Nucleus-Specific Expression of GABA_A Receptor Subunit mRNAs in Monkey Thalamus

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Expression of 10 GABA_A receptor subunit genes was examined in monkey thalamus by *in situ* hybridization using cRNA probes specific for α_1 , α_2 , α_3 , α_4 , α_5 , β_1 , β_2 , β_3 , γ_1 , and γ_2 subunit mRNAs. These displayed unique hybridization patterns with significant differences from rodents. α_1 , β_2 , and γ_2 transcripts were expressed at high levels in all dorsal thalamic nuclei, but expression was significantly higher in sensory relay nuclei—especially the dorsal lateral geniculate nucleus. Other transcripts showed nucleus-specific differences in levels of expression and in the range expressed. α_5 and α_4 subunit transcripts

were expressed in all nuclei except the intralaminar nuclei. Levels of α_2 , α_3 , β_1 , β_3 , and γ_1 expression were very low, except in intralaminar nuclei. In the reticular nucleus, most subunit transcripts were not expressed, and only γ_2 transcripts were consistently detected at modest levels. Thalamic GABA_A receptors may be assembled from nucleus-specific groupings of subunit polypeptides.

Key words: *in situ* hybridization; α , β , and γ subunits; inhibition; chromosomes; sensory nuclei; intralaminar nuclei

GABAergic inhibition in the mammalian thalamus affects rates and temporal patterns of discharge of relay neurons (Curtis et al., 1972; Duggan and McLennan, 1971; Curtis and Têbêcis, 1972; Hicks et al., 1986), receptive field properties (Sillito and Kemp, 1983; Pape and Eysel, 1986; Eysel et al., 1987; Sillito, 1992), and state-dependent rhythmic oscillations of the thalamic network (Steriade et al., 1991; Warren et al., 1994; Bal et al., 1995).

Thalamic inhibition is mediated by GABAergic neurons located in the reticular nucleus and by GABAergic intrinsic interneurons (Houser et al., 1980; Montero and Scott, 1981; Oertel et al., 1983; Spreafico et al., 1983; Montero and Singer, 1985; Yen et al., 1985; DeBiasi et al., 1986; Ohara et al., 1989; Cucchiari et al., 1991; Ralston, 1991; Ohara and Lieberman, 1993; Liu et al., 1995). In cats and monkeys, interneurons constitute ~25% of dorsal thalamic neurons (LeVay and Ferster, 1979; Weber and Kalil, 1983; Fitzpatrick et al., 1984; Montero and Zempel, 1985, 1986; Rinivik et al., 1987; Benson et al., 1991b; Hendry, 1991; Hunt et al., 1991).

The GABA_A receptor, a fast-acting, bicuculline-sensitive chloride channel is a heteropentameric complex composed of combinations of five or fewer polypeptide subunits (Macdonald and Olsen, 1994). The receptor has binding sites for benzodiazepines, barbiturates, and neurosteroids, all of which modify GABA-mediated chloride currents (DeLorey and Olsen, 1992). At least 15 genes encode GABA_A receptor subunits that share a high degree of sequence identity, classified as α , β , γ , δ , and ρ classes with subclass variants (α_{1-6} , β_{1-3} , γ_{1-3} , δ , $\rho_{1,2}$). α_1 , β_2 , and γ_2 subunits are expressed at high levels in most brain regions (Benke et al., 1991; Fritschy et al., 1992; Benke et al., 1994; Fritschy and

Möhler, 1995) of rodents (Gambarana et al., 1991; Persohn et al., 1992; Wisden et al., 1992) and monkeys (Huntsman et al., 1994, 1995a,b). GABA_A receptors may be assembled from specific combinations of individual subunits (Lüddens and Wisden, 1991; Angelotti et al., 1993; Angelotti and Macdonald, 1993; Backus et al., 1993; Perez-Velazquez and Angelides, 1993), but combinations of α , β , and γ subunits are necessary to produce fully functional GABA_A receptors (Pritchett et al., 1989a; Siegel et al., 1990; Verdoorn et al., 1990; Angelotti and Macdonald, 1993).

Previous radioligand binding studies of GABA_A receptor localization in the thalamus were confined to rats (Palacios et al., 1981; Unnerstall et al., 1981; Bowery et al., 1987; Olsen et al., 1990; Bureau and Olsen, 1993) in which intrinsic interneurons are largely absent (for review, see Benson et al., 1992), and to isolated nuclei in other species (Shaw and Cynader, 1986; Kultas-Ilinsky et al., 1988). Subunit-specific protein and mRNA expression has been briefly described in rat thalamus (Wisden et al., 1992; Fritschy and Möhler, 1995) but only in the dorsal lateral geniculate nucleus of monkeys (Huntsman et al., 1995a).

Preliminary reports of the present study have appeared elsewhere (Huntsman and Jones, 1993, 1995).

Abbreviations

<i>AD</i>	anterodorsal nucleus of the thalamus
<i>AM</i>	anteromedial nucleus of the thalamus
<i>AV</i>	anteroventral nucleus of the thalamus
<i>CeM</i>	central medial nucleus of the thalamus
<i>CL</i>	central lateral nucleus of the thalamus
<i>CM</i>	centre médian nucleus of the thalamus
<i>CN</i>	caudate nucleus
<i>Gpi</i>	globus pallidus, internal segment
<i>H</i>	habenular nuclei
<i>Hbm</i>	medial habenular nucleus
<i>L</i>	limitans nucleus of the thalamus
<i>LD</i>	lateral dorsal nucleus of the thalamus
<i>LGd</i>	dorsal lateral geniculate nucleus of the thalamus

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Figure 1. *A*, Digital scan of a section hybridized with a cRNA probe specific for α_1 subunit mRNA. Each sampled area is represented by a box within the borders of the relevant nucleus, which were confirmed from an adjacent Nissl-stained section. *B*, Section hybridized with sense-strand subunit riboprobe for α_1 subunit transcripts showed no labeling above background levels. Scale bar, 2 mm. *C*, Dark-field photomicrograph from an emulsion-dipped autoradiograph showing γ_2 probe hybridization in the reticular nucleus (*R*) and lateral posterior nucleus (*LP*). Scale bar, 100 μ m. *D*, Bright-field photomicrograph showing labeling of large and small cells in the mediodorsal nucleus with the γ_2 riboprobe; nuclei of neuroglial cells are not labeled. Scale bar, 10 μ m.

Table 1. GABA_A receptor mRNAs in monkey thalamus

	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\beta 1$	$\beta 2$	$\beta 3$	$\gamma 1$	$\gamma 2$
Anterior Group										
Intralaminar Nuclei										
Rostral										
Caudal										
Sensory Relay Nuclei										
Lateral Geniculate										
Reticular Nucleus										

< 100 nCi/g =
 101–200 nCi/g =
 201–400 nCi/g =

401–600 nCi/g =
 > 601 nCi/g =

Optical density readings were obtained from film autoradiograms in individual nuclei and averaged across serial sections from multiple runs of *in situ* hybridization histochemistry. Hybridization levels of ³³P-labeled cRNA probes were converted from density units to units of radioactivity using ¹⁴C standards exposed on the same sheet of film (see Materials and Methods). In some cases, values represent individual nuclei, and for others they represent average values in groups of nuclei. Hybridization levels are schematically illustrated in ascending order of intensity ranging from levels <100 nCi/gm to levels >600 nCi/gm. Note the high levels of hybridization for the $\alpha 1$, $\beta 2$, and $\gamma 2$ cRNA probes, especially in the lateral geniculate and other sensory relay nuclei.

LGmc dorsal lateral geniculate nucleus of the thalamus, magnocellular layers
LGpc dorsal lateral geniculate nucleus of the thalamus, parvocellular layers
LP lateral posterior nucleus of the thalamus
MD mediodorsal nucleus of the thalamus
MG medial geniculate nucleus of the thalamus
MGd medial geniculate nucleus, dorsal
MGv medial geniculate nucleus, ventral
Pc paracentral nucleus
Pf parafascicular nucleus of the thalamus
Pla anterior pulvinar nucleus of the thalamus
Pli inferior pulvinar nucleus of the thalamus
Pll lateral pulvinar nucleus of the thalamus
Plm medial pulvinar nucleus of the thalamus
Po posterior nucleus of the thalamus
Prg pregeniculate nucleus
PT pretectum
Pv paraventricular nucleus of the thalamus
R reticular nucleus of the thalamus
Rh rhomboid nucleus of the thalamus
Sb subthalamic nucleus
SG supragenulate nucleus of the thalamus
SNr substantia nigra, pars reticulata
VA ventral anterior nucleus of the thalamus
VL ventral lateral nucleus
VLa anterior ventral lateral nucleus of the thalamus

VLp posterior ventral lateral nucleus of the thalamus
VMb basal ventral medial nucleus of the thalamus
VPI ventral posterior inferior nucleus of the thalamus
VPL ventral posterior lateral nucleus of the thalamus
VPM ventral posterior medial nucleus of the thalamus
ZI zona incerta

MATERIALS AND METHODS

Generation of cRNA probes. Complementary RNA (cRNA) probes were prepared from monkey-specific cDNAs for localization of the $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\beta 1$, $\beta 2$, $\beta 3$, $\gamma 1$, and $\gamma 2$ GABA_A receptor subunit mRNAs and for 67 kDa glutamic acid decarboxylase (GAD) mRNA. These include the majority of the GABA_A receptor subunits that are expressed in the CNS. The methods used to subclone, purify, and characterize the cDNAs, including Northern blot analysis, were identical to those used in a previous study (Huntsman et al., 1994). Synthetic oligonucleotide primers were designed to flank a heterogeneous domain of each chosen GABA_A receptor subunit DNA that was then amplified against a monkey cDNA template using the PCR. The cDNA was sequenced and inserted into the pBS II cloning vector (Stratagene, La Jolla, CA). The monkey-specific cDNA for GAD encodes the 67 kDa protein product (GAD₆₇) and was generated and fully characterized in a previous study (Benson et al., 1991a).

Three additional monkey-specific cDNA subclones with sequences corresponding to the intracellular cytoplasmic loops of $\alpha 3$, $\beta 3$, and $\gamma 1$ GABA_A receptor subunit polypeptides were isolated and characterized using the same methods used to isolate $\alpha 1$, $\alpha 2$, $\alpha 4$, $\alpha 5$, $\beta 1$, $\beta 2$, and $\gamma 2$ cDNAs in the previous study (Huntsman et al., 1994). The cDNAs were then used as templates for the generation of riboprobes for *in situ* hybridization histochemistry as described previously (Benson et al., 1994; Huntsman et al., 1994, 1995a,b).

All the monkey-specific subclones used were similar in length (341–497

Figure 2. Photomicrographs from film autoradiograms of adjacent sections through anterior levels of the thalamus hybridized with the α_1 , α_2 , α_3 , α_4 , α_5 , γ_1 , and γ_2 GABA_A receptor subunit-specific cRNA probes (A–E, G, H), a GAD₆₇ cRNA probe (F), and a photomicrograph of an adjacent Nissl-stained section (I). These show the patterns of distribution of 7 of the 10 subunit-specific mRNAs in relation to each nucleus. All autoradiograms were exposed for the same time. Scale bar, 2 mm.

Figure 3. Photomicrographs from film autoradiograms of sections adjacent to those in Figure 2 through anterior levels of the thalamus, hybridized with β_1 , β_2 , and β_3 GABA_A receptor subunit-specific cRNA probes (A–C). Scale bar, 2 mm.

bp) and in G/C content and shared a high degree of sequence identity with previously cloned cDNAs of human (where available) and rat sequences, as determined by Genbank searches. Radiolabeled RNA probes for *in situ* hybridization histochemistry were generated by *in vitro* transcription of the linearized, subcloned cDNAs in the presence of [α - 33 P]UTP, using either T3 or T7 RNA polymerase for the production of antisense and sense strands.

The riboprobes had similar specific activities. Northern blot analysis (Huntsman et al., 1994) using the same cDNA templates identified transcripts with molecular weights that were the same as those reported in other studies (Garrett et al., 1988; Lolait et al., 1989; Ymer et al., 1989a,b; Khrestchatsky et al., 1989, 1991; Malherbe et al., 1990). Further verification of probe specificity is seen in the distinct patterns of expression of the α subunit variants. The riboprobes that identified the α_1 , α_2 , α_3 , α_4 , and α_5 subclass variants gave very distinctive and sometimes nonoverlapping patterns of labeling in the dorsal thalamus (e.g., α_2 and α_4) even though they share >70% sequence identity in pairwise residue comparisons (Seeburg et al., 1990).

In situ hybridization histochemistry. The brains of four monkeys were used: one *Macaca fuscata*, one *Macaca fascicularis*, and two *Macaca mulatta*. All animals were given an overdose of Nembutal and perfused through the ascending aorta with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were blocked and post-fixed overnight in 4% paraformaldehyde and then infiltrated with 30% sucrose in 4% paraformaldehyde. The blocks were frozen in dry ice and those containing the diencephalon were cut at 25 μ m on a sliding microtome. Sets of eleven sections were collected in cold, sterile 4% paraformaldehyde for *in situ* hybridization histochemistry; the two succeeding sections were collected in cold phosphate buffer for Nissl and cytochrome oxidase (CO) (Wong-Riley, 1979) staining. Ten of each set of 11 sections collected in paraformaldehyde were hybridized with radiolabeled antisense cRNA probes for the GABA_A receptor subunit mRNAs. The remaining section of each set was used for repeat runs, if necessary, for hybridization with the GAD₆₇ riboprobe, or for hybridization with sense control riboprobes.

Free-floating sections were prepared for *in situ* hybridization by washing in 0.1 M glycine in 0.1 M phosphate buffer, pH 7.4, followed by incubation in 1 mg/ml of proteinase K in 0.1 M Tris-HCl buffer, pH 8.0, containing 0.05 M EDTA for 30 min at 37°C; they were then washed in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, and in 2 $\times$ SSC. Sections were next incubated in the hybridization solution containing 50% formamide, 10% dextran sulfate, 0.7% Ficoll, 0.7% polyvinyl pyrrolidone, 0.5 mg/ml yeast tRNA, 0.33 mg/ml denatured herring sperm DNA, and 20 mM dithiothreitol (DTT) for 1 hr at 60°C, then transferred to fresh hybridization solution containing an additional 20 mM DTT and 1 $\times$ 10⁶ cpm/1 ml of the 33 P-radiolabeled sense or antisense riboprobe.

After at least 20 hr of hybridization at 60°C, sections were washed in 4 $\times$ SSC at 60°C, digested with 20 mg/ml ribonuclease A, pH 8.0, for 30

min at 45°C, and washed through descending concentrations of SSC with 5 mM DTT to a final stringency of 0.1 $\times$ SSC at 60°C for 1 hr. Sections were mounted on gelatin-coated slides, dried, and exposed to Amersham β -max film for 8 d. After development of the film, the sections were lipid-extracted in chloroform, dipped in Kodak NTB2 emulsion (diluted 1:1), exposed for 8 d at 4°C, developed in Kodak D-19, fixed, and counterstained with cresyl violet.

Quantitative analysis. Film autoradiograms of hybridized sections were quantified by taking optical density readings using a microcomputer imaging device (MCID/M4; Imaging Research, St. Catherine's, Ontario, Canada). Quantitative sampling was obtained by calibrating the optical system to a set of radioactive standards before reading density values. The imaging system functions by sampling mean gray-level values of all the pixels within a defined sampling area and converts them to values of concentration termed integrated optical density (IOD) values. The IOD value is converted to units of radioactivity by taking readings from 14 C reference standards (Amersham) exposed on the same sheet of film as the sampled sections and converting the values from these standards to units of radioactivity (nCi/gm) within the sampled area. Multiple samples were taken from individual thalamic nuclei using a sampling tool adjusted to fit within the borders of the nucleus, as confirmed from the adjacent Nissl- and CO-stained sections (Fig. 1A). Each unit sampling was tracked and recorded on the digitized image to ensure that the samples taken within a single thalamic nucleus did not overlap. The threshold level for reporting the presence of hybridization signal was set at 50 nCi/gm, because at this level nuclear borders could not be discerned. Sections hybridized with sense strand subunit riboprobes showed no labeling above background levels (Fig. 1B). Nuclear delineations are those of Jones (1985). The nuclear distribution patterns were compared with the patterns of expression of GAD₆₇, the details of which were described by Benson et al. (1991b).

RESULTS

Distribution of subunit mRNAs

The dorsal thalamus

The 10 GABA_A receptor subunit mRNAs examined in this study (α_1 , α_2 , α_3 , α_4 , α_5 , β_1 , β_2 , β_3 , γ_1 , and γ_2) had a wide range of expression patterns in terms of both density and distribution throughout the dorsal thalamus. The distribution of most of the subunit mRNAs was overlapping. However, when examined in detail, each transcript displayed a characteristic pattern that differed from that of the other subunit mRNAs. The intensity of hybridization, reflecting different levels of expression, specifically varied for each subunit mRNA. In most dorsal thalamic nuclei, the α_1 , β_2 , and γ_2 subunit transcripts were expressed at higher

Figure 4. Photomicrographs from film autoradiograms of adjacent sections through levels of the thalamus posterior to those represented in Figures 2 and 3. Sections were hybridized with the α_1 , α_2 , α_3 , α_4 , α_5 , γ_1 , and γ_2 GABA_A receptor subunit-specific cRNA probes (A–E, G, H) or with a GAD₆₇ riboprobe (F). All subunit mRNA distribution patterns can be compared with an adjacent Nissl-stained section (I). Note increased levels of γ_2 transcripts in VLa (H). Scale bar, 2 mm.

Figure 5. Photomicrographs from film autoradiograms of sections adjacent to those shown in Figure 4. Adjacent sections were hybridized with β_1 , β_2 , and β_3 (A–C) subunit-specific cRNA probes. Scale bar, 2 mm.

levels than the α_5 transcript, which was in turn expressed at higher levels than the α_2 , α_3 , α_4 , β_1 , β_3 , and γ_1 transcripts. Among each of the nuclei there were individual differences, as described in detail below. Some nuclei expressed a wide variety of subunit transcripts, whereas other nuclei expressed a more limited range. In certain groups of nuclei, there was a complementarity of expression in the sense that expression of one set of transcripts was associated with nonexpression of another set. These differences indicate nucleus-specific patterns of gene expression for each of the subunits. The distribution and density of labeling for individual transcripts is summarized in Table 1. The following section deals with some of the unique features of the localization patterns.

Cellular localization of mRNAs

Cell labeling with radioactive riboprobes consisted of clusters of silver grains overlying the somata of individual cells. Specific labeling consisted of compact accumulations of grain clusters over cell somata, yielding a clear definition of individually labeled cells, contrasting with the light scattering of silver grains associated with background labeling (Fig. 1C,D). The sizes of the grain clusters could therefore be used as a guide to the relative sizes of the cell bodies, based on known sizes of the major population of cells within particular thalamic nuclei (Benson et al., 1991b). An example of hybridization of GABA_A receptor riboprobes specific for the γ_2 subunit in the reticular nucleus and neighboring lateral posterior nucleus (Fig. 1C) shows grain accumulations over Nissl-stained cell bodies in which the grain clusters in the lateral posterior nucleus range in size from small (10–15 μ m) to large (20–25 μ m). This was typical of all dorsal thalamic nuclei (Fig. 1D). It is therefore assumed that both relay cells and interneurons, which are consistently smaller, were labeled with the subunit riboprobes. Those in the reticular nucleus were all large, reflecting the unimodal size range of cells in this nucleus. Qualitatively, the number of labeled neurons detectable with the different riboprobes varied. In general, in nuclei showing a high density of labeling for a particular subunit mRNA, most of the neurons were labeled. In those showing weak labeling for a particular subunit mRNA, few individually labeled cells could be de-

tected. All riboprobes labeled only neurons, except for the α_4 , β_1 , and γ_1 riboprobes in which labeling of significant numbers of neuroglial cells could also be detected, leading to darkening of the internal capsule in some of the autoradiograms (data not shown).

Nuclear localization of mRNAs

In what follows, a nucleus is said to be labeled for subunit transcripts if integrated optical density measurements translated to more than the 50 nCi/gm threshold level.

The anterior nuclear complex

The AM, AV, and AD nuclei expressed all the subunit mRNAs with varying degrees of intensity (Figs. 2–5). The most abundant mRNAs in order of intensity were β_2 , α_5 , α_1 , α_3 , and γ_2 (Figs. 2E,A,C,H, 3B). In these nuclei, α_5 transcripts reached higher levels of hybridization than in any other nuclear group in the dorsal thalamus. Modest levels were observed for the β_3 and β_1 mRNAs (Fig. 3C,A) with still lower levels observed for α_4 , α_2 , and γ_1 mRNAs (Fig. 2D,B,G).

The ventral group of nuclei

Moderate levels of expression were evident for the α_1 , β_2 , and γ_2 mRNAs in the VA nucleus (Figs. 2A,H, 3B, 4A,H, 5B), with slightly lower levels for α_5 mRNAs (Figs. 2F, 3F) and reduced levels for the other subunit mRNAs. The VL nucleus had a similar distribution pattern with noticeably increased levels of γ_2 subunit transcripts in the VL_a (see Fig. 5H). In the ventral posterior nucleus, the α_1 , β_2 , γ_2 , and α_5 subunit transcripts were the most prominent subunit mRNAs expressed (see Figs. 6A,E,H, 7B, 8A,E,H, 9B). α_3 and α_4 transcripts were also labeled but with lower intensity (see Figs. 6C,D, 8C,D), and even lower levels of hybridization were observed for α_2 , β_1 , β_3 , and γ_1 mRNAs (see Figs. 6B,G, 7A,C, 8B,G, 9A,C). In the VPL and VPM nuclei, 8 of the 10 subunit mRNAs showed increased levels in VPM compared with VPL, but α_4 and γ_2 levels did not (see Figs. 6D,H). The VPI nucleus typically had moderate levels of hybridization with noticeable decreases for α_1 and β_2 in relation to neighboring VPL and VPM (see Figs. 6A, 7B). The VMb nucleus had the highest expression levels of the ventral group for almost all of the subunits

Figure 6. Photomicrographs from film autoradiograms of adjacent sections through middle levels of the thalamus. Each section was hybridized with the α_1 , α_2 , α_3 , α_4 , α_5 , γ_1 , and γ_2 GABA_A receptor subunit-specific cRNA probes (*A–E*, *G*, *H*) or with a GAD₆₇ riboprobe (*F*). mRNA distribution patterns can be compared with an adjacent Nissl-stained section (*I*). Note intensity of α_1 transcript levels in LGd and in the ventral nuclear group (*A*) in addition to the restricted hybridization of α_3 transcripts in the magnocellular layers of the LGd, indicated by an arrow (*C*). Scale bar, 2 mm.

Figure 7. Photomicrographs from film autoradiograms of sections adjacent to those in Figure 6, hybridized with β_1 , β_2 , and β_3 (A–C) GABA_A receptor subunit-specific cRNA probes. Scale bar, 2 mm.

examined, exceeding those for both VPM and VPL, with the exception of the α_1 subunit transcripts, the levels of which decreased (see Fig. 6A).

The MD nucleus

The β_2 , γ_2 , α_5 , α_3 , and α_1 mRNAs were present in the mediodorsal nucleus at moderate levels, with slightly lower levels observed for γ_1 , α_4 , β_3 , β_1 , and α_2 subunit mRNAs (Figs. 4–9). No obvious subnuclear differences could be detected.

The lateral group of nuclei

The LD nucleus expressed high levels of most subunit transcripts, especially β_2 , α_1 , α_5 , and γ_2 (see Figs. 6A,E,H, 7B, 8A,E,H, 9B), along with moderately high levels of α_3 , β_1 , and γ_1 (see Figs. 6C,G, 7A, 8C,G, 9A) but weaker levels of α_2 , α_4 , and β_3 mRNAs (see Figs. 6B,D, 7C, 8B,D, 9C). The LP nucleus similarly displayed high hybridization levels of β_2 , α_1 , α_5 , and γ_2 mRNAs (see Figs. 8A,E,H, 9B, 10A,E,H, 11B) that approached those observed in the LGd nucleus. Overall, LP maintained high levels of the remaining subunit transcripts, except for α_2 , α_3 , β_1 , and β_3 mRNAs (see Figs. 8B,C, 9A,C, 10B,C, 11A,C). In the nuclei of the pulvinar, the Pla, Pli, Plm, and Pll nuclei showed a heterogeneous distribution of receptor subunit transcripts in which α_1 clearly dominated, reaching high levels in Pli, Pll, and Plm (see Fig. 10A), although levels were still lower than in the adjacent lateral geniculate and lateral posterior nuclei. High levels of α_5 , γ_2 , and β_2 transcripts were also observed in Pli and Plm (see Figs. 10E,H, 11A), along with moderate levels of α_2 , α_3 , α_4 , β_1 , β_3 , and γ_1 mRNAs, primarily in Pli and Plm, in which levels never dipped below the weakly positive range (see Figs. 10B,C,D,G, 11A,C). Overall, Pla had the lowest transcript levels for all subunits examined in the pulvinar, except for α_3 and γ_1 , whose levels in Pll were slightly less (see Figs. 9C,G, 10C,G).

The intralaminar nuclei

The intralaminar nuclei showed moderate-to-high levels of almost all of the receptor subunit transcripts examined. The moderate levels of hybridization observed for the α_2 , α_3 , β_1 , β_3 , and γ_1 mRNAs were unusual because these transcripts were only de-

tected at very low levels in the majority of thalamic nuclei. The rostral group of intralaminar nuclei in particular displayed moderately high levels of the α_1 , α_2 , α_3 , α_5 , β_1 , β_2 , β_3 , γ_1 , and γ_2 transcripts, especially in Rh and CeM nuclei (Figs. 2–7). There were also very high levels in the Pf nucleus of the caudal group of intralaminar nuclei (see Figs. 8, 9). Labeling of α_4 transcripts was noticeably absent from every nucleus in the intralaminar group with the Rh and CeM nuclei being minor exceptions, showing just weakly positive levels (Fig. 4D). α_5 transcripts were also decreased in the caudal intralaminar group from the usual moderately high levels of expression seen in other dorsal thalamic nuclei; there was a dramatic drop in hybridization levels for α_5 transcripts in the CM and CL nuclei (see Figs. 6E, 8E). Another unusual feature of the intralaminar nuclei compared with other nuclei was the complementary nature of expression of α_4 and α_5 mRNAs and of α_2 , α_3 , β_1 , and γ_1 mRNAs in the CM and CL nuclei. The α_4 and α_5 subunit transcripts were present in many of the dorsal thalamic nuclei, with the exception of the intralaminar nuclei in which there was a dramatic drop in hybridization levels for them (Figs. 4D,E, 6D,E, 8D,E). This was in sharp contrast to the expression of α_2 , α_3 , β_1 , and γ_1 subunit mRNAs, which was virtually absent in most other nuclei but present at moderate-to-high levels in the CeM, CM, Pc, Pf, and CL nuclei of the intralaminar group (Figs. 4B,C,G, 5A, 6B,C,G, 7A).

The medial geniculate complex

All of the subunit transcripts were present at moderate levels in the medial geniculate complex. High levels of hybridization were observed for the α_1 , α_5 , β_2 , and γ_2 subunit transcripts in both the MGv and MGd nuclear divisions, with a slight increase in signal in MGv (see Figs. 8A,E,H, 9B). A distinctive increase in hybridization levels was also observed for α_1 subunit transcripts in the magnocellular nucleus (see Fig. 8A). Moderate levels were detected for β_1 and γ_1 subunit transcripts (see Figs. 8G, 9A) with slightly lower levels for β_3 , α_3 , α_4 , and α_2 transcripts (see Figs. 8B,C,D, 9C). The dorsal nucleus of the complex showed increased levels over the ventral nucleus for α_3 , α_4 , and α_5 subunit transcripts.

Figure 8. Photomicrographs from film autoradiograms of adjacent sections through posterior levels of the thalamus. Each section was hybridized with the α_1 , α_2 , α_3 , α_4 , α_5 , γ_1 , and γ_2 GABA_A receptor subunit-specific cRNA riboprobes (A–E, G, H) or with a GAD₆₇ riboprobe (F). Patterns can be compared with an adjacent Nissl-stained section (I). Scale bar, 2 mm.

Figure 9. Photomicrographs from film autoradiograms of sections adjacent to those in Figure 8 hybridized with β_1 , β_2 , and β_3 (A–C) GABA_A receptor subunit-specific cRNA probes. Scale bar, 2 mm.

The LGd nucleus

Of all nuclei in the thalamus, the LGd nucleus had the highest levels of hybridization for every subunit transcript. The expression patterns in this nucleus have been described in detail elsewhere (Huntsman et al., 1995a). Expression of the α_1 , β_2 , and γ_2 subunit mRNAs was particularly high. Moreover, the LGd nucleus also expressed all other transcripts at moderate-to-high levels, including transcripts that were usually expressed at low levels in other nuclei—a similar pattern observed for the other sensory relay nuclei. Expression of α_1 , α_5 , β_2 , and γ_2 mRNA was slightly higher in the magnocellular layers (Figs. 6A,H, 7B), whereas that of the α_2 , α_4 , β_1 , β_3 , and γ_1 mRNAs was equally high in all six layers (Figs. 6B,D,G, 7A,C). The distribution of α_3 mRNAs was the most unique because hybridization for this subunit mRNA was primarily located in the magnocellular layers (Fig. 6C).

The posterior group of nuclei

The posterior group had overall low levels of expression of all receptor subunit transcripts, even those normally expressed at high levels, such as α_1 , α_5 , β_2 , and γ_2 mRNAs. The SG–L nuclei expressed a wide variety of receptor subunit transcripts, including α_1 , α_2 , α_3 , β_1 , β_2 , γ_1 , and γ_2 (see Figs. 10A,B,C,G,H, 11A,B). The distribution pattern and variety of transcripts expressed in SGL was similar to that observed in the intralaminar nuclei, with a comparable increase in hybridization levels for the otherwise rare α_2 , α_3 , β_1 , and γ_1 subunit mRNAs and a reduction to negligible levels of α_4 subunit mRNAs.

The epithalamus

In the habenular nuclei, the most prominently expressed subunit mRNAs were the two representatives of the γ class, γ_1 and γ_2 (Figs. 8G,H), and α_3 (Fig. 8C). α_1 , α_2 , β_1 , and β_2 subunit mRNAs were present, but at much lower levels (Figs. 8A,B, 9A,B), and α_4 , α_5 , and β_3 mRNAs hybridized just above background (Figs. 8D,E, 9C). The highest transcript levels detected in the paraventricular nuclei were γ_2 , γ_1 , α_1 , α_3 , α_5 , and β_1 and, to a lesser degree, α_2 , β_2 , β_3 , and α_4 (Figs. 2, 3). All of the subunit mRNAs examined were present at relatively high levels, similar to those observed in the intralaminar nuclei. The paraventricular nuclei also had the

highest expression levels of γ_1 transcripts: almost double that observed in any other nucleus of the thalamus (Fig. 2G).

The ventral thalamus

The reticular nucleus was unique. Of all the transcripts studied, only the γ_2 subunit mRNA was expressed in significant amounts. α_2 , α_3 , α_5 , β_1 , β_3 , and γ_1 subunit expression could sometimes be identified at extremely low levels, but this was not consistent and always bordered on undetectability. α_3 was detected more often than the others. Labeling for the α_1 , α_4 , and β_2 transcripts was never detected in the reticular nucleus. In the fields of Forel and the ZI, mRNAs for γ_2 , α_1 , β_2 , and α_3 subunits were present at relatively low levels, and other transcripts were absent (Figs. 4A,C,H, 5B). The small dorsal extension of the ZI into the posteroventral part of the reticular nucleus showed expression typical of the rest of the ZI (Figs. 4, 5). The pregeniculate nucleus expressed most of the subunit transcripts at moderate levels, especially γ_2 and α_1 (Figs. 6A,H).

Quantitative observations

Relative levels of mRNA expression were determined by measuring the density of hybridization from film autoradiograms and converting them to units of radioactivity (nCi/gm). Density readings for each cRNA probe were collected and compared across thalamic nuclei to determine which groups of subunit mRNAs were expressed in particular nuclei and which nuclei maintained the highest levels of each subunit mRNA (Table 2). Optical density readings for all GABA_A receptor transcripts verified the observations shown in Figures 2–11 and were the source of numerical data used to construct Figures 12–14 and Tables 1 and 2. The α_1 , β_2 , and γ_2 subunit transcripts were present at extremely high levels in the sensory relay nuclei (Fig. 12) and exhibited high levels in most of the other nuclei (Fig. 13). The other transcripts showed varying patterns of which the greatest variations were found in the intralaminar nuclei (Fig. 13). In the reticular nucleus, levels of most subunit mRNAs did not reach threshold, except γ_2 and occasionally α_3 subunit transcripts (Fig. 14).

Figure 10. Photomicrographs from film autoradiograms of adjacent sections through the posterior pole of the thalamus, including all subdivisions of the pulvinar except Pla. Sections were hybridized with the α_1 , α_2 , α_3 , α_4 , α_5 , γ_1 , and γ_2 GABA_A receptor subunit-specific cRNA riboprobes (A–E, G, H) or with a GAD₆₇ riboprobe (F). Note increased levels in LP for the α_1 (A) and γ_2 (H) transcripts. Patterns can be compared with an adjacent Nissl-stained section (I). Scale bar, 2 mm.

Figure 11. Photomicrographs from film autoradiograms of sections adjacent to those shown in Figure 10 and hybridized with β_1 , β_2 , and β_3 (A–C) GABA_A receptor subunit-specific riboprobes. Scale bar, 2 mm.

Table 2. Relative intensity of hybridization in thalamic nuclei

	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\beta 1$	$\beta 2$	$\beta 3$	$\gamma 1$	$\gamma 2$
AV	0.45	0.47	0.51	0.48	0.76	0.26	0.68	0.60	0.22	0.23
AM	0.34	0.47	0.46	0.46	0.67	0.26	0.56	0.64	0.23	0.23
AD	0.36	0.24	0.62	0.29	0.51	0.40	0.45	0.64	0.22	0.47
VA	0.23	0.36	0.29	0.24	0.17	0.19	0.27	0.57	0.21	0.22
Vla	0.23	0.28	0.40	0.40	0.14	0.20	0.16	0.54	0.21	0.34
Vlp	0.38	0.32	0.39	0.38	0.29	0.24	0.22	0.47	0.19	0.21
VPL	0.39	0.45	0.37	0.55	0.42	0.26	0.45	0.47	0.29	0.43
VPM	0.48	0.51	0.43	0.65	0.48	0.28	0.47	0.54	0.36	0.49
VPI	0.20	0.28	0.32	0.57	0.38	0.17	0.24	0.37	0.21	0.11
VMb	0.26	0.67	0.64	0.83	0.57	0.31	0.45	0.59	0.28	0.45
MDm	0.20	0.52	0.71	0.59	0.43	0.25	0.36	0.55	0.36	0.31
MDl	0.21	0.49	0.68	0.64	0.48	0.27	0.39	0.41	0.30	0.37
Rh	0.21	0.68	0.70	0.47	0.54	0.47	0.35	0.85	0.40	0.25
CeM	0.24	0.64	0.79	0.49	0.35	0.77	0.48	0.59	0.45	0.31
Pc	0.15	0.52	0.53	0.36	0.18	0.38	0.17	0.43	0.24	0.22
CM	0.22	0.44	0.29	0.28	0.14	0.42	0.32	0.58	0.36	0.30
CL	0.27	0.65	0.66	0.31	0.10	0.36	0.33	0.72	0.37	0.41
Pf	0.40	1.0	1.0	0.38	0.22	1.0	0.62	0.78	1.0	0.71
LD	0.41	0.42	0.57	0.41	0.52	0.37	0.43	0.41	0.37	0.32
LP	0.87	0.93	0.64	0.90	0.81	0.67	0.83	0.78	0.55	0.79
LGmc	1.0	0.50	0.61	0.85	0.69	0.43	1.0	0.65	0.31	1.0
LGpc	0.91	0.68	0.28	0.84	0.59	0.54	0.95	0.64	0.43	0.97
MGad	0.34	0.68	0.68	0.77	0.65	0.23	0.35	0.51	0.29	0.36
MGv	0.41	0.51	0.55	0.56	0.69	0.22	0.46	0.45	0.17	0.47
Pla	0.20	0.31	0.37	0.52	0.34	0.21	0.26	0.47	0.27	0.26
Pli	0.81	0.87	0.57	0.98	1.0	0.54	0.51	0.84	0.32	0.69
Pll	0.52	0.62	0.31	0.59	0.52	0.40	0.33	0.82	0.22	0.41
Plm	0.77	0.85	0.41	1.0	0.77	0.56	0.48	1.0	0.39	0.63
Po	0.27	0.33	0.22	0.37	0.39	0.27	0.18	0.65	0.25	0.22
SGL	0.72	0.82	0.89	0.54	0.60	0.63	0.37	0.82	0.57	0.55
R	0.01	0.10	0.18	0.05	0.04	0.07	0.01	0.14	0.08	0.11

Optical density readings were obtained from film autoradiograms in individual nuclei. Values from multiple *in situ* hybridization runs were averaged for all cRNA probes, and individual values were compared against each other for the estimation of the intensity of cRNA probe labeling in individual nuclei. The optical density value for each nucleus was divided by the value found in the nucleus with the highest density value for each probe. The density in the nucleus with the highest intensity for each cRNA probe is thus 1.0, and the other values are fractions of this value. Hybridization levels of ^{33}P -labeled cRNA probes were converted from density units to units of radioactivity using ^{14}C standards exposed on the same sheet of film (see Materials and Methods).

Figure 12. Histogram of mean levels of receptor subunit mRNA distribution for all the subunit mRNAs examined in selected nuclei of the dorsal thalamus, including the sensory relay nuclei. There is a noticeable increase in subunit mRNA levels within sensory-related nuclei, especially for the α_1 , β_2 , and γ_2 subunit mRNAs. Optical density readings converted to measures of radioactivity (nCi/gm) by reference to ^{14}C standards exposed on the same sheet of film.

Figure 13. Histogram of mean density of hybridization taken from intralaminar nuclei for all subunit transcripts examined. Note the decreased levels of α_4 and α_5 subunit mRNAs when compared with neighboring nuclei of the ventral group. Density readings were converted to units of radioactivity (nCi/gm) by reference to ^{14}C standards exposed on the same sheet of film.

DISCUSSION

Ten GABA_A receptor subunit mRNAs were expressed throughout the thalamus in overlapping yet distinct nucleus-specific patterns. Overall, α_1 , β_2 , and γ_2 subunit mRNAs were expressed at much higher levels than α_2 – α_5 , β_1 , β_3 , and γ_1 . Sensory relay nuclei (LGd, VP, MG) and the pulvinar showed increased levels compared with other nuclei and expressed a greater variety of transcripts. In intralaminar nuclei, transcripts such as α_2 , α_3 , β_3 , and γ_1 , with low levels of expression in most other nuclei, were expressed at moderate-to-high levels, whereas others, such as α_4 and α_5 expressed at higher levels in other nuclei, were not expressed. In the reticular nucleus, with the exception of γ_2 and α_3 transcripts, virtually no GABA_A receptor transcripts were expressed.

Some subunit mRNAs predominate throughout the thalamus

Although transcripts were distributed unevenly throughout the dorsal thalamus, there was an order of expression intensity consisting of (from high to low): α_1 , β_2 , γ_2 , α_5 , β_1 , α_3 , γ_1 , β_3 , α_4 , and α_2 . The abundance of α_1 , β_2 , and γ_2 mRNAs parallels that found

in monkey cerebral cortex (Huntsman et al., 1994; 1995b) and in most regions of rat CNS (Gambarana et al., 1991; Persohn et al., 1992; Wisden et al., 1992). This may reflect their being primary contributors to native GABA_A receptors (Möhler et al., 1995). Wisden et al. (1992) propose likely native subtypes based on relative distributions of mRNAs in the rat: $\alpha_2\beta_3\gamma_2$, $\alpha_5\beta_1\gamma_2$, $\alpha_1\alpha_6\beta_2\delta$, and a putative thalamic receptor isoform, $\alpha_1\alpha_4\beta_2\delta$. Similar conjectures can be made from the nuclear distributions in the present study. Receptors formed from different combinations of subunit polypeptides may confer different pharmacological properties on GABA_A receptors associated with particular functional groups of thalamic nuclei. Confirmation of this will require examination at the level of the translated polypeptides themselves.

Mapping of GABA_A receptor genes on human chromosomes identified three clusters consisting of: $\alpha_1\beta_2\gamma_2$ subunit genes on chromosome 5 (5q31–5q35) (Wilcox et al., 1992; Russek and Farb, 1994), $\alpha_2\alpha_4\beta_1\gamma_1$ on chromosome 4 (p12–p13), (Buckle et al., 1989; Kirkness et al., 1991; McLean et al., 1995), and $\alpha_5\beta_3\gamma_3$ on chromosome 15 (15q11–15q13) (Wagstaff et al., 1991), suggesting an ancestral $\alpha\beta\gamma$ cluster. Hence, nucleus-specific expression patterns

Figure 14. Histogram of mean density of hybridization taken from the reticular nucleus at all levels of the thalamus for all subunit transcripts examined. Note the absence of α_1 and β_2 subunit mRNAs in this nucleus and the background levels of other subunit mRNAs, except for γ_2 and α_3 . Density readings were converted to units of radioactivity (nCi/gm) by reference to ^{14}C standards exposed on the same sheet of film.

of $\alpha\beta\gamma$ transcripts may indicate expression of grouped genes under the influence of similar regulatory elements (Russek and Farb, 1994).

Nucleus-specific expression of GABA_A receptor subunit genes

Expression of GABA_A receptor subunit mRNAs was greatest in sensory thalamic nuclei. The predominant transcripts in sensory nuclei were α_1 , α_5 , β_2 , and γ_2 , in which levels of these mRNAs were higher than in other thalamic nuclei. In addition, all transcripts examined were present at detectable levels, even α_2 – α_4 , β_1 , β_3 , and γ_1 mRNAs, whose expression was very low or absent in other thalamic nuclei. The restricted α_3 expression in the magnocellular layers of the LGd reflects the pattern of muscimol binding; others reflect that of flunitrazepam binding (Shaw and Cynader, 1986).

The over-abundant expression of GABA_A receptor subunit genes in sensory relay nuclei is puzzling because they do not contain increased numbers of GABA cells compared with other monkey dorsal thalamic nuclei (Hunt et al., 1991), and there is no evidence for increased density of GABAergic innervation in them. If enhanced mRNA levels can be taken as indicators of an increased relative number of GABA_A receptors, this may reflect the importance of inhibition in thalamic sensory processing. In sensory nuclei, enhanced center-surround antagonism (Sillito and Kemp, 1983), binocular interactions (Pape and Eysel, 1986), orientation bias (Vidyasager, 1984), receptive field size, temporal patterns of cell discharge (Hicks et al., 1986), and suppression of background discharge during passage of afferent volleys (Warren and Jones, 1994) all depend on GABA_A-mediated inhibition.

The intralaminar nuclei expressed a wide range of GABA_A receptor subunit mRNAs, including α_1 – α_3 , β_1 – β_3 , γ_1 , and γ_2 . mRNAs usually expressed at much lower levels in other thalamic nuclei, namely α_2 , α_3 , β_1 , β_3 , and γ_1 , were found at moderate

levels. The virtual lack of expression of α_4 and α_5 transcripts in the intralaminar nuclei contrasted with all other nuclei. Selective expression of particular subunit mRNAs within functionally distinct groups of nuclei may indicate that in these nuclei, unusual subunit combinations are used to form functional GABA_A receptors. The probability of GABA-mediated channel subtypes with combinations of either $\alpha_4\beta_1\gamma_x$ or $\alpha_5\beta_1\gamma_x$ within intralaminar nuclei, for example, would be extremely low compared with a subtype made up of $\alpha_3\beta_1\gamma_x$ subunits.

The expression profile of GABA_A receptor subunit mRNAs in the SG–L nuclei was similar to that of the intralaminar group. This may confirm SG–L as a posterior extension of the intralaminar system (Jones, 1985).

The reticular nucleus

The reticular nucleus of the ventral thalamus essentially expressed only γ_2 transcripts and at weak levels. α_1 and β_2 transcripts that were invariably coexpressed with γ_2 transcripts in other thalamic nuclei were noticeable by their absence. Reticular nucleus cells are a major source of GABAergic terminals in the dorsal thalamus and are linked to one another synaptically by intranuclear axon collaterals and in some species by dendrodendritic synapses (Ide, 1982; Deschênes et al., 1985; Yen et al., 1985). Both sets of synapses are GABAergic, bicuculline- and benzodiazepine-sensitive (Huguenard and Prince, 1994), and thus associated with GABA_A receptors. There is no precedent for functional GABA_A receptors formed by homomeric arrays of γ_2 subunits. Therefore, novel GABA_A receptor subunit mRNAs and subunit polypeptides that combine with γ_2 subunits to form functional GABA_A receptors may be discovered in the reticular nucleus.

In the rat reticular nucleus, there is low expression (Wisden et al., 1992) and lack of immunoreactivity for most subunits (Bentivoglio et al., 1990; Fritschy and Möhler, 1995). Wisden et al. (1992) reported equal levels of α_1 , α_3 , and γ_2 transcripts in rat reticular nucleus, with lower levels of α_2 , α_4 , β_2 , β_3 , γ_1 , and γ_3 transcripts, and nondetectable levels of α_5 , α_6 , β_1 , and δ transcripts. We did not examine γ_3 , δ , and α_6 transcripts, so we cannot rule out that they may be present in the monkey reticular nucleus, although that would represent a major species difference. Absence of α_1 and β_2 transcripts from the monkey reticular nucleus suggests that the pharmacological profile of GABA_A receptors would be different from that of the rat reticular nucleus in view of the known involvement of the α_1 subunit with $\beta_x\gamma_2$ subunits in forming benzodiazepine type I receptors (Pritchett et al., 1989b).

Comparison with rodent thalamus

Compared with the rat thalamus, there is a scarcity of α_4 transcripts and an abundance of α_5 transcripts in the monkey. α_4 transcripts are reportedly expressed at extremely high levels throughout the rat thalamus (Khrestchatsky et al., 1989; Wisden et al., 1991), yet only sensory relay nuclei in monkeys express α_4 at significant levels. Specificity of the monkey α_4 subunit cDNA was tested in additional *in situ* hybridization experiments using the monkey-specific cRNA probes on sections of rat thalamus in which it produced very high levels of labeling. The same monkey-specific cDNA, when used to probe Northern blots containing total RNA from monkey or rat, identified two transcripts at 4 and 11 kb for the monkey and a single transcript at 4 kb for the rat, in accord with previous reports (Ymer et al., 1989a; Huntsman et al., 1994). Another conflicting distribution pattern is the lack of α_5 expression reported in the rat. This subunit maintains moderately high levels in most monkey nuclei except the intralaminar. In

recombinant receptors containing α_1 , α_2 , α_3 , and α_5 subunits, the α subunit variant strongly affects affinity and efficacy of benzodiazepine binding (Macdonald and Olsen, 1994); α_5 subunit combinations show a lower affinity for zolpidem (Pritchett and Seeburg, 1990). α_4 subunits are associated with subtypes that lack benzodiazepine binding (Wieland et al., 1992), and the mRNA distribution in rat is associated with a lack of [³H]flunitrazepam binding (Olsen et al., 1990). [³H]flunitrazepam sites appear much more abundant in monkey LGd (Shaw and Cynader, 1986), reflecting the high levels of α_1 subunit transcripts. The dominating presence of α_1 and α_5 transcripts with virtual lack of α_4 in most monkey thalamic nuclei suggests that species-specific expression patterns may be associated with pharmacological differences.

A fundamental difference between monkey and rat thalamus is lack of GABAergic interneurons from rat dorsal thalamic nuclei except the LGd (Ohara et al., 1983; Webster and Rowe, 1984; Montero and Zempel, 1986; Benson et al., 1992). By contrast, GABAergic interneurons in dorsal thalamus of cats and monkeys comprise ~25% of all neurons in most nuclei (Fitzpatrick et al., 1984; Benson et al., 1991b; Hunt et al., 1991). GABAergic inhibition in cat and monkey thalamus thus arises from both the reticular nucleus and intrinsic interneurons, whereas in most dorsal thalamic nuclei of rodents, the primary source is the reticular nucleus. Whether lack of α_4 or addition of α_5 transcripts in the monkey reflects this fundamental difference needs to be examined further.

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