

## Immunocytochemical evidence suggests that taurine is colocalized with GABA in the Purkinje cell terminals, but that the stellate cell terminals predominantly contain GABA: a light- and electronmicroscopic study of the rat cerebellum

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**Summary.** The distributions of taurine-like and GABA-like immunoreactivities in the rat cerebellum were compared by analysis of consecutive semithin and ultrathin sections, postembedding labeled with the peroxidase-antiperoxidase technique or with an indirect immunogold procedure, respectively. Taurine-like immunoreactivity was selectively enriched in Purkinje cell bodies, dendrites and spines, and boutons in the cerebellar nuclei exhibiting ultrastructural features typical of Purkinje cell terminals. The stellate and basket cell bodies and terminals were very weakly labeled. A computer assisted quantitative assessment of the net immunogold labeling revealed that the mean gold particle density in the Purkinje cell terminals was about 70% higher than that in the Purkinje cell dendrites, and about 14 times higher than that in the stellate/basket cell terminals in the molecular layer. Stellate, basket and Purkinje cell terminals emerged as intensely immunoreactive in adjacent sections processed with an antiserum against conjugated GABA. These findings indicate, contrary to recent electrophysiological data, that GABA is a more likely transmitter candidate than taurine in the stellate cells. The apparent colocalization of GABA and taurine in the terminals of Purkinje cells raises the possibility that these terminals are capable of releasing two different inhibitory amino acids.

**Key words:** Taurine – GABA – Colocalization – Cerebellum – Purkinje cells

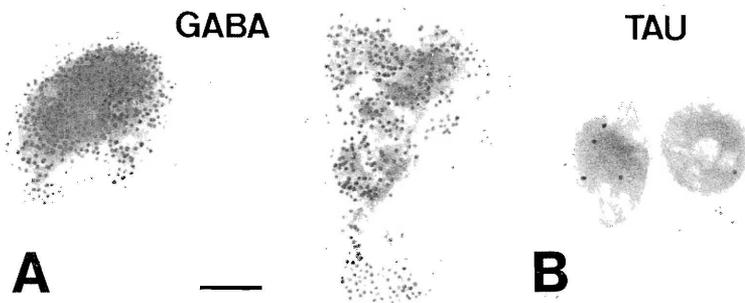
### Introduction

Early physiological studies established that all major neuronal classes in the cerebellar cortex, with the

exception of the granule cells, exert an inhibitory effect on their postsynaptic targets (Andersen et al. 1964; Eccles et al. 1967; Obata et al. 1970). Ensuing pharmacological investigations suggested that the inhibition was mediated by GABA, the strongest evidence being provided for the Purkinje and basket cells (Obata et al. 1970; Ito et al. 1970; Curtis et al. 1970; Curtis and Felix 1971). Release of GABA into the fourth ventricle was demonstrated on massive stimulation of Purkinje cells (Obata and Takeda 1969), and lesion studies and biochemical assay showed that the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) (Fonnum et al. 1970) as well as GABA are concentrated in Purkinje cell terminals (Otsuka et al. 1971). When it was later shown by immunocytochemistry that GAD is present in Golgi and stellate cells, as well as in the Purkinje and basket neurons (McLaughlin et al. 1974; Saito et al. 1974; Ribak et al. 1978; Oertel et al. 1981), GABA emerged as the most likely transmitter in all of the inhibitory cell types in the cerebellar cortex.

The situation became less clear in the early 1980's following the demonstration that subpopulations of the stellate and Purkinje cells contain the taurine-synthesizing enzyme, cysteine sulfinic acid decarboxylase (CSAD) (Chan-Palay et al. 1982a, b). Taurine is a potent inhibitory amino acid which has long been suspected to play a transmitter role in mammalian brain (Kuriyama et al. 1983). Indeed, electrophysiological and pharmacological data were interpreted to suggest that taurine was a more likely transmitter than GABA in the stellate cell synapses with the Purkinje cells (Yarbrough et al. 1981; Okamoto et al. 1983a, b, c).

In order to better understand the functional relationship between taurine and GABA in the stellate and Purkinje cells it is, inter alia, necessary to examine the fine distributions of the two amino acids with more direct methods than those referred to



**Fig. 5A, B.** Electron micrographs of conjugates prepared from GABA (A) or taurine (B), incubated with GABA antiserum 33 (1:200) under the same conditions as the tissue sections. The net gold particle densities over the GABA and taurine conjugates (calculated as in Fig. 4) were  $2310 \pm 275$  ( $N = 8$ ) and  $56.5 \pm 21.2$  ( $N = 10$ ), respectively. None of the remaining amino acid conjugates showed particle densities exceeding that of the taurine conjugate (also see Ottersen et al. 1988). Bar:  $0.2 \mu\text{m}$

It has previously been shown, using pre-embedding staining techniques, that taurine-LI is concentrated in Purkinje cell bodies and dendrites in the rat cerebellar cortex<sup>2</sup>, and in structures in the deep cerebellar nuclei interpreted as Purkinje cell terminals (Madsen et al. 1985; Campistrone et al. 1986; Yoshida et al. 1986). The present investigation, based on postembedding-stained semithin and ultrathin sections, substantiates the conclusions of the above studies and demonstrates that the taurine positive Purkinje terminals are also GABA positive. The selective staining of test antigens incubated together with the tissue sections and the results of inhibition experiments clearly show that the double labeling is not due to crossreactivity between the two amino acids. The observed distribution of taurine-LI contrasts with that of the taurine-synthesizing enzyme, CSAD, which according to immunocytochemical data is concentrated in a subpopulation of Purkinje cells forming sharply demarcated sagittal bands (Chan-Palay et al. 1982a).

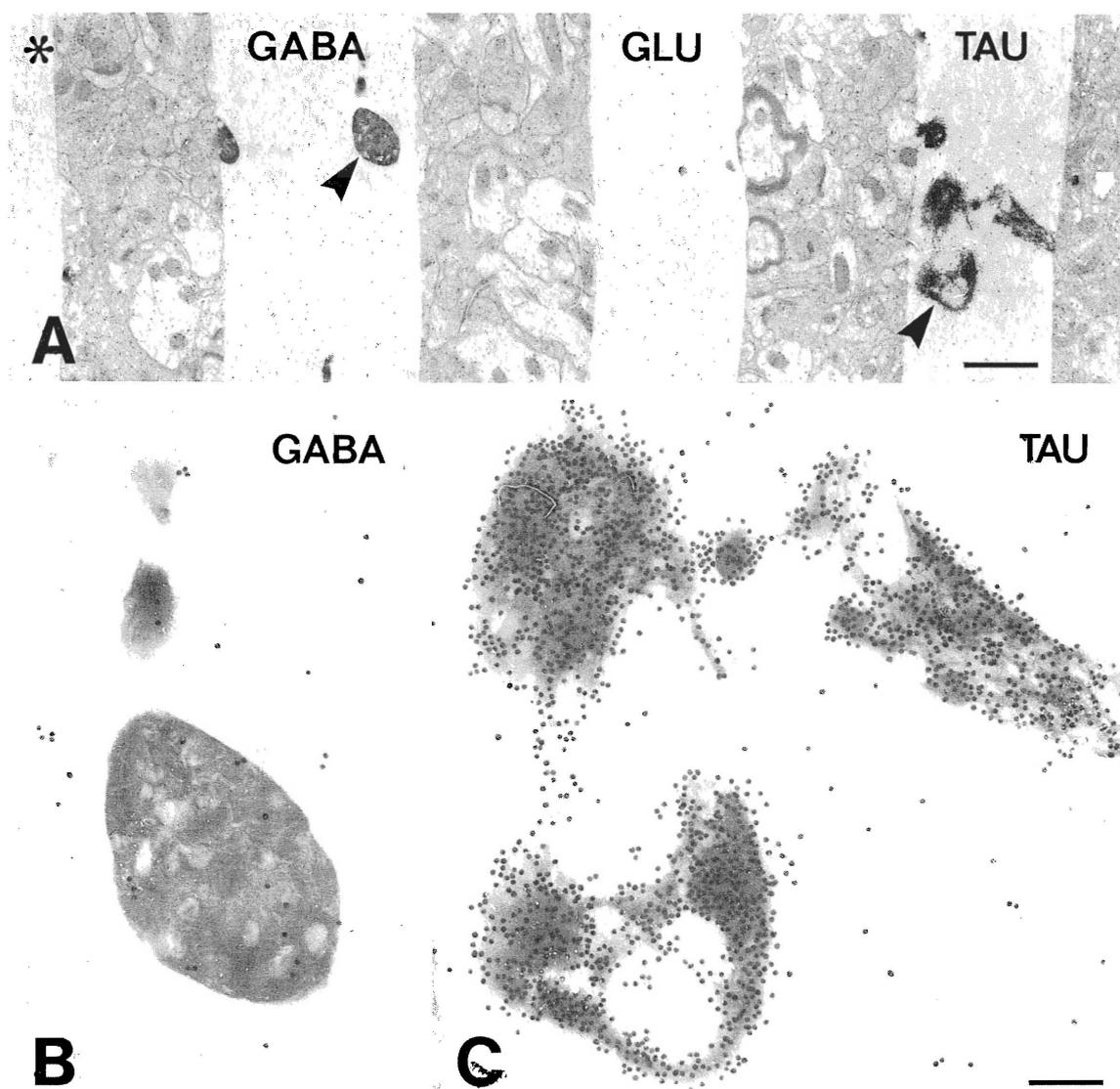
What is the functional significance of a colocalization of GABA and taurine? The finding that the level of taurine-LI is higher in the Purkinje cell terminals than in the respective dendrites (and cell bodies; Ottersen 1988) could be interpreted to suggest that taurine plays a role related to synaptic function. Although we cannot exclude the possibility that the distributions of the two amino acids within the nerve terminals are altered during the perfusion procedure, it is noteworthy that taurine-LI is found over clusters of synaptic vesicles (and over mitochondria), exactly as for GABA-LI (the resolution is not sufficient to decide whether the immunoreactive sites are actually located *within* the synaptic vesicles). Taurine has been found to depress potassium-induced release of radiolabeled GABA in cerebellar slices, possibly via

an interaction at the synaptic level (Namima et al. 1983), and could have a similar effect if released *in vivo*. The dendritic and perikaryal stores of taurine may serve various metabolic roles, the nature of which remains to be determined.

Several sets of experimental data have been interpreted to favor taurine as a transmitter in the stellate cells (review: Okamoto et al. 1983b). As mentioned in the Introduction, a subpopulation of these cells contain CSAD (Chan-Palay et al. 1982a, b). However, the strongest evidence for a transmitter role of taurine comes from pharmacological studies using the proposed taurine antagonist, 6-amino-methyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide hydrochloride (TAG). This compound blocked the inhibitory effect of taurine, but not that of GABA, on the Purkinje cell neurons in intact rats, and also reduced the synaptically evoked inhibitions of Purkinje cells produced by electrical stimulation of the cerebellar surface (Yarbrough et al. 1981). Similar results were obtained by Okamoto et al. (1983a) in guinea pig cerebellar slices. It was therefore surprising to find, in our original light microscopic study (Madsen et al. 1985), that the neuronal cell bodies in the molecular layer were generally very poor in taurine-LI. Similar negative results have been obtained more recently by other groups, using different antisera (Campistrone et al. 1986; Yoshida et al. 1986). In the present study we have taken advantage of the superior resolution in postembedding-stained semithin and ultrathin sections to show that taurine-LI is scarce in the stellate and basket cell terminals as well as in the respective cell bodies.

The possibility should be considered that the expression of taurine-LI in the stellate cell terminals could be impeded by the simultaneous occurrence of GABA. This explanation is made less likely by the fact that both immunoreactivities are readily demonstrated in the Purkinje cell terminals. Further, studies in model systems suggest that although two amino acids occurring in the same cell may interfere with each others fixation and antibody binding, the

<sup>2</sup> The distribution of taurine-LI in the cerebellar cortex appears to differ among species. Thus, the Purkinje cell bodies are less intensely stained in guinea pig and cat than in rat (unpublished). However, the stellate and basket cell bodies were almost invariably immunonegative in all three species studied



**Fig. 4A-C.** Electron micrographs of ultrathin test section incubated with taurine antiserum 20 (1:500) in the same drops of sera as the tissue sections. The low power view in **A** shows part of the "sandwich" composed of sections of Durcupan-infiltrated amino acid conjugates alternating with spacer sections from rat hippocampus (cf. Fig. 1). Asterisk denotes resin support. Arrowheads indicate clumps of conjugates enlarged in **B** and **C**. The net gold particle densities (expressed as particles/ $\mu\text{m}^2$ ) over the taurine and GABA conjugates were  $1946 \pm 100$  ( $N = 7$ ) and  $29.3 \pm 10.7$  ( $N = 4$ ), respectively, after subtraction of background (empty resin, 8.4 particles/ $\mu\text{m}^2$ ). The other amino acid conjugates were as weakly labeled as the GABA conjugates (see Ottersen 1987), and none of the conjugates (except that prepared from taurine) showed particle densities significantly different from that over glutaraldehyde-treated brain protein. The absolute particle densities over the test conjugates are not directly comparable with those over cell profiles. Thus, to achieve high sensitivity the test conjugates were made at an amino acid concentration corresponding to 200 mM in the brain. In addition, the conjugates were strongly condensed during embedding (Ottersen 1987). Bar: **A**, 1  $\mu\text{m}$ ; **B**, **C**, 0.2  $\mu\text{m}$

Mathisen 1984; Seguela et al. 1985; Somogyi et al. 1985; Gabbott et al. 1986) with largely consistent results. Although there is some controversy as to the exact level of immunoreactivity in the Purkinje cell bodies and dendrites, these structures are generally described as less intensely stained than the other presumed GABA-ergic neurons in the cerebellar cortex, i. e., the stellate, basket, and Golgi cells.

However, the Purkinje cell axons become strongly immunoreactive soon after leaving the parent cell bodies, and remain labeled throughout their course to the deep cerebellar nuclei (Ottersen and Storm-Mathisen 1984). The distribution of GABA-LI in the cerebellum shows close correspondence with that of GAD-LI (Mugnaini and Oertel 1985; Somogyi et al. 1985; Wu et al. 1986).

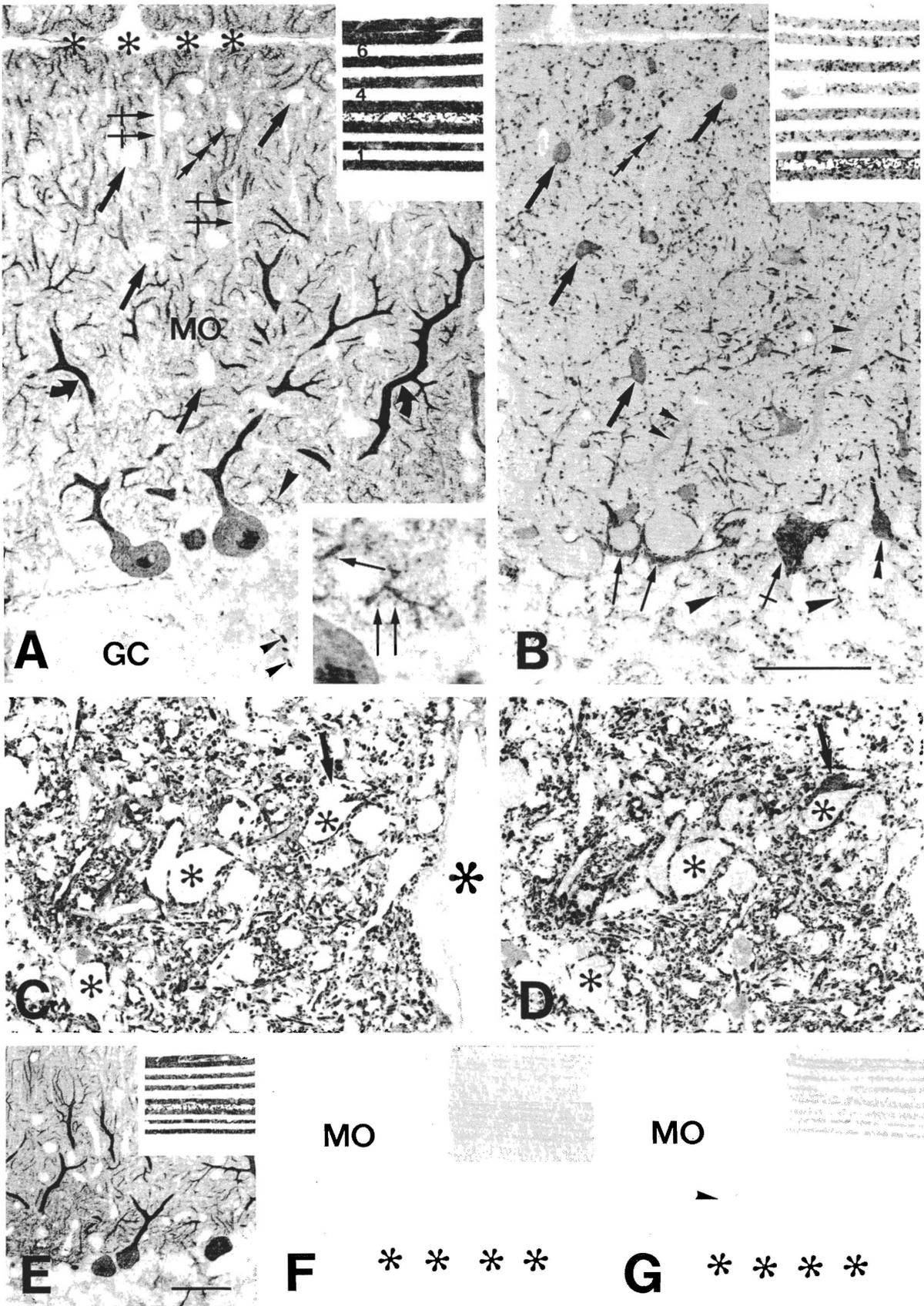
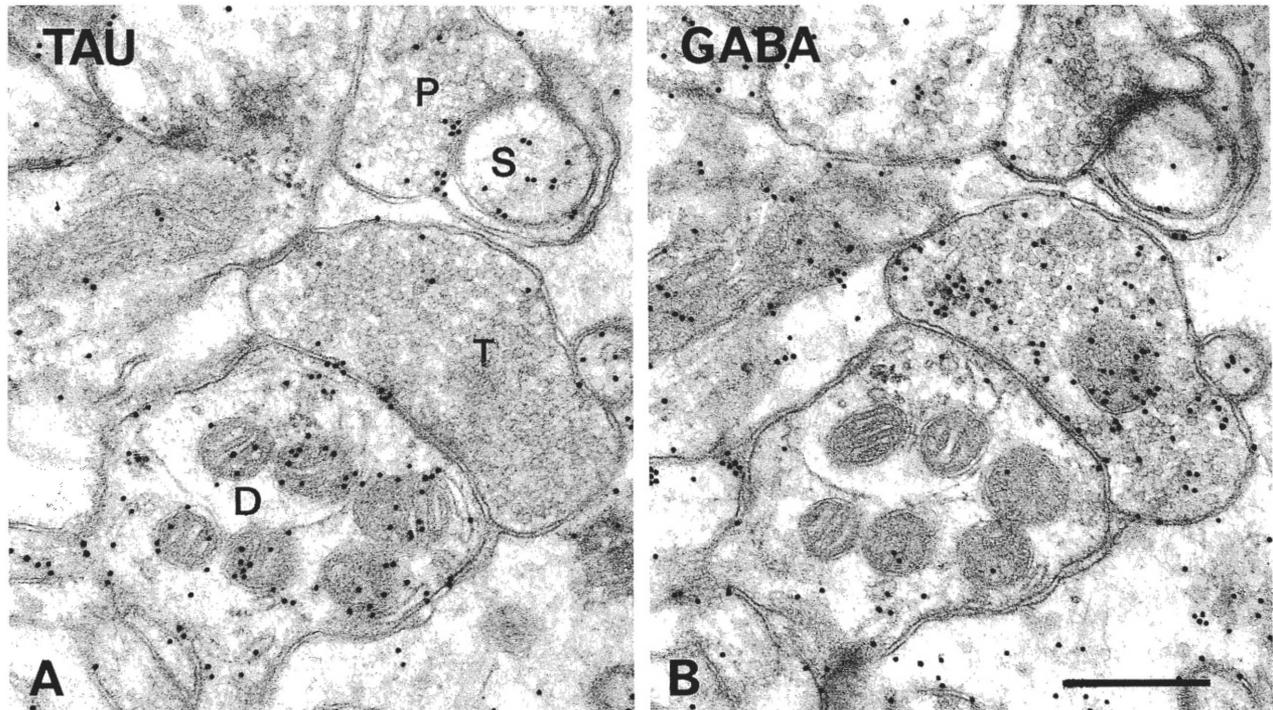


Fig. 1



**Fig. 2A, B.** Electron micrographs of consecutive sections incubated with taurine antiserum 20 diluted 1:500 (A) and GABA antiserum 09 diluted 1:2000 (B). The presumed stellate cell terminal (T) is immunonegative for taurine, but immunopositive for GABA. Conversely, the postsynaptic Purkinje cell dendrite (D) is strongly labeled with the taurine antiserum, but very weakly labeled with the GABA antiserum. From outer part of the molecular layer (vermis posterior). P, parallel fiber terminal; S, Purkinje cell dendritic spine. Bar: 0.4  $\mu$ m

(except for antiserum 09). For optimum results with GABA antiserum 33 it proved necessary to decrease the concentration of NaCl in the diluting buffer from 0.12 M (used for the other antisera) to 0.03 M, and to carry out the incubation at 25° C in a thermostat instead of at room temperature. The sections were thoroughly rinsed between the steps, except after steps 2 and 4, and were dried before staining with uranyl acetate and lead citrate.

#### *Specificity controls*

The tissue sections were incubated together with semithin (0.5  $\mu$ m) or ultrathin test sections (Figs. 1, 4, 5) containing a series of different amino acid-glutaraldehyde-brain protein conjugates (Ottersen 1987). For additional control, aliquots of the taurine antiserum were preadsorbed with glutaraldehyde fixation complexes of taurine, GABA, hypotaurine, cysteic acid, cysteine sulfinic acid or gamma-glutamyl taurine (complexes prepared according to Ottersen et al. 1986), whereas the GABA antisera were preadsorbed with similar complexes prepared from GABA or taurine. Some sections were incubated with a preimmune serum instead of the specific serum.

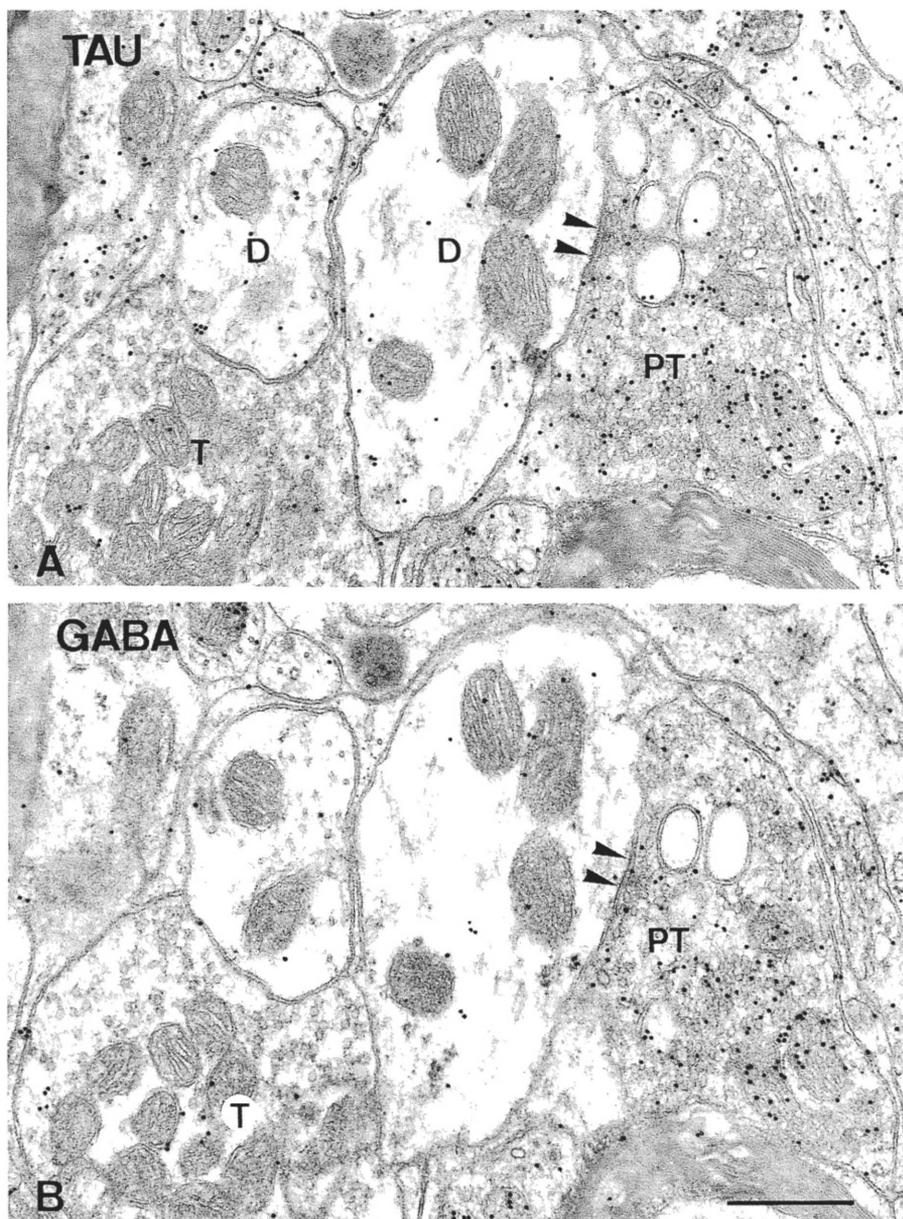
#### *Computer analysis*

Gold particle densities over cell profiles and test conjugates were assessed as previously described (Ottersen 1987) using a computer programme (Morforel, version 8) developed by Th. W. Blackstad.

## **Results**

### *Light microscopy*

Adjacent semithin sections through the cerebellar cortex treated with an antiserum against taurine (Fig. 1A) or GABA (Fig. 1B), respectively, showed very different patterns of labeling. The Purkinje cell bodies and dendrites, including the dendritic spines (inset, Fig. 1A), were strongly stained for taurine, but only weakly immunoreactive for GABA. Conversely, the cell bodies of the stellate and basket cells were generally poor in taurine-LI (the few exceptions noted amounted to less than 2% of the total number of cells in the molecular layer; cf. Madsen et al. 1985), but rich in GABA-LI. On very rare occasions we observed cell bodies in the molecular layer that appeared to lack both immunoreactivities. Matching the cell body staining, GABA-LI was enriched in punctate structures in the molecular layer interpreted as stellate and basket cell terminals, and in the basket plexus surrounding the Purkinje cell bodies. The GABA immunopositive baskets facilitated the identification of the Purkinje cell bodies and enabled us to confirm, after careful analysis of numerous pairs of



**Fig. 3A, B.** Electron micrographs of consecutive sections from nucleus interpositus anterior, incubated with taurine antiserum 20 (**A**) or GABA antiserum 33 (**B**), both diluted 1:500. The GABA antiserum has been preadsorbed with glutaraldehyde complexes of glycine and glutamate (200  $\mu$ M of each). The Purkinje cell terminal (PT) is labeled with both antisera and establishes a symmetric contact with one of the poorly labeled dendrites (D). The terminal with round vesicles (T) lacks both immunoreactivities. Bar: 0.4  $\mu$ m

transverse and sagittal sections, that all Purkinje cell bodies in the parts of the cerebellum investigated (vermis, intermediate zone, and the greater part of the hemispheres) contain taurine-LI. At the LM level of resolution it was not possible to discern any taurine immunopositive structures that could be interpreted as stellate or basket cell terminals.

In the granule cell layer, GABA-LI was found in the Golgi neurons and their circularly arranged terminals (Fig. 1B). The latter terminals could not be distinguished in sections treated with the taurine antiserum, but moderate taurine-LI could be observed in some of the Golgi cell bodies. With the

exception of traversing Purkinje cell axons and their recurrent collaterals in the infraganglionic plexus, none of the remaining constituents of the granule cell layer displayed noteworthy levels of taurine-LI.

In the cerebellar nuclei the distribution of taurine-LI was very similar to that of GABA-LI (Fig. 1C, D). With both antisera, intensely labeled terminals were found to encircle immunonegative cell bodies and dendrites. The two labeling patterns differed, however, in one notable respect: immunopositive cell bodies were commonly seen after incubation with the GABA antiserum, but only occasionally observed with the taurine antiserum.

### Electron microscopy

In agreement with the observations in semithin sections, ultrathin sections incubated with the taurine antiserum showed a high density of gold particles over Purkinje cells, including their dendrites and spines (Fig. 2A), whereas adjacent sections treated with a GABA antiserum displayed only weak labeling of these profiles (Fig. 2B). The electron microscopic analysis further established that similar to their parent cell bodies, the stellate and basket cell terminals were poorly labeled for taurine but strongly GABA positive (Fig. 2B)<sup>1</sup>. A quantitative assessment of the immunolabeling in a neighbouring section to that in Fig. 2A, incubated with the taurine antiserum diluted 1:500, showed that the average net particle density in the stellate/basket cell terminals in the molecular layer amounted to 14.3% of that in the Purkinje cell dendrites. The actual values, expressed as particles/ $\mu\text{m}^2 \pm \text{SEM}$ , after subtracting the particle density over empty resin (8.0 per  $\mu\text{m}^2$ ), were  $16.3 \pm 1.4$  (N = 22) and  $114.0 \pm 6.0$  (N = 33), respectively. The highest net value observed for an individual stellate/basket cell terminal was 29 particles/ $\mu\text{m}^2$ . The sample included stellate/basket cell terminals from the entire thickness of the molecular layer. In an identically treated section from another animal, the gold particle density in stellate/basket cell terminals was 12.0% of that in the Purkinje cell dendrites (net values  $11.4 \pm 1.9$  [N = 14] and  $95.0 \pm 6.7$  [N = 10], respectively). The differences were highly significant ( $P < 0.001$ , Student's t-test) in both cases. The values given above were representative for the ten different animals that were subjected to analysis.

In the cerebellar nuclei, taurine-LI was concentrated in a majority of the terminals. The labeled terminals contained rather densely packed pleomorphic vesicles in a distinct axoplasmic matrix, and established symmetric or intermediate type contacts with poorly labeled dendrites or somata. On the basis of their abundance and ultrastructural appearance (cf. Chan-Palay 1977), the labeled terminals were interpreted as originating from Purkinje cells. Similar to the Purkinje cell bodies, all Purkinje cell terminals appeared to be labeled with the taurine antiserum.

<sup>1</sup> The basket cell terminals in the molecular layer are not easily distinguished from the stellate cell terminals on morphological grounds. However, stellate cell terminals prevail in the outer part of the molecular layer (from which Fig. 2B was taken). Observations in the present material and previous quantitative data (Ottersen 1988) suggest that terminals that establish symmetric contacts with dendrites in the deep part of the molecular layer (or with the Purkinje cell bodies) are equally poor in taurine-LI as more superficially located terminals of similar type. This suggests that the stellate and basket cell terminals are similar with respect to their contents of taurine

Whenever these terminals could be identified in adjacent sections treated with a GABA antiserum, they turned out to be double labeled (Fig. 3). With both antisera, the gold particles were found over vesicles as well as over mitochondria. Terminals with round vesicles (Fig. 3) contained neither taurine-LI, nor GABA-LI.

A quantitative analysis in a representative section subjected to the same treatment as that shown in Fig. 3A (taurine antiserum diluted 1:500) revealed that the net gold particle density in the Purkinje cell terminals was about 70% higher than that in the Purkinje cell dendrites ( $161.4 \pm 10.4$  [N = 12] vs.  $95.0 \pm 6.7$  [N = 10],  $P < 0.001$ ). In the same section (also referred to above), the average net density over stellate/basket cell terminals was  $11.4 \pm 1.9$  particles/ $\mu\text{m}^2$  (N = 14), i. e., about 7% of that in the Purkinje cell terminals.

### Control procedures

Semithin or ultrathin test sections incubated together with the tissue sections showed that the GABA and taurine antisera reacted strongly with glutaraldehyde-brain protein conjugates of GABA and taurine, respectively, but not with similar conjugates prepared from other abundant amino acids in the CNS (insets in Fig. 1A, B, Figs. 4, 5). In a test section treated with the taurine antiserum, the net gold particle density over the taurine conjugates was two orders of magnitude higher than that over any of the other conjugates (Fig. 4). A similar high selectivity was obtained with the GABA antisera (Fig. 5). Preadsorption of the GABA and taurine antisera with glutaraldehyde complexes of the respective amino acids (200  $\mu\text{M}$  with respect to the amino acid) or substitution of the specific sera by a preimmune serum, virtually eliminated staining of test and tissue sections, both at the light microscopic (Fig. 1) and electron microscopic levels. In contrast, preadsorption of the taurine antiserum with GABA complexes (Fig. 1E) or the GABA antisera with taurine complexes at the same concentrations (not shown) had no effect on the immunolabeling. The pattern of taurine-LI was also unchanged by adding glutaraldehyde complexes of hypotaurine (200  $\mu\text{M}$ ) or of a mixture of hypotaurine, cysteic acid, cysteine sulfinic acid and gamma-glutamyl taurine (50  $\mu\text{M}$  of each) to the specific antiserum (not illustrated).

### Discussion

The distribution of GABA-LI in the cerebellum has been studied by many authors (Ottersen and Storm-

magnitude of this effect is hardly sufficient to produce false negative staining (Ottersen et al. 1986).

Although the level of taurine-LI in the stellate cell terminals was very low as compared to that in the Purkinje cell terminals, the labeling was significantly different from background labelling (over empty resin) and from the most weakly labeled cell profiles (the mossy fibers; Ottersen 1988). This suggests that even the stellate cell terminals do contain small amounts of taurine. It is usually assumed that all transmitters are enriched in the terminals from which they are released. Strictly speaking, however, compounds occurring at low concentrations in the terminals could well act as transmitters, provided they can be rapidly replenished by synthesis *de novo* or by reuptake. Thus, the present results do not exclude the possibility that taurine subserves a transmitter role in the stellate cell terminals. Alternatively, the TAG-sensitive effects observed by Yarbrough et al. (1981) and Okamoto et al. (1983a) could have been caused by a compound that shares the pharmacological properties of taurine, but which is not detected by our specific antibodies. It is relevant in this context that several metabolites of taurine, including taurocyamine, hypotaurine, and homotaurine, dose dependently suppress the spontaneous spike discharge of Purkinje cells (Okamoto and Sakai 1981). However, the fact that the stellate neurons consistently display three important markers of GABAergic neurons, i. e., GABA-LI, GAD-LI (Mugnaini and Oertel 1985), and GABA uptake (Hökfelt and Ljungdahl 1970; Schon and Iversen 1972), implies that GABA should still be regarded as the most likely transmitter candidate in these cells.

The present findings, and observations made with GABA and taurine antisera in other parts of the brain, indicate that whereas GAD can still safely be regarded as a valid marker of GABA containing neurons, CSAD is not generally useful as a marker of neurons containing taurine. The poor correlation between the distributions of CSAD and taurine may simply reflect the fact that the overall rate of taurine synthesis is extremely low (average turnover in brain 5.5 days [Huxtable 1981] compared to about 1 h for GABA [Collins 1972]), allowing ample time for redistribution caused by release, active uptake, or passive diffusion. It should also be recalled that part of the taurine pool in the brain may originate from biosynthetic pathways that do not involve CSAD (Cavallini et al. 1976), or from the diet (Sturman et al. 1985).

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above. Although the distributions of GABA (Ottersen and Storm-Mathisen 1984; Seguela et al. 1985; Somogyi et al. 1985; Gabott et al. 1986) and taurine (Madsen et al. 1985; Campistrone et al. 1986; Yoshida et al. 1986; Ottersen 1988) in the cerebellum have recently been examined by means of specific antisera raised against the conjugated amino acids themselves, no direct comparison between the two has been made. In the present report, the distributions of taurine-like and GABA-like immunoreactivities (taurine-LI and GABA-LI) are compared by analysis of consecutive semithin and ultrathin sections obtained from rat cerebella.

## Material and methods

### Experimental material

Ten male Wistar rats (200–300 g) were deeply anaesthetized with pentobarbital (50 mg/kg) and perfused through the ascending aorta at a rate of 50 ml/min with 2% dextran (mw 70,000) in 0.1 M sodium phosphate buffer pH 7.4 (4° C, 10–15 s), followed by a mixture of 2.5% glutaraldehyde and 1% paraformaldehyde in the same buffer (room temperature, 15 min). Less than 90 s elapsed from thoracotomy until neck stiffness and other signs of fixation appeared. Tissue blocks from the cerebellum were osmicated, dehydrated in graded ethanols and propylene oxide, and embedded in Durcupan (ACM, Fluka). Serial semithin (0.5 µm) and ultrathin sections were cut in the transverse or sagittal plane with a Reichert ultramicrotome and mounted on gelatinized glass slides, and nickel mesh grids, respectively.

### Sera

All primary antisera used in the present study were raised against amino acids coupled to carrier proteins by glutaraldehyde. The sera have been thoroughly characterized previously and found to

be highly selective after appropriate purification (taurine antiserum 20: Madsen et al. 1985; Ottersen et al. 1985; Ottersen 1988; GABA antiserum 26: Ottersen and Storm-Mathisen 1984; GABA antiserum 33: Ottersen et al. 1988; GABA antiserum 09: Hodgson et al. 1985). The batch of GABA antiserum 33 used here was preadsorbed in liquid phase with 300 µM of glutamate-glutaraldehyde complexes or 200 µM each of glutaraldehyde complexes of glutamate and glycine (concentrations with respect to the amino acid; Ottersen et al. 1986), whereas GABA antiserum 26 and taurine antiserum 20 were preadsorbed in solid phase as previously described (Ottersen and Storm-Mathisen 1984; Madsen et al. 1985).

### Immunocytochemical procedures

**A. Postembedding staining of semithin sections.** The sections were treated essentially as described by Somogyi et al. (1984). Following etching in a saturated solution of NaOH in ethanol and immersion in 1% NaIO<sub>4</sub> in H<sub>2</sub>O the sections were exposed to 1) 20% normal sheep serum, 2) taurine antiserum 20, diluted 1:3000, or GABA antiserum 26, diluted 1:100 (18 h at room temperature), 3) sheep anti-rabbit IgG (own produce, 1:10), 4) rabbit peroxidase-antiperoxidase complex (DAKOPATTS, 1:100), and 5) diaminobenzidine/H<sub>2</sub>O<sub>2</sub>. The staining was intensified by treating the sections with OsO<sub>4</sub> (2 drops of 1% OsO<sub>4</sub> in 50 ml 0.1 M sodium phosphate buffer, pH 7.4, 5 min).

**B. Postembedding immunogold staining of ultrathin sections.** The sections were immersed in the following solutions: 1) 1% HIO<sub>4</sub> in H<sub>2</sub>O (7 min) followed by 1% NaIO<sub>4</sub> in H<sub>2</sub>O (7 min), 2) 1% human serum albumin (10 min), 3) taurine antiserum 20, diluted 1:500, GABA antiserum 33, diluted 1:200 or 1:500, or GABA antiserum 09, diluted 1:2000, 4) polyethylenglycol (50 mg/100 ml 0.05 M Tris buffer, pH 7.4, 5 min), 5) goat anti-rabbit IgG coupled to colloidal gold particles (mean diameter 15 nm; Janssen) diluted 1:20 in the solution used in the preceding step (1 h), 6) 1% uranyl acetate (20 min) followed by lead citrate (1–3 min). The primary antisera were diluted in Tris-phosphate buffered saline, and were applied to the sections for 60 min to 3 h. This procedure is similar to that of Somogyi and Soltész (1986) with the notable difference that normal serum was presently omitted from the blocking and rinsing solutions and from the diluting buffer for the primary antisera

**Fig. 1.** Photomicrographs of semithin (0.5 µm) tissue and test sections incubated with taurine antiserum 20 diluted 1:3000 (**A, C, E, F**) or GABA antiserum 26 diluted 1:100 (**B, D, G**). **A** In the cerebellar cortex, the taurine antiserum stains Purkinje cell bodies, dendrites (curved arrows), and dendritic spines (arrows in inset). The radial processes of Bergmann glia (crossed arrows) and the interneurons in the molecular layer (thick arrows) stand out as negative profiles. Small arrowheads, immunolabeled Purkinje cell axons; asterisks, pial surface. Large arrowhead indicates dendrite enlarged in lower inset. Upper inset shows test section incubated together with the tissue specimen. Only the taurine conjugates (third from below) show noteworthy staining. (Dark zones between conjugates represent spacer sections obtained from rat hippocampi.) The other conjugates were: 1, GABA; 2, glutamate; 4, glycine; 5, glutaraldehyde-treated brain protein (no amino acid added); 6, aspartate; 7, glutamine (section displaced, only small part represented). Abbreviations: MO, molecular layer; GC, granule cell layer. **B** The GABA antiserum produces strong staining of interneurons in the molecular layer (thick arrows), of basket cell terminals (thin arrows), and of Golgi cell bodies (double arrowhead) and their processes (large arrowheads), and weak staining of Purkinje cell dendrites (small arrowheads). In addition, the molecular layer contains numerous intensely stained dots representing stellate cell terminals. Triple arrow, cell body lacking GABA-LI as well as taurine-LI. Crossed arrow, tangentially cut basket plexus. Inset shows test section incubated together with tissue specimen: note selective staining of GABA conjugate. **C, D** In the cerebellar nuclei, both antisera produce intense labeling of bouton-like dots that partially encircle negative dendrites and somata (smaller asterisks). Thick arrow shows small neuron that is labeled for GABA, but not for taurine. Large asterisk indicates a vessel. **E, F** Staining with the taurine antiserum is unaffected by preadsorption with 200 µM GABA-glutaraldehyde (GABA-G) complexes (**E**), but abolished by similar complexes (200 µM) prepared from taurine (**F**). **G** GABA-G (200 µM) suppresses staining with the GABA antiserum, except for some residual labeling (probably unspecific) in the Purkinje cell dendrites (arrowhead). Asterisks in **F** and **G** indicate position of Purkinje cell layer. **C** and **D** are from nucleus interpositus anterior, the remaining photomicrographs are from vermis posterior. Bar: 50 µm

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