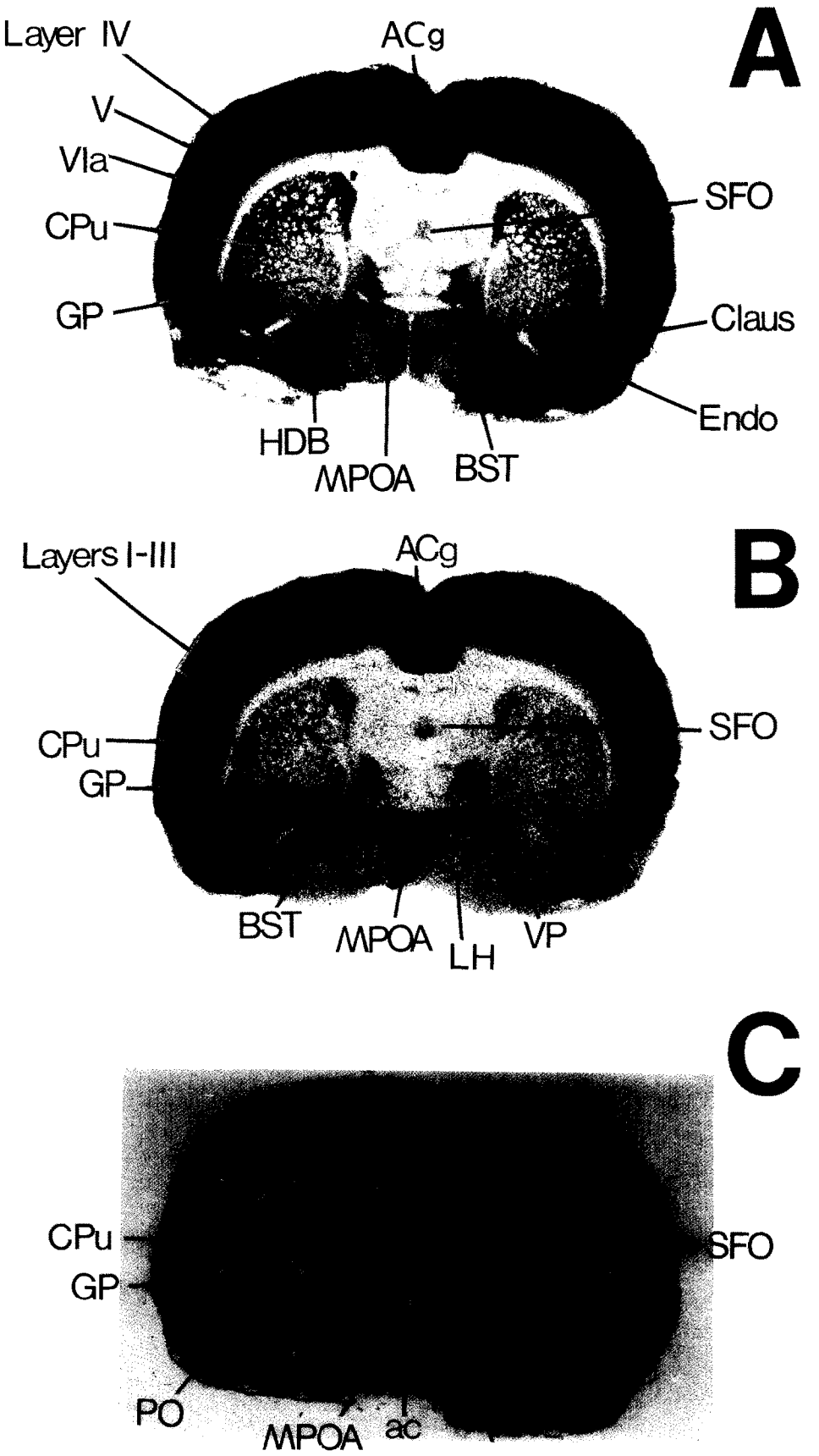

Fig. 5. Binding at the level of the anterior commissure.

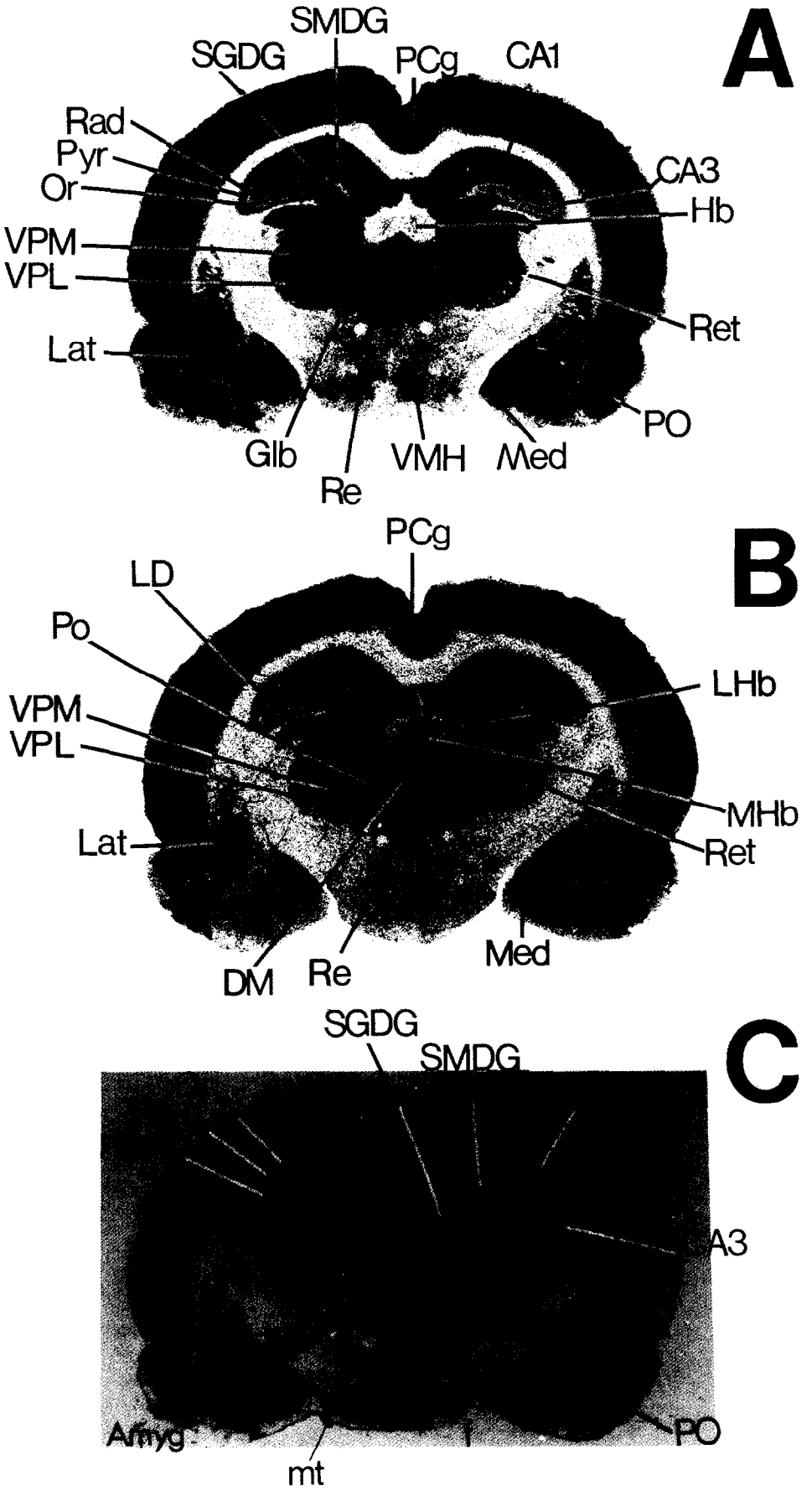

Fig. 6. Binding at the level of the dorsal hippocampus.

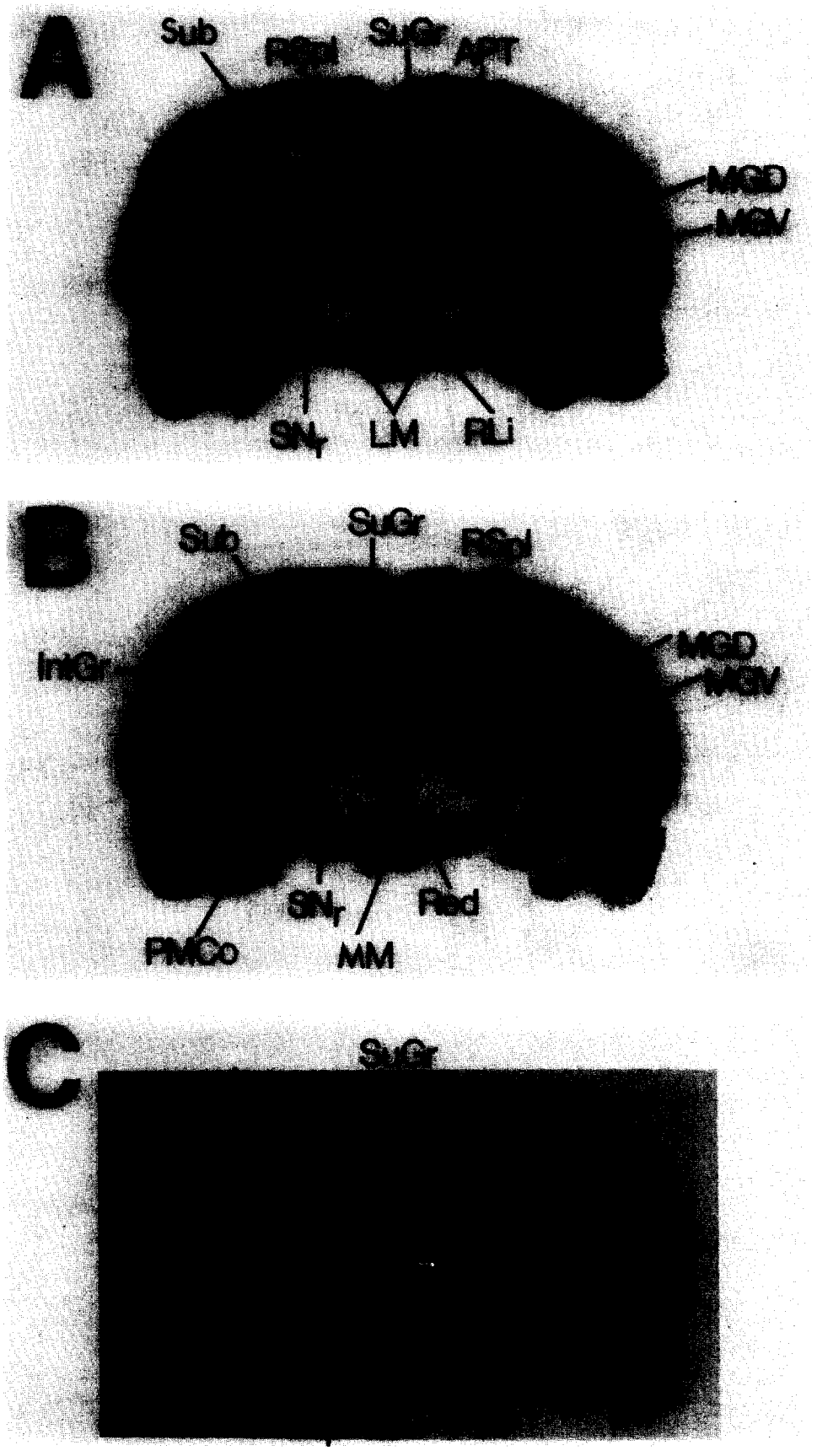

Fig. 7. Binding at the level of the medial geniculate body.

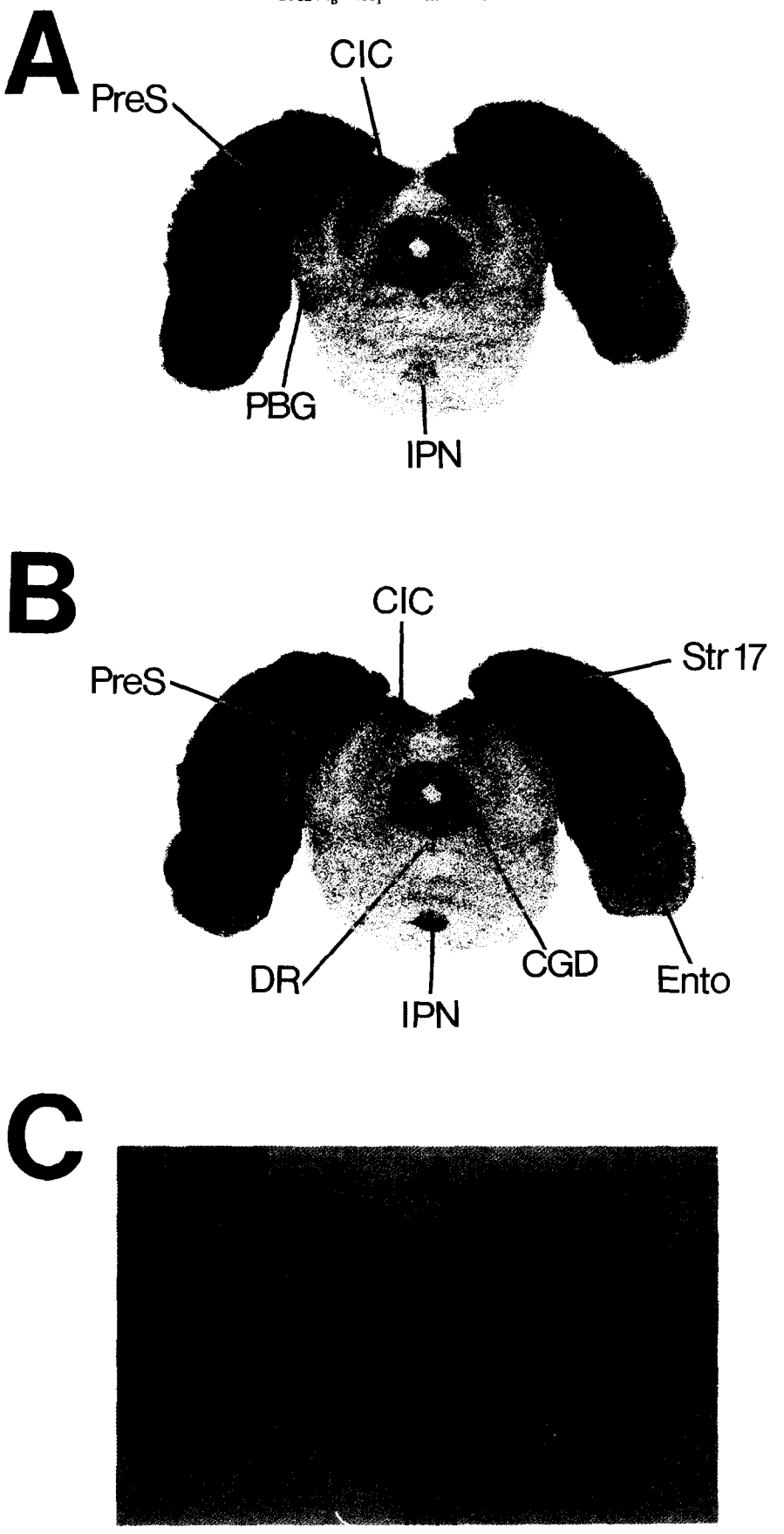

Fig. 8. Binding at the level of the inferior colliculus.

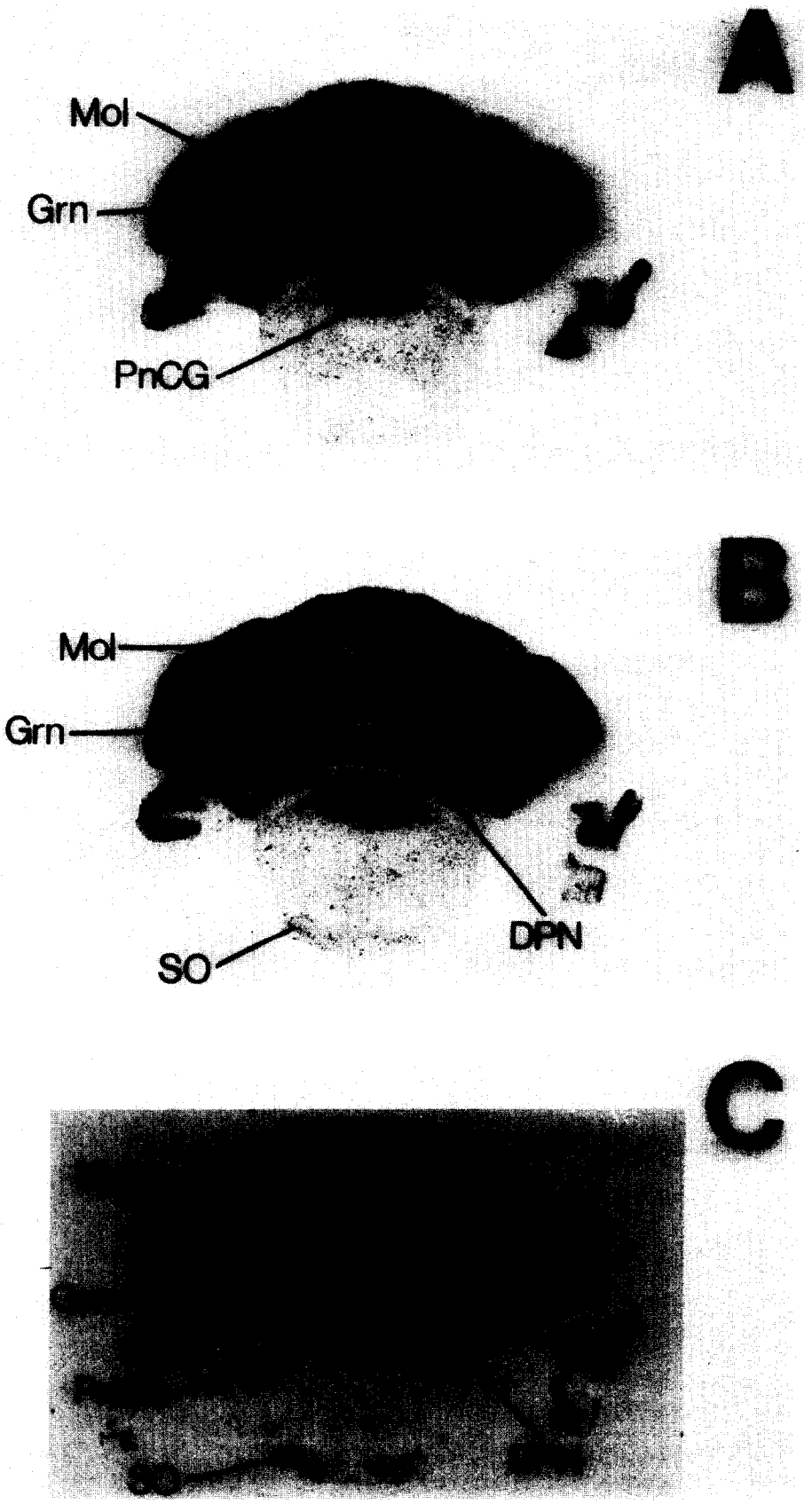

Fig. 9. Binding at the level of the rostral pons and cerebellum.

were performed in hypotonic (50 mM) Tris buffer, which reduces uptake by lysis of membrane-bound saccules.[47]

The binding constants obtained in this study were consistent with those reported previously for $GABA_B$ sites in homogenate studies using [^{3}H]GABA[54] and [^{3}H]baclofen.[13] In the presence of 2.5 mM $CaCl_2$ and 10 μM isoguvacine, Scatchard plots of $GABA_B$ binding were linear and yielded Hill coefficients close to unity. Likewise, [^{3}H]GABA appeared to bind to a single $GABA_A$ site in the presence of 100 μM (±)baclofen. Thus, [^{3}H]GABA appeared to bind to homogeneous $GABA_A$ and $GABA_B$ receptor populations. However, other populations of $GABA_A$ and $GABA_B$ sites demonstrating much lower affinity or more rapid dissociation kinetics may have gone undetected by our assay conditions.

Using similar ionic conditions, others have shown monophasic $GABA_B$ binding in tissue homogenates.[12] Some studies, however, have resolved two baclofen-sensitive [^{3}H]GABA sites.[48] [^{3}H](−)Baclofen itself had been shown to bind to a single site in rat forebrain sections[34] and in rat cerebellar membranes.[24,50] Bowery *et al.*[13] have found that [^{3}H](−)baclofen binds to two sites in rat brain membranes with high and low affinities of 19 and 304 nM, respectively. They also describe two baclofen-sensitive [^{3}H]GABA binding sites with affinities of 29 and 1242 nM, in contrast to their previous findings of a single site.[12] This discrepancy was attributed to the use of excess radioligand (> 10 nM) in the earlier study which would have revealed only the low affinity site. A higher affinity $GABA_B$ site in our assay may also have gone undetected, but such a high affinity site was investigated by using [^{3}H]GABA concentrations from 5 to 20 nM for the lower points in the saturation curve.

Although the $GABA_B$ binding site exhibited a K_D of approximately 300 nM, it displayed rather slow association and dissociation kinetics. These two features are not incompatible, as the K_D derived from the kinetic experiments agreed very well with K_D values obtained from saturation studies. The relatively slow on and off rates for [^{3}H]GABA may reflect the low temperature (4°C) at which the assay was conducted. It is unlikely that these values reflect artifactual kinetics, as a discrepancy between K_D values obtained from kinetic and saturation studies would be expected in that case.

Distribution of [^{3}H]*GABA binding*

The present study extends previous autoradiographic descriptions of the localization of $GABA_B$ and $GABA_A$ receptors[15,56,81] by providing direct quantitative comparison of the affinity and number of $GABA_B$ versus $GABA_A$ receptors in discrete brain regions (Tables 1 and 2, Figs 4–9), and by obtaining kinetic constants within the same animals using quantitative autoradiography (Fig. 1). We also report detailed displacement curves for baclofen and isoguvacine from data obtained in serial autoradiographic sections (Fig. 3).

Our findings are in close agreement with an earlier study by Bowery *et al.*[15] However, differences in methodology between the present and the earlier study should be pointed out. Bowery *et al.*[15] performed assays in brains fixed by paraformaldehyde perfusion. They used 10-μm-thick sections, room temperature incubations in hypotonic buffer, brain paste standards for calibration of optical densities, and reported bound values in pmol/g wet weight tissue. Furthermore, K_D and B_{max} values were reported for $GABA_A$ and $GABA_B$ binding to whole brain sections and not from distinct areas of the brain. Despite these differences in methods, however, the regional localization of $GABA_A$ and $GABA_B$ binding sites demonstrated by our assay is in excellent agreement with that of Bowery's earlier report.

The general trend apparent from regional distribution results is that the ratio of $GABA_B$ vs $GABA_A$ binding at a single concentration of [^{3}H]GABA is roughly 1:3–1:4. This ratio may primarily reflect differences in receptor affinity, as $GABA_A$ receptors exhibit three- to four-fold higher affinities for [^{3}H]GABA than $GABA_B$ receptors (Table 1). Regions where differences in receptor number may outweigh those in receptor affinity typically exhibit other ratios. For instance, in the cerebellar granule cell layer, $GABA_A$ receptors are four times more numerous than $GABA_B$ receptors; but in the molecular layer, $GABA_B$ receptors outnumber $GABA_A$ receptors (Table 1).

The greater affinity and apparently greater number of $GABA_A$ than $GABA_B$ receptors in several brain regions may account for certain electrophysiological observations. Activation of $GABA_B$ receptors by GABA may be easier to detect *in vitro* when $GABA_A$ receptors are masked by bicuculline.[32] The differential affinity may also contribute to differences in the onset of $GABA_B$- vs $GABA_A$-mediated membrane events. $GABA_B$-mediated membrane effects are usually slower in onset and longer in duration than $GABA_A$ membrane activation.[32,60] Since $GABA_B$ receptors have a higher K_D than do $GABA_A$ receptors, GABA released into the synaptic cleft might first interact with $GABA_A$ receptors. As the amount of GABA released into the synaptic cleft accumulates over time, $GABA_B$ receptor activation could be recruited. The difference in time of onset of observable electrophysiological effects of GABA could also be related to the slow association time at $GABA_B$ receptors and the fact that $GABA_B$ receptors are coupled to inhibitory guanine-nucleotide binding proteins.[2]

Table 2 details the existence of $GABA_B$ sites previously undetected by biochemical, behavioral or physiological methods. The highest density of $GABA_B$ sites was found in the medial habenula. It is not clear if $GABA_B$ receptors in the medial habenula are innervated by local interneurons or by an extrinsic GABAergic source. Putative extrinsic GABAergic

afferents to the medial habenula may come from the nucleus of the diagonal band of Broca.[38] Efferent projections from the medial habenula innervate the interpeduncular nucleus.[37] The interpeduncular nucleus has the second highest level of $GABA_B$ binding in the rat brain. Habenular stimulation has resulted in analgesia in animal models of chronic pain.[22] That $GABA_B$ receptors are highest in the medial habenula and interpeduncular nucleus may correlate with some of the analygesic effects of baclofen observed clinically[30,78] and experimentally.[7,20,36,53] However, whether baclofen affects transmission along the habenulo-interpeduncular tract remains to be investigated.

Price *et al.*,[71] suggested that interpeduncular $GABA_B$ receptors may exist on presynaptic terminals of fasciculus retroflexus afferents from the medial habenula. In their study, kainate- or ibotenate-induced lesions of intrinsic interpeduncular neurons did not cause reductions in $GABA_B$ receptor binding, while electrolytic habenular lesions result in 85% loss of interpeduncular $GABA_B$ sites 12 days post-lesion.

Other brain regions possess $GABA_B$ receptors whose physiological function is unknown. These include the lateral and medial geniculate bodies, various thalamic nuclei and the superior colliculus. These regions, however, do exhibit glutamate decarboxylase-positive reaction product[58] and dendrodendritic synapses.[29,57] $GABA_B$ receptors in these areas could be involved in dendrodendritic inhibition.

In general, GABA receptor distribution parallels the topography of glutamate decarboxylase-positive[58] and GABA-immunoreactive terminals.[64] An exception to this trend is observed in the globus pallidus, where little [^{3}H]GABA binding occurs despite well-documented presynaptic markers. This discrepancy could in part reflect quenching of tritium label by the rich network of white matter tracts which traverse this structure.[33]

Possible functions of $GABA_B$ receptors

Electrophysiological studies of $GABA_B$ receptors in the hippocampal slice have shown both pre- and postsynaptic effects of baclofen.[4,8,44,66] Baclofen blocks synaptic transmission across various excitatory hippocampal pathways,[5,52,63] presumably by decreasing presynaptic release of glutamate or aspartate.[49] Baclofen completely blocks conduction across the CA3–CA1 synapse and partially blocks transmission across the medial perforant pathway.[5] Baclofen also directly mediates postsynaptic hyperpolarization of hippocampal granule and pyramidal cells.[6,32,60,80] This hyperpolarizing effect of baclofen appears to involve an outward barium-sensitive potassium current[32,43] and is more readily elicited from the dendritic zones of hippocampal pyramidal cells than from the soma.[60]

The high density of $GABA_B$ receptors in the CA1 region and in stratum moleculare of the dentate gyrus is consistent with findings that baclofen depresses synaptic transmission across the CA3–CA1 synapse and across the perforant pathway.[52] Interestingly, the presubiculum and parasubiculum demonstrate high densities of $GABA_B$ binding sites, although baclofen's electrophysiological effects in these regions have not been documented. Lastly, $GABA_B$ and $GABA_A$ receptor distribution in rat hippocampal formation parallels that in human hippocampal formation.[18]

In a previous study, dendrites and somata of granule cells in the external plexiform layer and periglomerular neurons in the glomerular layer displayed dense glutamate decarboxylase-positive immunoreactivity.[74] These cells apparently subserve dendrodendritic inhibition of mitral and tufted cells activity following stimulation of major olfactory pathways.[61,72] In the present study, $GABA_A$ receptors were present within the external plexiform layer of the olfactory bulb. The $GABA_A$ antagonists, picrotoxin and bicuculline, block dendrodendritic inhibition by granule cells.[62] Thus, $GABA_A$ receptors may mediate inhibition of mitral cells by GABAergic granule cells. $GABA_B$ receptors were present in the glomerular layer. Although the electrophysiology of baclofen and the pharmacology of dendrodendritic inhibition by periglomerular cells have not been directly examined in the olfactory bulb, a bicuculline-resistant inhibitory postsynaptic potential has been observed previously in mitral cells following orthodromic stimulation of the olfactory nerve.[45] Therefore, we suggest that $GABA_B$ receptors may be responsible for inhibition of mitral and tufted cells by periglomerular neurons.

Much evidence supports the existence of $GABA_B$ receptors within the cerebral cortex. Baclofen inhibits the release of endogenously incorporated glutamate,[49,69] aspartate[49,70] and serotonin[77] from cortical slices. Cortical $GABA_B$ receptors are decreased after lesion of the dorsal adrenergic bundle.[48] These data suggest that $GABA_B$ receptors may exist as presynaptic heteroreceptors on cortical afferents where they attenuate release of excitatory amino acids and biogenic amines. $GABA_B$ receptors are localized primarily in outer layers (I–III) of cerebral cortex. The outer layers of cortex receive afferents predominantly from cortico-cortical association fibers,[68] which most likely use glutamate or aspartate as a neurotransmitter.[1,40] The laminar distribution of serotonin[10] and noradrenergic[31] input to the neocortex is more uniform and not confined to the outer layers of cortex. Therefore, if cortical $GABA_B$ binding sites represent true heteroreceptors, they are likely to be largely on glutamatergic cortico-cortical afferents. However, the possibility that $GABA_B$ receptors may exist on postsynaptic intrinsic cortical neurons cannot be disregarded.

Striatal $GABA_B$ receptors are few in number when compared to the density of striatal $GABA_A$ receptors. Baclofen, acting at presumptive presynaptic $GABA_B$ receptors in the striatum, reduces the evoked release of dopamine[73] and potentiates the release of [Met]-enkephalin from rat striatal slices.[76] $GABA_B$

receptors may also exist on corticostriatal terminals, based on preliminary anatomical lesion data.[51] In addition, baclofen reduces field potentials evoked by stimulation of cortical efferents.[21] However, intracellular recordings from caudate neurons[82] have shown that systemic or intracaudate baclofen does not reduce excitatory postsynaptic potentials in response to cortical or thalamic stimulation, but blocks evoked hyperpolarizations. This suggests that baclofen has no effect upon striatal afferents, but that it exerts a direct effect on intracaudate circuitry. Thus, baclofen-sensitive $GABA_B$ receptors may exist on postsynaptic neurons within the striatum. Lesion studies from our laboratory indicate that striatal $GABA_B$ receptors are unchanged after decortication but are markedly reduced after striatal ibotenate lesions.[19]

The differential laminar localization of $GABA_B$ and $GABA_A$ receptors in the cerebellum confirms previous reports.[15,81] We also find the molecular layer to be enriched in $GABA_B$ receptors, and the granular layer to have the greatest density of $GABA_A$ receptors in rat brain. Studies in mutant mice indicate that $GABA_B$ receptors in the molecular layer of the cerebellum may be localized on Purkinje cell dendrites[16] and on parallel fibers from granule cells.[83] Kato and Fukuda[50] found that lesions of the inferior olive result in decrements of cerebellar $GABA_B$ receptors and proposed that $GABA_B$ receptors may exist on climbing fiber afferents to the cerebellum.

CONCLUSION

The regional distribution and kinetic properties of $GABA_B$ binding in rat brain are in excellent agreement with previous $GABA_B$ binding studies. The distribution of $GABA_B$ sites within rat brain correlates well with sites where baclofen has been shown to have biochemical and electrophysiological actions. In addition, $GABA_B$ sites are highest in the medial habenula and interpeduncular nucleus, where they may be involved in central pain pathways.

Acknowledgements—We would like to thank Suyin Liang for help in preparation of the manuscript. This work was supported by USPHS grants NS 19613 and T32 GM 07863.

REFERENCES

1. Addae J. I. and Stone T. W. (1986) Effects of topically applied excitatory amino acids on evoked potentials and single cell activity in rat cerebral cortex. *Eur. J. Pharmac.* **121,** 337–343.
2. Andrade R., Malenka R. C. and Nicoll R. A. (1986) A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science* **234,** 1261–1265.
3. Asano T. and Ogasawara N. (1986) Uncoupling of γ-amino butyric acid B receptors from GTP-binding proteins by *N*-ethylmaleimide: effect of *N*-ethylmaleimide on purified GTP-binding proteins. *Molec. Pharmac.* **29,** 244–249.
4. Ault B. and Nadler J. V. (1983) Anticonvulsant-like actions of baclofen in the rat hippocampal slice. *Br. J. Pharmac.* **78,** 701–708.
5. Ault B. and Nadler J. V. (1983) Effects of baclofen on synaptically-induced cell firing in the rat hippocampal slice. *Br. J. Pharmac.* **80,** 211–219.
6. Ault B., Gruenthal M., Armstrong D. R., Nadler J. V. and Wang C. M. (1986) Baclofen suppresses bursting activity induced in hippocampal slices by differing convulsant treatments. *Eur. J. Pharmac.* **126,** 289–292.
7. Bartolini A., Bartolini R., Biscini A., Giotti A. and Malmberg P. (1980) Investigations into baclofen analgesia: effect of naloxone, bicuculline, atropine and ergotamine. *Proc. Br. pharmac. Soc.* 156P–157P.
8. Blaxter T. J. and Carlen P. L. (1985) Pre- and postsynaptic effects of baclofen in the rat hippocampal slice. *Brain Res.* **341,** 195–199.
9. Blaxter T. J., Carlen P. L., Davies M. F. and Kujtan P. W. (1986) γ-Aminobutyric acid hyperpolarizes rat hippocampal pyramidal cells through a calcium-dependent potassium conductance. *J. Physiol.* **373,** 181–194.
10. Bobillier P., Seguin S., Petitjean F., Salvert S., Touret M. and Jouvet M. (1976) The raphe nuclei of the cat brain: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res.* **11,** 449–486.
11. Bowery N. G. (1984) Classification of GABA receptors. In *The GABA Receptors* (ed. Enna S. J), pp. 177–213. The Humana Press, Clifton, NJ.
12. Bowery N. G., Hill D. R. and Hudson A. L. (1983) Characteristics of $GABA_B$ receptor binding on rat whole brain synaptosomes. *Br. J. Pharmac.* **78,** 191–206.
13. Bowery N. G., Hill D. R. and Hudson A. L. (1985) [^{3}H](−)Baclofen: an improved ligand for $GABA_B$ sites. *Neuropharmacology* **24,** 207–210.
14. Bowery N. G., Hill D. R., Hudson A. L., Doble A., Middlemiss D. N., Shaw J. and Turnbull M. J. (1980) (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283,** 92–94.
15. Bowery N. G. Hudson A. L. and Price G. W. (1987) $GABA_A$ and $GABA_B$ receptor site distribution in rat central nervous system. *Neuroscience* **20,** 365–383.
16. Bowery N. G., Price G. W., Turnbull M. J. and Wilkin G. P. (1983) Evidence for the presence of $GABA_B$ receptors on cerebellar Purkinje dendrites. *Br. J. Pharmac.* **79,** Suppl. 189P.
17. Chu D. C. M., Penney J. B. and Young A. B. (1987) Cortical $GABA_B$ and $GABA_A$ receptors in Alzheimer's disease: a quantitative autoradiographic analysis. *Neurology* **37,** 1454–1459.
18. Chu D. C. M., Penney J. B. and Young A. B. (1987) Quantitative autoradiography of hippocampal $GABA_B$ and $GABA_A$ receptor changes in Alzheimer's disease. *Neurosci. Lett.* **82,** 246–252.
19. Chu D. C. M., Penney J. B. and Young A. B. (1987) Autoradiographic evidence for postsynaptic GABA-B receptors in rat striatum and hippocampus. *Soc. Neurosci. Abstr.* **13,** 952.
20. Cohen S. R. and Melzack R. (1986) Habenular stimulation produces analgesia in the formalin test. *Neurosci. Lett.* **70,** 165–169.

21. Cordingley G. and Weight F. (1982) Noncholinergic, excitatory transmission in rat neostriatum. *Soc. Neurosci. Abstr.* **8,** 373.
22. Dauth G. W., Frey K. A. and Gilman S. (1983) A densitometer for quantitative autroadiography. *J. Neurosci. Meth.* **9,** 243–251.
23. Dolphin A. C. and Scott R. H. (1986) Inhibition of calcium currents in cultured rat dorsal root ganglion neurones by (−)baclofen. *Br. J. Pharmac.* **88,** 213–220.
24. Drew C. A., Johnston G. A. R. and Weatherby R. P. (1984) Bicuculline-insensitive GABA receptors: studies on the binding of (−)baclofen to rat cerebellar membranes. *Neurosci. Lett.* **52,** 317–321.
25. Dunlap K. (1984) Functional and pharmacological differences between two types of GABA receptor on embryonic chick sensory neurons. *Neurosci. Lett.* **47,** 265–270.
26. Dunlap K. and Fischbach G. D. (1978) Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature* **276,** 837–839.
27. Enna S. J. and Gallagher J. P. (1983) Biochemical and electrophysiological characteristics of mammalian GABA receptors. *Int. Rev. Neurobiol.* **24,** 181–212.
28. Falch E. and Krogsgaard-Larsen P. (1982) The binding of the GABA agonist ^{3}H-THIP to rat brain membranes. *J. Neurochem.* **38,** 1123–1129.
29. Famigletti E. V. (1970) Dendro-dendritic synapses in the lateral geniculate nucleus of the cat. *Brain Res.* **20,** 181–191.
30. Fromm G. H., Terrence C. F. and Chattha A. S. (1984) Baclofen in the treatment of trigeminal neuralgia: double-blind study and long-term follow up. *Ann. Neurol.* **15,** 240–244.
31. Fuxe K., Hamberger B. and Hökfelt T. (1968) Distribution of noradrenaline nerve terminals in cortical areas of the rat. *Brain Res.* **8,** 125–131.
32. Gähwiler B. H. and Brown D. A. (1985) $GABA_B$-receptor-activated K^+ current in voltage-clamped CA_3 pyramidal cells in hippocampal cultures. *Proc. natn. Acad. Sci. U.S.A.* **82,** 1558–1562.
33. Geary W. A. and Wooten G. F. (1985) Regional tritium quenching in quantitative autoradiography of the central nervous system. *Brain Res.* **336,** 334–336.
34. Gehlert D. R., Yamamura H. I. and Wamsley J. K. (1985) γ-Aminobutyric $acid_B$ receptors in the rat brain: quantitative autoradiographic localization using [^{3}H](−)baclofen. *Neurosci. Lett.* **56,** 183–188.
35. Guidotti A., Corda M. G., Wise B. C., Vaccarino F. and Costa E. (1983) GABAergic synapses; supramolecular organization and biochemical regulation. *Neuropharmacology* **22,** 1471–1479.
36. Hammond D. L. and Drower E. J. (1984) Effects of intrathecally administered THIP, baclofen and muscimol on nociceptive threshold. *Eur. J. Pharmac.* **103,** 121–125.
37. Herkenham M. and Nauta W. J. H. (1979) Efferent connections of the habenular nuclei in the rat. *J. comp. Neurol.* **187,** 19–48.
38. Herkenham M. and Nauta W. J. H. (1980) Afferent connections of the habenuclar nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. *J. comp. Neurol.* **173,** 123–146.
39. Herkenham M. and Sokoloff L. (1984) Quantitative receptor autoradiography: tissue defatting eliminates differential self-adsorption of tritium radiation in gray and white matter of brain. *Brain Res.* **321,** 363–368.
40. Hicks T. P., Ruwe W. D., Veale W. L. and Veenhuizen, J. (1985) Aspartate and glutamate as synaptic transmitters of parallel visual cortical pathways. *Expl Brain Res.* **58,** 421–425.
41. Hill D. R. (1985) $GABA_B$ receptor modulation of adenylate cyclase activity in rat brain slices. *Br. J. Pharmac.* **84,** 249–257.
42. Hosli E., Mohler H., Richards J. G. and Hosli L. (1985) Autoradiographic localization of binding sites for [^{3}H]γ-aminobutyrate, [^{3}H]muscimol, (±)[^{3}H]bicuculline methiodide and [^{3}H]flunitrazepam in cultures of rat cerebellum and spinal cord. *Neuroscience* **5,** 1657–1665.
43. Inoue M., Matsuo T. and Ogata N. (1985) Possible involvement of K^+-conductance in the action of γ-aminobutyric acid in the guinea-pig hippocampus. *Br. J. Pharmac.* **86,** 515–524.
44. Inoue M., Matsuo T. and Ogata N. (1985) Characterization of the pre- and postsynaptic actions of (−)-baclofen in the guinea-pig hippocampus *in vitro*. *Br. J. Pharmac.* **84,** 843–851.
45. Jahr C. E. and Nicoll R. A. (1982) An intracellular analysis of dendrodendritic inhibition in the turtle *in vitro* olfactory bulb. *J. Physiol.* **326,** 213–234.
46. Johnston G. A. R., Beart P. M., Curtis D. R., Game F. J. A., McCulloch R. M. and Maclachlan R. M. (1972) Bicuculline methochloride as a GABA antagonist. *Nature* **240,** 219–220.
47. Kanner B. I. (1983) Bioenergetics of neurotransmitter transport. *Biochim. biophys. Acta* **726,** 293–316.
48. Karbon E. W., Dunman R. and Enna S. J. (1983) Biochemical identification of multiple $GABA_B$ binding sites: association with noradrenergic terminals in rat forebrain. *Brain Res.* **274,** 393–396.
49. Kato K., Goto M. and Fukuda H. (1982) Baclofen: inhibition of release of L-[^{3}H]glutamate and L-[^{3}H]aspartate from rat whole brain synaptosomes. *Gen. Pharmac.* **13,** 445–447.
50. Kato K. and Fukuda. H. (1985) Reduction of $GABA_B$ receptor binding induced by climbing fiber degeneration in the rat cerebellum. *Life Sci.* **37,** 279–288.
51. Kilpatrick G. J., Muhyaddin M. S., Roberts P. J. and Woodruff G. N. (1983) $GABA_B$ binding sites on rat striatal synaptic membranes. *Br. J. Pharmac.* **78,** Suppl., 6P.
52. Lanthorn T. H. and Cotman C. W. (1981) Baclofen selectively inhibits excitatory synaptic transmission in the hippocampus. *Brain Res.* **225,** 171–178.
53. Levy R. A. and Proudfit H. K. (1979) Analgesia produced by microinjection of baclofen and morphine at brain stem sites. *Eur. J. Pharmac.* **57,** 43–55.
54. Majewska M. D. and Chuang D.-M. (1984) Modulation by calcium of γ-aminobutyric acid (GABA) binding to $GABA_A$ and $GABA_B$ recognition sites in rat brain. *Molec. Pharmac.* **25,** 352–359.
55. Mathers D. A. (1987) The $GABA_A$ receptor: new insights from single-channel recording. *Synapse* **1,** 96–101.
56. McCabe R. T. and Wamsley J. K. (1986) Autoradiographic localization of subcomponents of the macromolecular GABA receptor complex. *Life Sci.* **39,** 1937–1945.
57. Morest D. K. (1971) Dendrodendritic synapses of cells that have axons: the fine structure of the Golgi type II cell in the medial geniculate body of the cat. *Z. Anat. EntwGesch.* **133,** 216–246.

58. Mugnaini E. and Oertel W. H. (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In *Handbook of Chemical Neuroanatomy*, Vol. 4: *GABA and Neuropeptides in the CNS*, Part 1 (eds Björklund A. and Hökfelt T.), pp. 436–608. Elsevier, Amsterdam.
59. Munson P. J. (1983) LIGAND: a computerized analysis of ligand binding data. *Meth. Enzym.* **92,** 543–576.
60. Newberry N. R. and Nicoll R. A. (1984) Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. *Nature* **308,** 450–452.
61. Nicoll R. A. (1969) Inhibitory mechanisms in the rabbit olfactory bulb: dendrodendritic mechanisms. *Brain Res.* **14,** 157–172.
62. Nicoll R. A. (1971) Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb. *Brain Res.* **35,** 137–149.
63. Olpe H. R., Baudry M., Fagni L. and Lynch G. (1982) The blocking action of baclofen on excitatory transmission in the rat hippocampal slice. *J. Neurosci.* **2,** 698–703.
64. Ottersen O. and Storm-Mathisen J. (1984) Neurons containing or accumulating transmitter amino acids. In *Handbook of Chemical Neuroanatomy*, Vol. 3: *Classical Transmitters and Transmitter Receptors in the CNS*, Part 2 (eds Björklund A., Hökfelt T. and Kuhar M. J.), pp. 141–246. Elsevier, Amsterdam.
65. Pan H. S., Frey K. A., Young A. B. and Penney J. B. (1983) Changes in [^{3}H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. *J. Neurosci.* **3,** 1189–1198.
66. Peet M. J and McLennan H. (1986) Pre- and postsynaptic actions of baclofen: blockade of the late synaptically-evoked hyperpolarization of CA1 pyramidal neurones. *Expl Brain Res.* **61,** 567–574.
67. Penney J. B., Pan H. S., Young A. B., Frey K. A. and Dauth G. W. (1981) Quantitative autoradiography of [^{3}H]muscimol binding in rat brain. *Science* **214,** 1036–1038.
68. Porter R. (1981) Internal organization of the motor cortex for input–output arrangements. In *Handbook of Physiology*, section I. *The Nervous System*, Vol II. *Motor Control*, Part 2 (ed. Brooks V. B.), pp. 1063–1081. Waverly Press, Baltimore.
69. Potashner S. J. (1979) Baclofen: effects on amino acid release and metabolism in slices of guinea pig cerebral cortex. *J. Neurochem.* **32,** 103–109.
70. Potashner S. J. and Gerard D. (1983) Kainate-enhanced release of D-[^{3}H]aspartate from cerebral cortex and striatum: reversal by baclofen and pentobarbital. *J. Neurochem.* **40,** 1548–1557.
71. Price G. W., Blackburn T. P., Hudson A. L. and Bowery N. G. (1984) Presynaptic $GABA_B$ sites in the interpeduncular nucleus. *Neuropharmacology* **23,** 861–862.
72. Rall W., Shepherd G. M., Reese T. S. and Brightman M. W. (1966) Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Expl Neurol.* **14,** 44–56.
73. Reimann W. (1983) Inhibition by GABA, baclofen and gabapentin of dopamine release from rabbit caudate nucleus: are there common or different sites of action? *Eur. J. Pharmac.* **94,** 341–344.
74. Ribak C. E., Vaughn J. E., Saito K., Barber R. and Roberts E. (1977) Glutamate decarboxylase localization in neurons of the olfactory bulb. *Brain Res.* **126,** 1–18.
75. Rogers A. W. (1979) *Techniques of Autoradiography*, Elsevier/North Holland, New York.
76. Sawynok J. and LaBella F. S. (1981) GABA and baclofen potentiate the K^+-evoked release of methionine-enkephalin from rat striatal slices. *Eur. J. Pharmac.* **70,** 103–110.
77. Schlicker E., Classen K. and Göthert M. (1984) $GABA_B$ receptor mediated inhibition of serotonin release in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmac.* **326,** 99–105.
78. Steardo L., Leo A. and Marano E. (1984) Efficacy of baclofen in trigeminal neuralgia and some other painful conditions. *Eur. Neurol.* **23,** 51–55.
79. Stephenson F. A. and Olson R. W. (1983) Biochemical pharmacology of the GABA receptor–ionophore protein complex. In *CNS Receptors—From Molecular Pharmacology to Behavior* (eds Mandel P. and DeFeudis F. V.), pp. 71–80. Raven Press, New York.
80. Swartzwelder H. S., Bragdon A. C., Sutch C. P., Ault B. and Wilson W. A. (1986) Baclofen suppresses hippocampal epileptiform activity at low concentrations without suppressing synaptic transmission. *J. Pharmac. exp. Ther.* **237,** 881–887.
81. Wilkin G. P., Hudson A. L., Hill D. R. and Bowery N. G. (1981) Autoradiographic localization of $GABA_B$ receptors in rat cerebellum. *Nature* **294,** 584–587.
82. Wilson J. S. and Wilson J. A. (1985) Baclofen attenuates hyperpolarizing not depolarizing responses of caudate neurons in cat. *Brain Res.* **342,** 396–400.
83. Wojcik W. J. and Neff N. H. (1984) γ-Aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells. *Molec. Pharmac.* **25,** 24–28.

(*Accepted* 5 *June* 1989)