Research Report

Distribution of $\text{GABA}_A$ and $\text{GABA}_B$ binding sites in the cochlear nucleus of the guinea pig

Jose M. Juiz $^a$,*, Roger L. Albin $^b$, Robert H. Helfert $^a$, Richard A. Altschuler $^a$

$^a$ Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109, USA
$^b$ Department of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48104, USA

(Accepted 19 October 1993)

Abstract

We compared the distribution of $\text{GABA}_A$ and $\text{GABA}_B$ binding sites in the cochlear nucleus using quantitative receptor autoradiography with [$^3\text{H}$]GABA. To visualize $\text{GABA}_A$ binding sites, $\text{GABA}_B$ binding sites were blocked with $+\text{baclofen}$. To visualize $\text{GABA}_B$ binding sites, isoguvacine was used to block $\text{GABA}_A$ binding sites. $\text{GABA}_A$ binding sites predominated over $\text{GABA}_B$, although there were marked regional differences in the distribution of binding. In the ventral cochlear nucleus, $\text{GABA}_A$ and $\text{GABA}_B$ binding sites were concentrated in the peripheral granule cell cap, with low binding levels in the central region. In the dorsal cochlear nucleus, binding was concentrated in the superficial (fusiform and molecular) layers, with a distinct laminar pattern. $\text{GABA}_A$ binding sites predominated in the fusiform cell layer. The molecular layer contained the highest level of $\text{GABA}_B$ binding sites in the entire cochlear nucleus. These results suggest that GABAergic inhibition in the cochlear nucleus is mediated both by $\text{GABA}_A$ and $\text{GABA}_B$ receptors, particularly in the dorsal cochlear nucleus. However, low levels of binding in areas such as the magnocellular regions of the ventral cochlear nucleus, known to contain abundant GABAergic synapses, suggest heterogeneity of GABA receptors in this auditory nucleus.

Key words: GABA; GABA receptor; Auditory pathway; Cochlear nucleus; Neurotransmitter

I. Introduction

There is much evidence supporting gamma-aminobutyric acid (GABA) as a major inhibitory neurotransmitter in the cochlear nucleus (CN) (extensively reviewed in [3,19,82]). GABAergic inhibition of CN neurons arises from multiple ‘non-primary’ intrinsic and extrinsic (descending) inputs from a variety of sources [4,62,67,74]. Such extensive GABA-mediated inhibition would be crucial for CN processing of the primary acoustic input carried by the auditory nerve. The way in which GABA-mediated inhibition could modify or modulate the response of CN neurons to auditory stimuli would depend in good part on the type of GABA receptors and their distribution on CN neurons.

Two major classes of GABA receptors have been identified to date [8,24,27,72,75], termed respectively $\text{GABA}_A$ and $\text{GABA}_B$. They have different biochemical, physiological and pharmacological characteristics as well as distinct patterns of anatomical distribution [11,20,34,60]. At the cellular level, $\text{GABA}_A$ receptors are located preferentially in postsynaptic regions [2,21,35,64,73] contain an integral chloride channel [22,49,70,75,76] and mediate ‘fast’ inhibitory responses which can be blocked with the specific antagonist bicuculline. $\text{GABA}_B$ receptors may be pre- or postsynaptic and regulate $K^+$ or $Ca^{2+}$ channels through a guanyl nucleotide-binding protein mechanism which is linked to some second messenger systems [1,24,27,33,53,71,83]. $\text{GABA}_B$ receptors produce slow and sustained inhibition insensitive to bicuculline blockade [7,9,23,53].

It is very likely that $\text{GABA}_A$ receptors are involved in inhibition in the CN, as GABA-mediated inhibition of spontaneous and tone-evoked activity of CN neurons is selectively blocked by bicuculline [15,16,18,19,25], which also alters auditory evoked potentials [47].
Also, GABA_A receptors have been localized in the CN by receptor autoradiography with the agonist [3H]muscimol [26,28] and by immunocytochemistry with an antibody against the β subunit of the GABA_A receptor [35].

In contrast, much less is known about the function and distribution of GABA_B receptors in the CN. The finding that the prototype GABA_B agonist baclofen [9,10], alters auditory evoked potentials [46] and reduces tone-evoked activity of CN neurons [17,19,25] suggests involvement of GABA_B receptors in CN inhibition. However, if GABA_B receptors are present in the CN, their precise distribution in comparison to GABA_A receptors is unknown.

This study addresses the question of the localization and comparative distribution of GABA_A and GABA_B receptors in the CN. For this purpose, we have used receptor autoradiography with [3H]GABA under binding conditions which allow visualization of either GABA_A or high affinity-GABA_A binding sites [20].

2. Materials and methods

Six adult guinea pigs with normal pinna reflex were used in this study. The animals were deeply anesthetized with chloral hydrate (300 mg/kg b.w.). Their brain stems were dissected and immediately frozen in dry ice. Serial sections (20 μm thick) in the coronal plane were obtained in a Lipshaw cryostat. Sections containing the CN complex were identified and thaw-mounted onto gelatin coated slides. Slides containing the sections of interest were dried on a warming plate and stored at -80°C until use.

The autoradiography technique followed the procedure of Chu et al. [20]. Sections were washed for 30 min in 50 mM Tris-2.5 mM CaCl_2 buffer (pH 7.4) at 4°C followed by drying under a stream of cool air. Serial sections were assayed for either GABA_A or GABA_B binding sites. In both cases [3H]GABA (Amersham, specific activity 99.6 Ci/mmol) was used as radioligand at a concentration of 20 nM, together with 100 μM (+)baclofen (Ciba-Geigy) to block GABA_B binding sites. To study the distribution of GABA_A receptors, [3H]GABA was used in the presence of isoguvacine (10 μM) to block GABA_A binding sites. Non-specific [3H]GABA binding was determined in sections incubated with a mixture of the radioligand, isoguvacine (100 μM) and baclofen (100 μM). After incubation, sections received 3 quick 3-ml rinses with buffer, followed by one 3-ml rinse with 2.5% glutaraldehyde in acetone and immediately dried under a stream of warm air. The slides were placed in X-ray cassettes and apposed to tritium-sensitive film (Ultrascreen, LKB) for 3 weeks at 4°C along with known radioactive 14C standards (American Radiochemical Co., St. Louis, Mo., USA), after which the films were developed and dried. To facilitate identification of CN subdivisions and regions, selected sections were stained with Neutral red after film exposure.

Optical densities of specific binding in the different subdivisions of the CN were quantified using computer-assisted densitometry with a computer-generated fourth-degree polynomial regression equation, as described in detail elsewhere [59]. The 14C standards were calibrated against brain paste standards containing known amounts of tritium and protein [59,79]. Optical densities of regions of interest were expressed in fmol of [3H]GABA bound per mg of protein using the standard curve derived from the standards [59].

3. Results

To describe the density of GABA_A and GABA_B binding sites in the different regions of the CN, binding levels in the cerebellar cortex were used as reference values (see Table 1). Using an identical binding assay, Chu et al. [20] reported levels of GABA_A and GABA_B binding sites in the rat cerebellar cortex which were among the highest found in the rat central nervous system. As the values that we obtained in guinea pig were comparable to those reported by these authors, it was considered convenient to use the cerebellar cortex as a general reference to describe findings in the CN (Table 1).

<table>
<thead>
<tr>
<th>CN area</th>
<th>GABA_A binding (fmol of GABA bound/mg of protein)</th>
<th>GABA_B binding (fmol of GABA bound/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVCN-Central region</td>
<td>74 ± 10</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>AVCN-Gran. cell cap</td>
<td>931 ± 12</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>PVCN-Central region</td>
<td>54 ± 1</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>PVCN-Gran. cell cap</td>
<td>807 ± 100</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>DCN-Deep region</td>
<td>77 ± 15</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>DCN-Fus. cell layer</td>
<td>662 ± 115</td>
<td>39 ± 4.8</td>
</tr>
<tr>
<td>DCN-Mol. layer</td>
<td>220 ± 3</td>
<td>154 ± 13.8</td>
</tr>
<tr>
<td>Cerebellum-Granule cell layer</td>
<td>1794 ± 7.5</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>Cerebellum-Molecular layer</td>
<td>449 ± 3</td>
<td>237 ± 4.5</td>
</tr>
</tbody>
</table>

Values represent mean±S.E.M. for three animals. Binding levels in the cerebellar cortex are given as reference.

<table>
<thead>
<tr>
<th>CN area</th>
<th>GABA_A binding (fmol of GABA bound/mg of protein)</th>
<th>GABA_B binding (fmol of GABA bound/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVCN-Central region</td>
<td>74 ± 10</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>AVCN-Gran. cell cap</td>
<td>931 ± 12</td>
<td>63 ± 12</td>
</tr>
<tr>
<td>PVCN-Central region</td>
<td>54 ± 1</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>PVCN-Gran. cell cap</td>
<td>807 ± 100</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>DCN-Deep region</td>
<td>77 ± 15</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>DCN-Fus. cell layer</td>
<td>662 ± 115</td>
<td>39 ± 4.8</td>
</tr>
<tr>
<td>DCN-Mol. layer</td>
<td>220 ± 3</td>
<td>154 ± 13.8</td>
</tr>
<tr>
<td>Cerebellum-Granule cell layer</td>
<td>1794 ± 7.5</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>Cerebellum-Molecular layer</td>
<td>449 ± 3</td>
<td>237 ± 4.5</td>
</tr>
</tbody>
</table>

Values represent mean±S.E.M. for three animals. Binding levels in the cerebellar cortex are given as reference.

For GABA_A binding sites, values over 600 fmol of GABA bound per mg of protein were considered ‘very high’, values between 600 and 300 fmol/mg were considered ‘high’, ‘moderate’ between 300 and 100 fmol/mg, ‘low’ between 100 and 50 fmol/mg and ‘very low’ below 50 fmol/mg of protein. For GABA_B binding sites, which usually occur at lower levels than GABA_A sites, values above 300 fmol/mg were considered ‘very high’, values between 300 and 150 fmol/mg were referred to as ‘high’, ‘moderate’ between 150 and 75 fmol/mg, ‘low’ between 25 and 75 fmol/mg and ‘very low’ below 25 fmol/mg of protein.

Ventral cochlear nucleus (VCN). The distribution and levels of GABA_A and GABA_B binding sites was very similar in the anteroventral and posteroverentral CN (AVCN and PVCN respectively). In both subdivisions of the VCN, the central region which contains
the main projection neurons (spherical, globular, multipolar and octopus cells), had low levels of GABA_A binding sites and very low, but detectable levels of binding to GABA_B sites (Figs. 1 and 4, Table 1). In contrast, the peripheral granule cell cap of the VCN, contained higher levels of both GABA_A and GABA_B binding.

Fig. 1. False color images of autoradiography films showing the distribution of GABA_A and GABA_B binding sites in adjacent sections of the (A) anteroventral (AVCN) and (C) posteroverntral (PVCN) cochlear nucleus. Red and yellow indicate high levels of binding, while different tones of blue code for lower binding levels. Drawings with the outlines of the magnocellular (Mag) and granule/small (Gran) cell regions are shown next to each photograph for orientation purposes (B, D).
binding sites. In this region, the level of GABA_A binding sites was very high, and the level of GABA_B binding sites low to moderate (Figs. 1 and 4, Table 1). Overall, the level of GABA_A binding sites in the VCN was 5 to 15 times higher than that of GABA_B binding sites (Table 1).

**Dorsal cochlear nucleus (DCN).** In the superficial layers of the DCN, GABA_A and GABA_B binding sites were abundant, and their distribution showed a clear laminar pattern. In the fusiform/granule cell layer, the level of GABA_A binding sites was very high, while that of GABA_B sites was low (Figs. 2 and 4, Table 1). In this layer, the level of GABA_A sites was 17 times higher than that of GABA_B sites. In the molecular layer, however, the density of GABA_A binding sites was moderate and the density of GABA_B binding sites high, the former being only 1.4 times more abundant than the latter (Figs. 2 and 4, Table 1).

In contrast, in the deep DCN the level of GABA_A binding sites was low and the level of GABA_B sites

---

**Fig. 2.** (A) False color images of two autoradiography films from adjacent sections of the dorsal cochlear nucleus showing the comparative distribution of GABA_A and GABA_B binding sites. Color coding is described in Fig. 1. The drawing in B outlines the layers of the dorsal cochlear nucleus identified in sections stained with Neutral red. Mol: molecular layer; Gran, Gran/FC: granule cell regions of the DCN, including the fusiform/granule cell layer; Deep: deep region of the DCN.

**Fig. 3.** This figure illustrates the similarities in the distribution of GABA_A and GABA_B binding sites in the DCN and cerebellar cortex. In this figure the cerebellum was taken as reference for color coding. (A) GABA_A binding sites predominate in the fusiform cell layer and granule cell regions of the DCN. In the cerebellar cortex, GABA_A binding sites predominate in the Purkinje cell layer. (B) The density of GABA_B binding sites is higher in the molecular layer of the DCN and the molecular layer of the cerebellar cortex, although the latter shows comparatively higher binding levels.
Levels of GABA-A and GABA-B Binding in CN (fmol/mg prot.)

Fig. 4. Summary sketch of the distribution of GABA_A and GABA_B binding sites in the cochlear nucleus. The levels of binding shown in Table 1 have been collapsed into a single, idealized sagittal section through the cochlear nucleus to give a better idea of the distribution of binding.

very low, similar to those found in the central or magnocellular regions of the VCN (Figs. 2 and 4, Table 1).

Summarizing, under the conditions of this assay GABA_A binding sites were concentrated in granule cell regions of the CN, including the fusiform/granule cell layer of the DCN (Figs. 1–3, 4). GABA_B binding sites were found at moderate levels in the granule cell cap of the VCN (Figs. 1, 4) while the level was high in the molecular layer of the DCN (Figs. 2–4). GABA_A binding sites clearly predominated over GABA_B sites across the entire CN (Fig. 4), except in the molecular layer of the DCN, which contained the highest density of GABA_B binding sites in the CN, approaching the density of GABA_A sites. It is interesting to note that the distribution of binding in the superficial layers of the DCN was strikingly similar to that in the cerebellar cortex, where GABA_A binding sites predominated in the granule and Purkinje cell layers, and the molecular layer was characterized by a high level of GABA_B binding sites. However, the density of both GABA_A and GABA_B binding sites was higher in the cerebellar cortex than in the DCN (Table 1, Fig. 3).

4. Discussion

The general distribution of GABA_A binding sites in the CN using [3H]GABA as radioligand does not differ much from the pattern of [3H]muscimol binding reported by Frostholm and Rotter [26] and Glendenning and Baker [28]. With either ligand, GABA_A binding sites are highly concentrated in the granule/small cell cap of the VCN and are also abundant in the superficial layers (fusiform/granule cell layer and molecular layer) of the DCN, while binding levels are much lower in the magnocellular regions of the VCN and deep DCN. There are, however, interesting new data derived from the use of quantitative densitometry of [3H]GABA binding, which help to define better the pattern of distribution of GABA_A binding sites in the CN. The great concentration of GABA_A binding sites in the granule/small cell cap of the VCN compared with the rest of the nucleus is illustrated by the fact that levels of [3H]GABA binding to GABA_A binding sites in this VCN region probably are among the highest in the brain, extrapolating from the results obtained by Chu et al. [20]. Autoradiography with [3H]GABA also reveals an abundance of GABA_A binding sites in the superficial layers of the DCN, but with important differences between the fusiform/granule cell layer and molecular layer that have not been considered in previous studies [26, 28]. With our binding assay, levels of GABA_A binding sites are higher in the fusiform/granule cell layer than in the molecular layer. This finding suggests that there are potentially important differences in the distribution of GABA_A receptors between the two layers that make up the ‘cortex’ of the DCN. The relevance of these differences in relation to auditory processing mechanisms in this nucleus needs to be evaluated.

Besides receptor autoradiography, immunocytochemistry also has been used to localize GABA_A receptors in the CN [35]. The distribution of GABA_A binding sites reported here and in previous receptor autoradiography studies [26, 28] is in fact comparable to immunocytochemical findings in the CN [35] using an antibody against the β2 subunit of the GABA_A/benzodiazepine receptor complex [21, 35, 81]. With this antibody, immunolabeling was intense in neurons in the superficial layers of the DCN, and very light in the central regions of the VCN [35]. Granule cells in granule cell domains also were intensely immunolabeled (unpublished observations).

In agreement with the high levels of GABA_A receptors found in the granule/small cell cap of the VCN and the superficial layers of the DCN, GABA is abundant in both regions as determined by microneurochemical methods [29–31]. Accordingly, these same regions contain numerous GAD or GABA immunoreactive endings and cell bodies (reviewed in refs. 38 and 82). Pharmacological data also are consistent with these findings, as neurons in the superficial DCN are inhibited by GABA, and this inhibition is antagonized by bicuculline [15, 19, 25]. In contrast, the paucity of GABA_A binding sites and GABA_A receptor immunolabeling in the magnocellular regions of the VCN and the deep DCN does not match with the high levels of GABA found in these regions [29, 30, 31], or with the abundance of GABA or GAD immunoreactive endings and cell bodies, particularly in the magnocellular regions of the VCN (see refs. 4, 38 and 82 for reviews).
The presence of strong inhibitory responses to GABA, especially in magnocellular regions of the VCN [15,19,46] also is in contrast with this apparently low concentration of GABA_A receptors. It should be kept in mind that conventional receptor autoradiography methods do not provide resolution at the synaptic level. Therefore, we cannot disregard the possibility that the low levels of GABA_A binding sites found in the magnocellular regions of the VCN relative to other CN regions could be due in part to potential differences in the concentration of GABAergic synapses. However, in regions like the rostral part of the AVCN, where GABA_A binding levels are very low, GABA immunopositive endings seem to be at least as abundant as in the superficial layers of the DCN [38], where GABA_A binding levels are dramatically high. It is therefore unlikely that differences in synaptic concentration could account for all the observed mismatch between the distribution of GABA and GABA_A binding sites in the CN. Another possible explanation for this mismatch is that GABA_B rather than GABA_A receptors could be associated with many GABAergic synapses in the central region of the VCN and in the deep DCN. The levels of GABA_B binding sites in these regions, however, are too low to support this hypothesis (see discussion on GABA_B receptors below). Furthermore, considering that GABA-mediated inhibition is essentially bicuculline-sensitive [15,16,19], one would expect to find indications of a greater concentration of GABA_A receptors in these regions than actually found by autoradiography or immunocytochemistry. Therefore, another possibility to explain this mismatch is that there may be GABA_A receptors in magnocellular regions of the VCN and deep DCN different from those visualized in granule cell domains and superficial layers of the DCN. Several lines of evidence support this possibility. First, [3H]muscimol and [3H]GABA under the conditions of our assay bind preferentially to the so-called ‘high-affinity’ GABA_A sites [8,12,55,56,58]. Reflecting their complex pharmacology, GABA_A receptors can also be identified with assays for ‘low affinity’ binding sites, for the benzodiazepine binding sites, and for the picrotoxin binding sites [39,55,57,58,64,65]. In several instances, the regional distribution of these binding sites differs from that of ‘high affinity’ GABA_A binding sites [50,58,64,66,68,78,85]. Application of these autoradiographic assays to the CN might reveal the distribution of GABA_A binding sites not visualized with our assay. Second, the possibility of heterogeneous populations of GABA_A receptors among different anatomical regions of the CN is further strengthened if one considers the structural diversity of GABA_A receptors at the molecular level [41,70]. There are multiple isoforms of each of the subunits which constitute the GABA_A receptor and they probably combine in many different ways to give receptors with different structural and functional characteristics [22,43,56,63,70,80]. Mapping by in situ hybridization histochemistry the GABA_A receptor subunits expressed in different CN regions and neurons [32] is another promising approach to address the possibility of GABA_A receptor diversity in this nucleus.

Several pharmacological studies have addressed the inhibitory effects of the GABA_B agonist baclofen in the CN [17,19,25,48]. However, the distribution of GABA_B receptors in this nucleus was so far unknown. The heterogeneous distribution of GABA_B binding sites in the CN and the high binding levels in the molecular layer of the DCN found in the present study, raise questions about the function of GABA_B receptors in this auditory nucleus. Based on iontophoretic application of the prototype GABA_A agonist baclofen [9,10] Caspary and coworkers have hypothesized that this substance may inhibit units in the VCN by blocking the release of glutamate or aspartate from auditory nerve endings [17,19], as suggested in other systems [9,14,37,54,61]. According to our results, the main territory of distribution of auditory nerve endings, including the central region of the VCN, deep DCN and fusiform/granule cell layer of the DCN contained low to very low levels of GABA_B binding sites. The low level of GABA_B binding sites found in these areas, does not necessarily argue against a role for GABA_B receptors in synaptic events in the central region of the VCN. In most hippocampal regions, the level of GABA_B binding sites is not particularly high [11,20], but physiological studies have demonstrated that presynaptic GABA_B receptors have significant effects on the control of excitatory transmission in this region [40,53,54].

In contrast with findings in magnocellular regions of the VCN, levels of GABA_B binding sites were remarkably higher in other areas of the CN, where they may be involved in inhibitory activity together with GABA_A receptors. GABA_B binding was moderate in the granule cell cap of the ventral CN and high in the molecular layer of the DCN. A feature common to both areas is the endings of granule cells [51,52]. Therefore, one possibility is that GABA_B receptors might be presynaptic at the level of granule cell synapses, where they could modulate the release of glutamate, apparently used by CN granule cells as neurotransmitter [36,44,45]. However, there are also abundant GABA immunoreactive axo-dendritic endings in the granule cell cap of the VCN and the molecular layer of the DCN [38], and GABA_B receptors might be postsynaptic to many of them. GABA_B binding sites in the molecular layer of the cerebellum, whose organization shares analogies with the molecular layer of the DCN [5,6,51,52,84] are predominantly postsynaptic and located on Purkinje cells [69,77]. It is obvious that the ascertainment of pre-
or postsynaptic localization of GABA$_B$ receptors in the CN requires further experiments. In any case, the high level of GABA$_B$ binding sites in the molecular layer of the DCN, suggests that they have an important role in the integrative activity of this CN division. The apical dendrites of fusiform cells [6,13,42], the main projection neurons of the DCN, are located in the molecular as well as in the fusiform cell layers, which makes it likely that both GABA$_A$ and GABA$_B$ receptors contribute to the control of fusiform cell activity.

Acknowledgements: Supported by Grants NIDCD DC00383 (R.A.A.), NS 01300, NS 19613 (R.L.A.) and CICYT PB/0523/C02/00 (J.M.J.)

5. References


[71] Sekiguchi, M., Sakuta, H., Okamoto, K. and Sakai, Y., GABA B receptors expressed in Xenopus oocytes by guinea pig cerebral mRNA are functionally coupled with Ca2+-dependent Cl- channels and with K+ channel through GTP-binding proteins, Brain Res.-Mol. Brain Res., 8 (1992) 301-309.


