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RESEARCH ARTICLE

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Glutamate, aspartate and co-localization with calbindin in the medial thalamus

An immunohistochemical study in the rat

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Abstract Topographical and quantitative features of medial thalamic neurons in which aspartate (ASP) or glutamate (GLU) might act as neurotransmitters were investigated in the rat. The calcium-binding protein calbindin D-28k (CB) was exploited as a marker of neuronal subsets, thus allowing us to study also the relationships between the CB-containing neurons and those immunoreactive to excitatory amino acids. Double immunocytochemistry of ASP and CB or GLU and CB was performed in 40-µm-thick sections. The three markers were distributed in the thalamic midline, mediodorsal, anterior intralaminar and ventromedial nuclei, with regional variations. ASP-immunoreactive neurons appeared more numerous than the GLU-immunoreactive ones throughout these structures; ASP-CB or GLU-CB double-immunostained neurons were evident. ASP-, GLU- and CB-immunoreactive cells were then quantitatively evaluated in 5-µm-thick consecutive sections. Interindividual variations and different anti-ASP and anti-GLU antibodies did not result in significant differences. ASP and GLU were not co-localized. Single ASP- or GLU-immunoreactive neurons accounted for 60% of the total number of immunostained cells, and single ASP-immunopositive cells represented more than half of these neurons. Among the CB-immunoreactive cells (40% of the total), half were double immunostained; the proportion of double CB-ASP-immunopositive neurons was sevenfold higher than that of the CB-GLU-immunoreactive ones. These results indicate that ASP may act as excitatory neurotransmitter in a relatively high proportion of medial thalamic neurons, in which ASP frequently coexists with CB. Approximately 50% of the CB-immunoreactive cells did not contain either ASP or GLU, suggesting that some medial thalamic neurons may utilize a different neurotransmitter.

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Introduction

Several lines of evidence indicate that the excitatory amino acids glutamate (GLU) and aspartate (ASP) are involved in thalamic neurotransmission. The occurrence of glutamatergic and/or aspartatergic neurons in the thalamus was pointed out on the basis of immunohistochemistry (Ottersen and Storm-Mathisen 1984a,b; Aoki et al. 1987; Rustioni et al. 1988) and of the selective retrograde transport of D-[^{3}H]ASP (Streit 1980; Baughman and Gilbert 1981; Cuénod et al. 1983; Ottersen et al. 1983; Christie et al. 1987; Fuller et al. 1987; Johnson and Burkhalter 1992; Pirot et al. 1994), which labels neurons that contain the high-affinity uptake mechanisms for both GLU and ASP (Streit 1980). Iontophoretically applied GLU antagonists were found to block thalamocortical transmission (Hicks et al. 1991), but these effects could be also mediated by intracortical mechanisms. Stronger evidence in favour of excitatory amino acidergic neurotransmission has been provided by the ultrastructural findings of thalamocortical terminals enriched in GLU (Montero and Wenthold 1989; Kharazia and Weinberg 1993, 1994; Weinberg and Kharazia 1996). However, the proportion of thalamic relay cells that might utilize GLU and/or ASP has not been hitherto determined. The present study was aimed at investigating this issue in the rat medial thalamus, exploiting the expression of the calcium-binding protein calbindin D-28k (CB), which in the rat occurs selectively in neuronal subsets of the thalamic medial nuclei (Celio 1990; Frassoni et al. 1991; Andersen et al. 1993; Arai et al. 1994).

The immunocytochemical detection of CB has provided an effective marker for populations of neurons (Celio 1990). In the pulvinar and lateral posterior nuclei of the cat thalamus, GLU was found to be co-localized with CB and GABA was found to coexist with the calcium-binding protein parvalbumin (Palestini et al. 1993).

However, parvalbumin and GABA are not expressed in cell bodies of the rat medial thalamus (Celio 1990; Bentivoglio et al. 1991), where data on the neurotransmitter(s) coexisting with CB are still lacking.

Double and single immunocytochemical approaches were used in the present study to investigate GLU-immunoreactive (ir) and ASP-ir neurons in the rat medial thalamic nuclei and their relationships with CB-containing cells.

Materials and methods

Six male (150–200 g) Wistar rats were deeply anaesthetized with 4% chloral hydrate (1 ml/100 g i.p.) and rapidly perfused through the ascending aorta with saline followed by the fixative solution. The brains were immediately removed from the skull, postfixed for several hours in the fixative solution and processed as outlined below. The sections adjacent to the immunoreacted ones were stained with thionin for cytoarchitectonic identification. All sections were dehydrated, coverslipped with DPX and studied under bright-field microscopy.

Double immunocytochemistry

In order to evaluate the distribution of excitatory amino acids and their relationships with CB, double immunocytochemistry was performed as a first step in two rats (rats 1 and 2). Fixation with paraformaldehyde, which was also used in previous studies exploiting the same antibodies (e.g. Helpler et al. 1988; Conti et al. 1987a,b; Giuffrida and Rustioni 1988; Battaglia and Rustioni 1988), was adopted in this set of experiments, since perfusion with glutaraldehyde (ranging from 0.2% to 2.5%) had been found in pilot experiments to result in background staining unsuitable for double immunocytochemistry. Thus, these animals were perfused with 1% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.2, followed by a more concentrated fixative solution (4% paraformaldehyde in PB). The brains were cut with a vibratome into 40-μm-thick serial sections. Free-floating sections through the diencephalon were incubated with 10% normal goat serum for 45 min to mask nonspecific adsorption sites. Two adjacent series of sections were subsequently incubated for 16 h at 4°C with either anti-GLU (dilution 1:20000) or anti-ASP (dilution 1:30000) polyclonal primary antibodies raised in rabbit and previously characterized (Helpler et al. 1988). The sections were then rinsed in TRIS-buffered saline (TBS), incubated for 2 h with goat anti-rabbit IgGs conjugated with colloidal gold particles (Amersham; diluted 1:100) and, after repeated washes in TBS, processed for silver intensification (Intense TM M kit; Amersham). The sections were then rinsed in TBS for 2 h, pretreated in 0.4% Triton X-100 in TBS to increase the penetration of the reagents, pre-incubated in 10% normal horse serum and incubated with anti-CB (dilution 1:5000) monoclonal antibodies (Swant; characterized by Celio 1990) for 16 h at room temperature. After rinsing for 1 h in TBS, the sections were incubated for 2 h with biotinylated horse anti-mouse IgGs (Vector; diluted 1:200), washed in TBS and then processed with the avidin-biotin-peroxidase protocol (ABC kit, Vector). In the final step of the procedure, the sections were reacted with 0.075% 3–3′-diaminobenzidine (DAB) and 0.002% hydrogen peroxide in TRIS-buffer (pH 7.6). In order to control the specificity of the antibodies, series of sections were processed omitting primary antibodies or replacing them with normal sera (diluted 1:100). No specific staining was detected in these sections.

Immunocytochemistry in consecutive thin sections

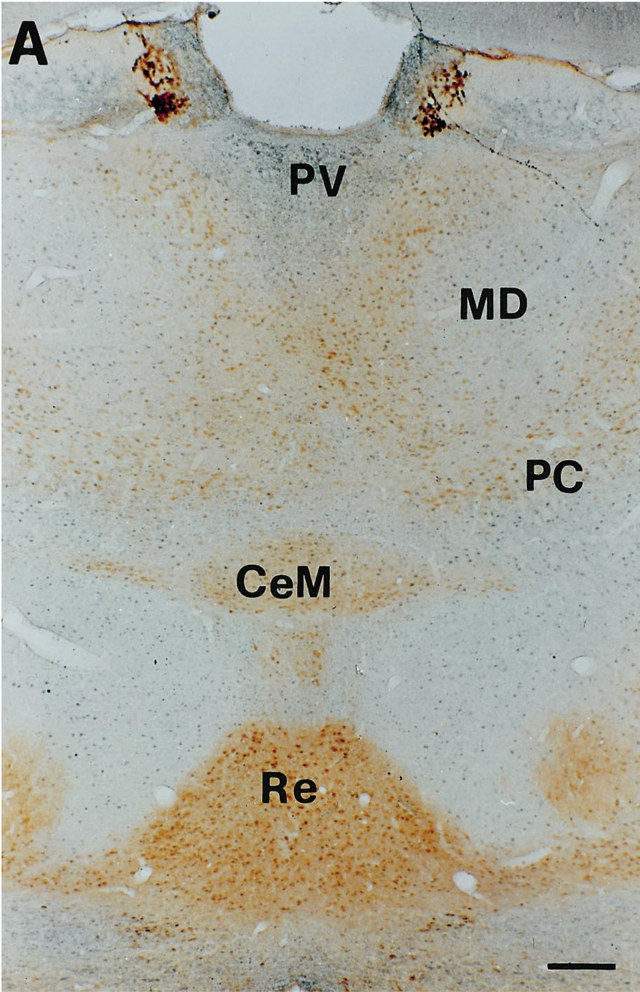
GLU, CB and ASP immunocytochemistry was performed in four animals in serial sections. Two of these animals (rats 3 and 4) were perfused with 4% paraformaldehyde as rats 1 and 2. The other two animals (rats 5 and 6) were perfused with a mixture of 2.5% glutaraldehyde and 0.5% paraformaldehyde in PB. The blocks containing the diencephalon were paraffin-embedded and cut into 5-μm-thick serial sections on gelatin-coated slides. After paraffin removal and rinsing in TBS, adjacent sections were processed for immunocytochemistry with different antibodies. Sections from rats 3 and 4 were incubated for 24 h at 4°C with the same primary antibodies (anti-ASP, diluted 1:1000, anti-GLU 1:2500, and anti-CB 1:2500) used for the double immunocytochemical procedure. These sections were then incubated with biotinylated secondary antibodies and processed with the avidin-biotin peroxidase protocol using a solution of 0.08% nickel ammonium sulphate and 0.02% DAB in the final step of the procedure. The immunocytochemical specificity was assessed by omitting the primary antibodies from the outlined staining sequence. In rats 5 and 6, the same immunocytochemical procedure was performed using different anti-GLU (dilution 1:1000) and anti-ASP (dilution 1:300) polyclonal primary antibodies (kindly supplied by J. Storm-Mathisen and O.P. Ottersen), previously characterized (Storm-Mathisen et al. 1983; Storm-Mathisen and Ottersen 1986).

Data analysis

In rats 1 and 2, the distribution of immunopositive neurons in the thalamus was charted from the double-immunostained sections with a computerized *x-y* plotting system attached to the microscope stage by means of transducers. Quantitative analysis was performed only on the sections processed for single immunocytochemistry. To this purpose, in rats 3–6 samples were selected in serial 5-μm-thick sections from the following nuclear territories of the thalamus: the mediodorsal nucleus (MD), the midline thalamic paraventricular nucleus (PV) and nucleus reuniens (Re), the ventromedial nucleus (VM) and the rostral intralaminar complex (paracentral, PC, and central medial, CeM, nuclei). The mediolateral boundaries between CeM and the interanteromedial nucleus (IAM) were difficult to delineate and the two nuclei were therefore analysed together (CeM-IAM). An example of the extent and location of the selected areas is shown in Fig. 3. CB is expressed in neurons located throughout these structures in the rat thalamus (Frassoni et al. 1991; Arai et al. 1994) and was thus used as a marker of neuronal subsets. The sampled areas were photographed with a ×40 objective. All the single- and double-immunostained neurons were counted in these areas on the microphotographs from three sections through different anteroposterior levels. Blood vessels and fibre bundles provided effective landmarks for the identification of the same cell bodies in adjacent sections (see Fig. 2).

For the purposes of the present investigation, three series of sections were processed, in the following order, for GLU, CB and ASP immunocytochemistry, respectively, and every fourth section was stained with thionin. In an initial pilot study, the total number of neurons in thionin-stained sections, which should have corresponded to the total number of cells in the examined regions, was found to be about 10% lower than the total number of immunostained cells. The interval between the three immunoreacted sec-

Fig. 1A–E Photomicrographs from thalamic sections (40 μm thick) processed with double immunocytochemistry with calbindin D-28k (CB; brown immunostaining) and glutamate (GLU; black granular immunoreactivity) antibodies. **A** Low-power view showing the distribution of GLU and CB in the midline nuclei and in the medial part of the intralaminar nuclei, **B** Distribution of GLU and CB single- and double-immunostained neurons in the anterior intralaminar nuclei. **C–E** Higher power photographs showing single GLU-ir neurons (*arrowheads*), CB-ir neurons (*long arrowheads*) and double-labelled GLU-CB neurons (*arrows*) in nucleus reuniens (*Re*) (**C**) and paracentral nucleus (*PC*) (**D, E**). *Scale bars* **A** 200 μm; **B** 150 μm; **C–E** 25 μm (*CeM* central medial nucleus, *MD* mediodorsal nucleus, *PV* paraventricular nucleus) ▶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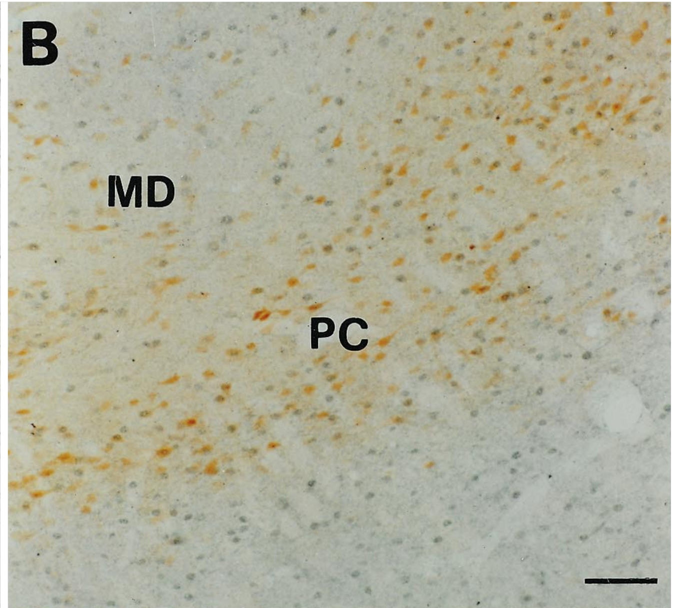
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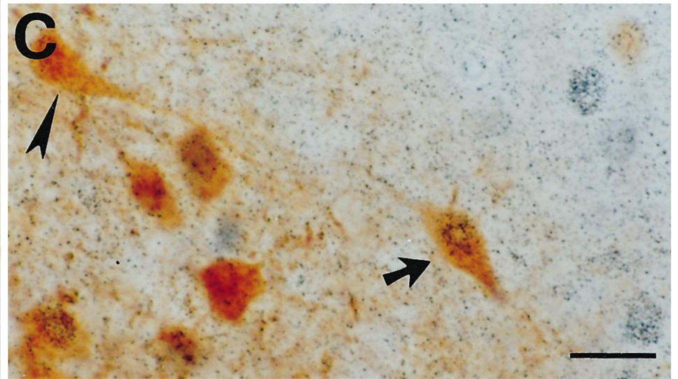
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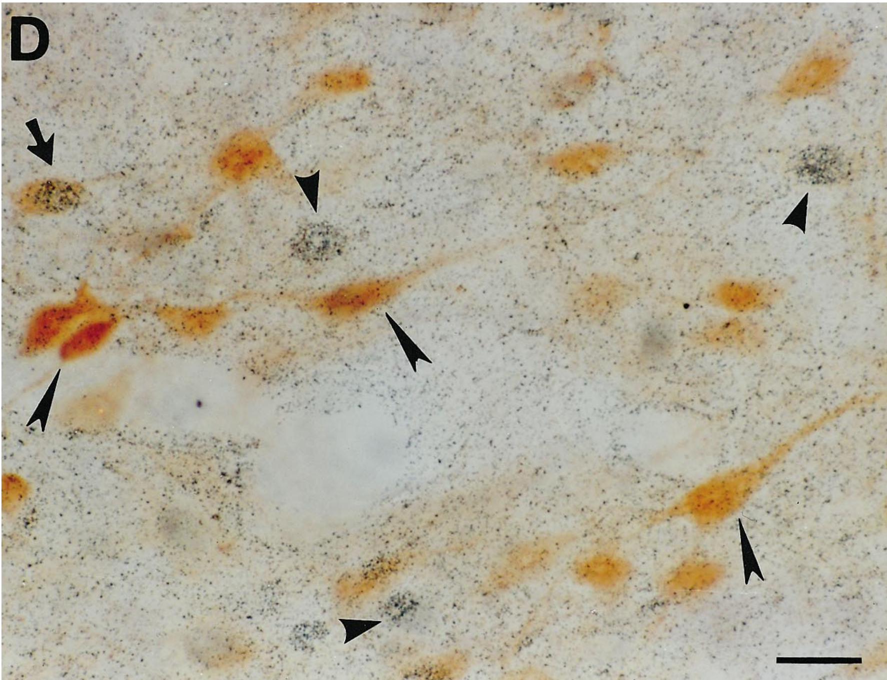
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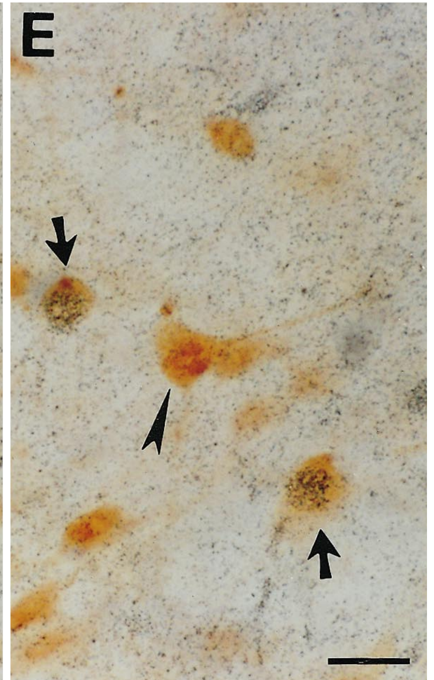
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tions and the thionin-stained ones (a total of 15 μm) could have accounted for this discrepancy, considering that some neuronal cell bodies might have not extended through the thionin-stained section. Therefore, the proportion of each single- or double-immunopositive cell population (%GLU, %CB, %ASP, %ASP-CB, %GLU-CB) was evaluated with respect to the total number of immunostained cells (ASP+GLU+CB+ASP-CB+GLU-CB). In addition, the proportion of double-immunostained neurons was calculated with respect to the total number of CB-ir cells (%ASP-CB/CB and %GLU-CB/CB). The variations among different rats were statistically evaluated using the chi-square test.

Results

Double immunocytochemistry

GLU or ASP immunoreactivity, revealed by silver grains, and CB immunoreactivity, visualized by DAB reaction products, were simultaneously detected in the thalamus of rats 1 and 2. The colour contrast and the different features of labelling (black granular GLU or ASP immunoreactivity versus brown diffuse CB immunostaining) allowed a clear distinction between single- and double-immunopositive neurons (Fig. 1C–E). In the sections processed for ASP and CB immunocytochemistry, a high background hampered, in some instances, unequivocal detection of the single CB-ir neurons. However, single ASP-ir and double ASP-CB-ir neurons, as well as single GLU-ir or CB-ir and double GLU-CB immunostained ones (Fig. 1), were clearly recognized in the sections processed for double immunohistochemistry.

The distribution of CB-ir cells in the thalamic midline, MD, VM and anterior intralaminar nuclei was consistent with previous descriptions (Frassoni et al. 1991; Arai et al. 1994). GLU- or ASP-positive cells were densely distributed in the dorsal components of the thalamic midline, especially in PV (Fig. 1A), and ASP-ir neurons appeared more numerous than the GLU-ir ones throughout the examined medial thalamic regions. On the other hand, along the midline, CB-ir cell bodies and neuropil prevailed in Re, where double GLU-CB-ir (Fig. 1A,C) and ASP-CB-ir neurons were also present. ASP-ir or GLU-ir neurons were intermingled with CB-ir or double-immunostained cells in the anterior intralaminar nuclei (Fig. 1B,D).

Although the present study was focused on the medial thalamus, at the inspection of the sections processed with double immunocytochemistry, GLU-ir neurons were seen to prevail in the lateral and ventral (e.g. the ventrolateral and ventroposterior nuclei) thalamic domains, in contrast with the prevalence of ASP-ir cells observed in the medial thalamic regions. In addition, both ASP and GLU were abundant in the anterior nuclei.

Immunocytochemistry in consecutive thin sections

A total of more than 1500 immunostained neurons were counted in rats 3 and 4 in the sampled areas, and their distribution was consistent with the findings observed in the previous set of experiments by means of double immunohistochemistry. ASP-ir neurons were in general intensely labeled (Fig. 2A), and it was confirmed that their density was highest in PV, CeM-IAM, PC and MD, and relatively low in VM and Re (Fig. 3A). GLU immunostaining, although less intense than the ASP immunostaining, was clearly detectable above background (Fig. 2C). GLU-ir neurons were distributed throughout the examined territories, with a slight prevalence along the midline in both PV and Re (Fig. 3B, Table 1). CB-ir

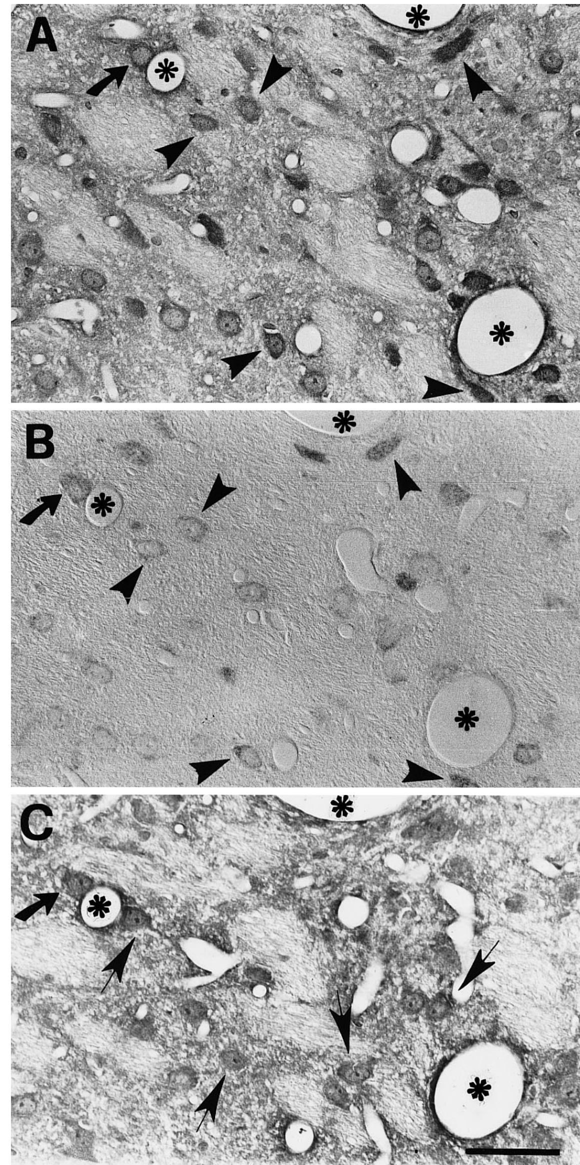

Fig. 2A–C Photographs from serial 5-μm-thick adjacent sections reacted with aspartate (ASP) (**A**), CB (**B**) and GLU (**C**) antisera, respectively. *Asterisks* mark the same blood vessels as reference landmarks in the different sections. *Arrowheads* in **A** and **B** point to some of the neurons immunostained by both ASP and CB. The *arrows* in **C** point to neurons immunopositive only to GLU antiserum. The *large arrow* close to the blood vessel in the *upper left corner* in **A–C** points to a neuron labelled by all three antibodies. *Scale bar* 55 μm

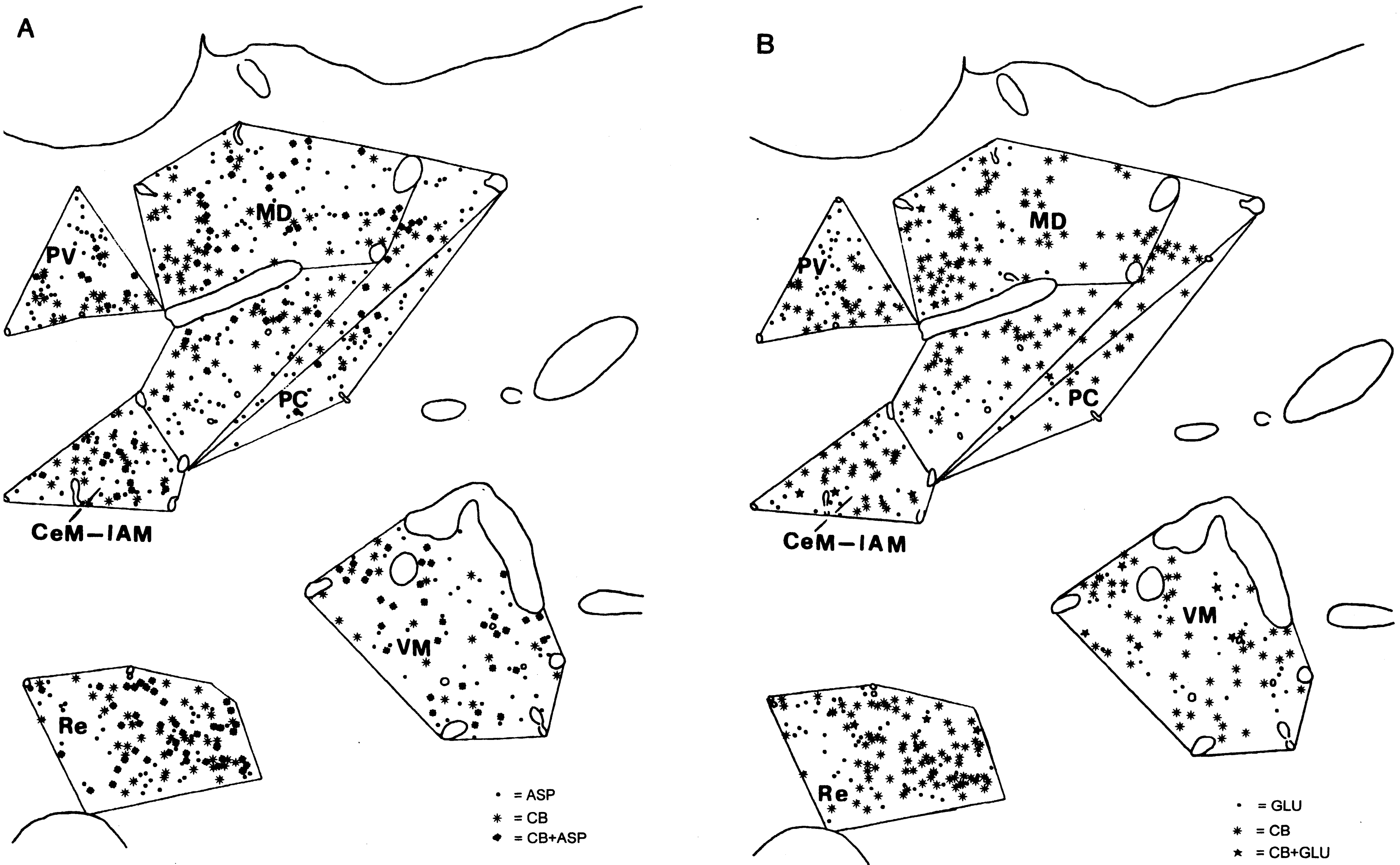

Fig. 3A, B Distribution of immunopositive neurons in the sampled areas of the medial thalamus, shown in two 5-μm-thick serial sections processed for ASP (**A**) or GLU (**B**) immunohistochemistry; the single- and double-immunostained CB neurons are evaluated from the 5-μm-thick section interposed between **A** and **B**. Each symbol corresponds to one neuron (*CeM-IAM* central-medial-interanteromedial nuclei, *VM* ventromedial nucleus)

Table 1 Percentage of single aspartate (*ASP*), glutamate (*GLU*) and calbindin D-28k (*CB*)-immunopositive neurons in the thalamic nuclei in two different animals (*PV* paraventricular nucleus, *MD* mediodorsal nucleus, *PC* paracentral nucleus, *CeM-IAM* central medial-interanteromedial nuclei, *VM* ventromedial nucleus, *Re* nucleus reuniens)

	ASP (%)	GLU (%)	CB (%)
Rat 3			
PV	34	31	26
MD	32	23	21
PC	58	24	8
CeM-IAM	45	20	20
VM	29	21	26
Re	32	22	26
Rat 4			
PV	41	32	19
MD	41	11	27
PC	61	20	11
CeM-IAM	45	21	19
VM	32	23	14
Re	21	29	22

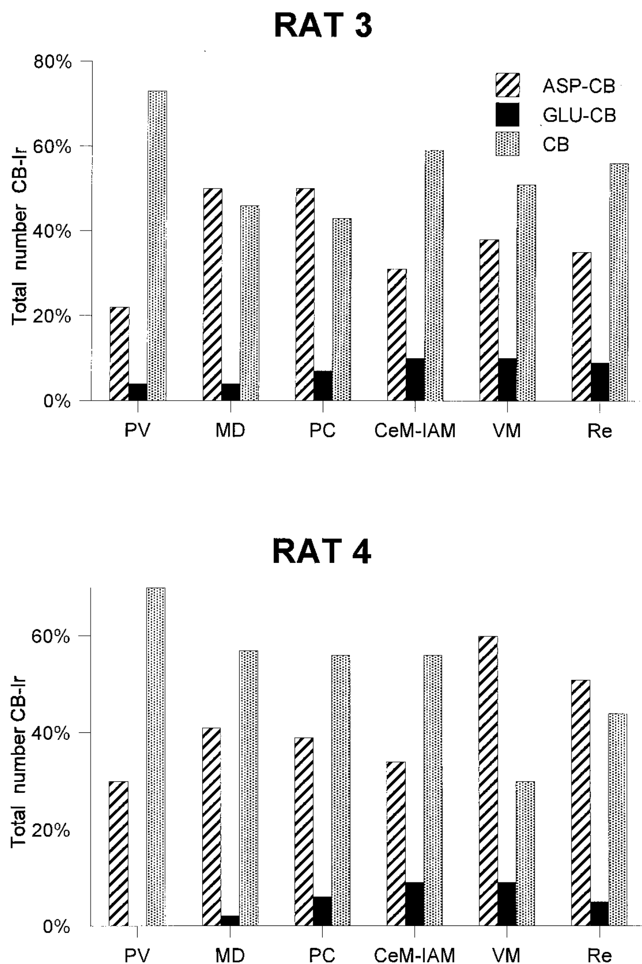

Fig. 4 Histograms showing the percentage of single- (CB) and double (ASP-CB and GLU-CB)-immunopositive neurons in respect to the total number of CB-ir (*CB-Ir*) cells (*y*-axis) in the examined nuclei of the medial thalamus (*x*-axis) evaluated in two representative animals

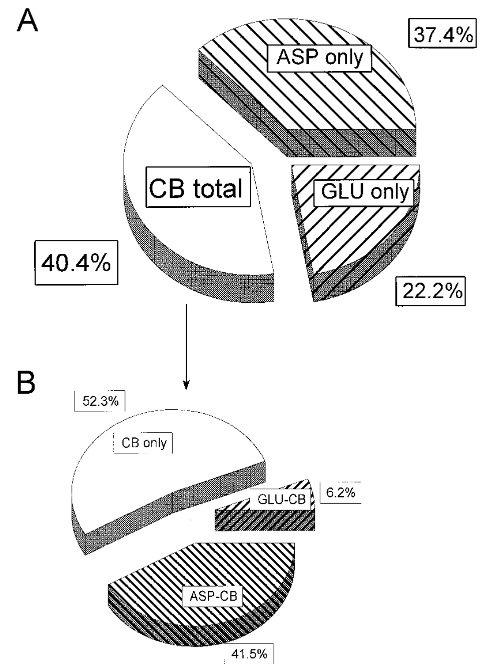

Fig. 5A, B Graphic representation of the percentage of immunopositive cell populations considering the proportion of the total number of CB-ir neurons (**A**); note that more than 50% of the immunostained cells are CB-immunonegative. The CB-immunopositive neurons include single-stained cells and double-immunostained (ASP-CB and GLU-CB) ones (**B**)

neurons displayed an intense, coarse cytoplasmic immunoreactivity (Fig. 2B).

ASP-ir neurons were overall more numerous than the GLU-ir ones (Table 1, Figs. 4, 5). ASP and GLU were found to immunostain almost exclusively different cells in the thalamus: only two neurons displayed both ASP and GLU immunopositivity; the same neurons were also CB-ir and one of them is shown in Fig. 2A–C. On the other hand, co-localization of ASP with CB, as well as of GLU with CB, was detected in adjacent thin sections (Fig. 2A–C), consistently with the findings observed in the double immunocytochemical experiments. Double ASP-CB-ir neurons were relatively numerous throughout the sampled areas (Fig. 3A), where they ranged in the different nuclei from 20–60% of the CB-ir cells (Fig. 4). Double GLU-CB-ir neurons were relatively infrequent (Fig. 3B) and ranged from 0 to 10% of the CB-ir cells (Fig. 4).

The proportion of the single GLU-ir, CB-ir, ASP-ir and double-immunostained neurons in the different nuclei displayed some variability in rats 3 and 4 processed with the same antibodies (Fig. 4, Table 1). However, the statistical evaluation indicated that the interindividual differences among the considered percentage values were not significant. Similar data were also obtained in rats 5 and 6, and the variations between the quantitative values obtained from the sections processed with different anti-ASP and anti-GLU antibodies (rats 3 and 4 compared with rat 5) were not significant.

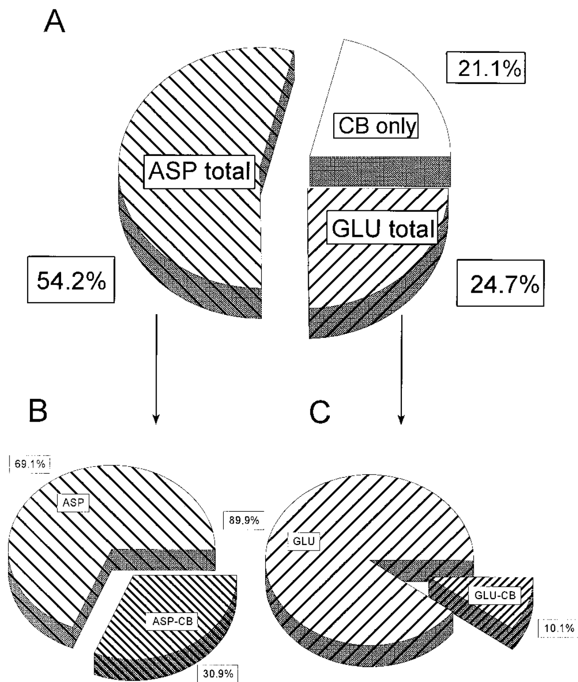

Fig. 6A–C Graphic representation of the proportion of the immunostained cell populations considering the proportion of the total number of ASP-ir and GLU-ir neurons (**A**); note that approximately 20% of the immunostained cells do not contain either amino acid and contain only CB. The relative proportion of the single-ASP-ir and ASP-CB double-immunopositive neurons is shown in **B**, and the same values of GLU-ir cells are shown in **C**

Therefore, the data observed by both double and single immmunohistochemical procedures pointed out the occurrence of five immunostained (single CB-ir, single ASP-ir, single GLU-ir, double GLU-CB-ir, double ASP-CB-ir) cell populations. In addition, single immunohistochemistry allowed a quantitative evaluation. When considering the total proportion of CB-ir cells (both single and double immunostained), these accounted for 40% of the immunopositive neurons (Fig. 5A). About 48% of the CB-ir cells were double immunostained: approximately 6% of these neurons were GLU-CB-ir, whereas the ASP-CB-ir cell population represented the vast majority (Fig. 5B). In addition, more than 50% of the CB-ir neurons (Fig. 5B), accounting for more than 20% of the total immunostained cells (Fig. 6A), did not display immunoreactivity for either amino acid. On the other hand, the neurons single immunopositive for ASP and GLU represented about 60% of the total number of cells counted in the sampled areas, and the proportion of the single ASP-ir neurons was about 1.5 times higher than that of the single GLU-ir ones (Fig. 5A). When considering the total proportion (about 80%) of ASP-ir and GLU-ir medial thalamic neurons (Fig. 6A), ASP was co-localized with CB in about 30% of the ASP-ir cells (Fig. 6B), whereas GLU was co-localized with CB in a minor proportion (10%) of the GLU-ir neurons (Fig. 6C). Thus, the majority of cells immunopositive for either amino acid were single immunostained.

Discussion

All cells of the rat medial thalamus, where no GABA-ergic intrinsic neurons are detected, are represented by projection neurons (Bentivoglio et al. 1991). The present study strongly implicates that the excitatory amino acids GLU or ASP might act as neurotransmitters in a large proportion of these relay neurons. Our data point out a considerable selectivity of the ASP and GLU immunoreactivities in the rat thalamic midline and medial nuclei, where ASP and GLU were not co-localized, and the ASP-ir neurons were more numerous than the GLU-ir ones. In addition, CB was found to coexist with ASP in a relatively high proportion of medial thalamic neurons, whereas CB was co-localized with GLU in a relatively low proportion of these cell bodies. The present findings also point out the occurrence of CB-containing neurons that did not display immunoreactivity for either ASP or GLU. Therefore, neurons in the medial thalamus might include aspartatergic and glutamatergic neuronal subsets, as well as neurons that might utilize a different neurotransmitter. This chemical diversity is especially striking considering the morphological homogeneity of neurons in the rat dorsal thalamus.

Methodological remarks

The antisera to GLU and ASP used in the present investigation have been extensively characterized by immunoblots and immunoadsorption on nervous tissue sections and showed a high degree of specificity for their respective antigen (Conti et al. 1987a,b; Helpler et al. 1988; Battaglia and Rustioni 1988). However, the significance of GLU or ASP immunoreactivity has long been debated, since these amino acids serve many metabolic routes in the central nervous system (McGeer et al. 1987). It has been suggested that, in perfusion-fixed material, GLU and ASP immunoreactivities may reflect both the transmitter and the metabolic pools (Ottersen and Storm-Mathisen 1985). In addition, GLU and ASP might readily interconvert (McGeer et al. 1987), and the biochemical features underlying the immunoreactivity to these amino acids in vivo might, therefore, be difficult to unravel. However, the present finding of a lack of co-localization of ASP and GLU in the medial thalamus rules out an eventual cross-reactivity of the antibodies. It should also be considered that GLU and ASP immunoreactivities were consistently reproducible in our material, even when using different anti-GLU and anti-ASP antibodies. Altogether these data strongly implicate that GLU and ASP immunopositivity of medial thalamic neurons could either reflect different neurochemical pathways or the use of different excitatory amino acids as neurotransmitter. In addition, the selectivity of ASP or GLU immunolabelling, which was restricted to subsets of neurons in the present study, could be ascribed to a higher rate of synthesis of the neurotransmitter pool of these amino acids with respect to the metabolic pool (Storm-Mathisen and Ottersen 1986).

The possibility that in the medial thalamus GLU and ASP immunoreactivities reflect neurotransmitter pools of relay neurons is supported by a number of observations. Studies based on the retrograde transport of D-[^{3}H]ASP (Streit 1980; Baughman and Gilbert 1981; Cuènod et al. 1983; Ottersen et al. 1983; Christie et al. 1987; Fuller et al. 1987; Johnson and Burkhalter 1992; Pirot et al.1994) and quantitative autoradiography of [^{3}H]glutamate binding sites (Halpain et al. 1984; Monaghan and Cotman 1985) have also suggested the presence of glutamatergic or aspartategic neurons in the rat thalamus. More compelling evidence derives from the enrichment, in GLU of thalamocortical terminals (de Felipe et al. 1988; Kharazia and Weinberg 1993, 1994; Weinberg and Kharazia 1996). A similar evidence has not been hitherto reported for ASP, requiring further investigation in cortical and subcortical target areas of medial thalamic efferents.

Excitatory amino acids in the medial thalamus

The present data demonstrating GLU-positive neurons scattered through thalamic nuclei including PV, Re and MD is in agreement with previous findings (Ottersen and Storm-Mathisen 1984a,b). In addition, phosphate-activated glutaminase, a major synthetic enzyme of the transmitter GLU in the central nervous system, was found to be expressed in thalamic intralaminar and midline neurons (Kaneko and Mizuno 1988). The occurrence of ASP-ir neurons was also previously reported in many thalamic nuclei of the rat (Aoki et al. 1987). The present finding of ASP-ir neurons in the rat MD is at variance with a previous negative report in the same nucleus (Aoki et al. 1987), but technical parameters could have accounted for this discrepancy. Our data reveal a preponderance of aspartatergic neurons over glutamatergic ones in the rat medial thalamus. The present observations in vibratome-cut sections also suggest that ASP may predominate in the medial thalamic territories and GLU in the lateral and ventral thalamic domains. It has been mentioned that, in the ventroposterior nucleus of the rat thalamus, all neurons stain above background for both GLU and ASP (Rustioni et al. 1988). Although the present investigation was focused on the medial thalamic regions, in our hands such antibodies did not result in ubiquitous staining in the ventral thalamic nuclei, in which GLU seemed to prevail over ASP immunoreactivity.

Even taking into account that the immunostained neurons could have been underestimated due to technical limitations, the present finding that a relatively large neuronal cell population was immunonegative for both GLU and ASP suggests that a neuronal subset of the medial thalamus might utilize a different neurotransmitter. In the cerebral cortex, the prevalence of ASP-ir neurons in infragranular layers, as well as the occurrence of non-ASP and non-GLU pyramidal neurons, were reported (Conti et al. 1987a,b; Dori et al. 1992; Giuffrida and Rustioni 1988). It has been suggested that some cortical projection neurons may use the dipeptide *N*-acetylaspartylglutamate (Tieman et al. 1987), but the significance and distribution of immunoreactivity to this peptide in the forebrain is still debated (Henderson and Salt 1988; Tsai et al. 1993; Moffett and Nambodiiri 1995) and its occurrence in the medial thalamus has not been investigated in detail.

Glutamatergic and non-glutamatergic (possibly aspartatergic) pyramidal neurons in layer VI of the rat cerebral cortex exhibit different electrophysiological properties (Kaneko et al. 1995). Thus, the present observation of the lack of coexistence of ASP and GLU in medial thalamic neurons could indicate an electrophysiological diversity of amino acidergic excitatory cells in the medial thalamus.

CB is a selective immunochemical marker for a discrete medial thalamic cell population in the rat. However, the significance of CB expression in neurons is still unclear and has not been hitherto investigated in the thalamus. CB-ir GABAergic nonpyramidal neurons of the rat frontal cortex have been found to be characterized by low-threshold spikes, in contrast with fast-spiking neurons immunostained by the calcium-binding protein parvalbumin (Kawaguchi and Kubota 1993). Low-threshold spikes have also been demonstrated in thalamocortical neurons (Llinas and Jahnsen 1982), and the colocalization of CB with excitatory amino acids in the medial thalamus could thus represent an additional indication of functional selectivity of medial thalamic cell populations.

Functional considerations

The effects imposed by excitatory amino acids on target neurons are mainly determined by postsynaptic receptors. Two distinct subtypes of excitatory amino acid ionotropic receptors have been identified on the basis of pharmacological and physiological responses to selective antagonists: the *N*-methyl-D-aspartate (NMDA) and non-NMDA (kainate, KA, and alpha-amino-3-hydroxy-5-methyl-4-isoxozolone proprionic acid, AMPA) receptors. A variety of agonists and antagonists of these receptors have been studied in detail (Watkins and Evans 1981; Foster and Fagg 1984). Physiological studies on cerebellar granule cells have proposed a model based on the existence of multiple conductance channels activated by receptors that are preferentially activated by distinct excitatory amino acids (Cundy et al. 1988). Data from the thalamic Reticular nucleus (de Curtis et al. 1989) have suggested that NMDA receptors might be activated by ASP, while GLU might preferentially act on the non-NMDA receptors. Cortical neurons bear receptors sensitive to excitatory amino acid agonists (Thomson 1986; Artola and Singer 1987; Sutor and Hablitz 1989) and to antagonists of their receptors (Hicks and Guedes 1981; Miller et al. 1989; Hablitz and Sutor 1990). Both the NMDA receptor subunit 1, a constant component of the

NMDA receptor complex (Conti et al. 1994; Petralia et al. 1994) and different AMPA receptor subpopulations (Petralia and Wenthold 1992) have been reported in the cerebral cortex, where these receptors could serve both the excitatory corticocortical and subcortical inputs. Our findings on the existence of distinct populations of glutamatergic and aspartatergic medial thalamic neurons raises the possibility that thalamocortical fibres could elicit different postsynaptic effects depending on the preferential activation of glutamatergic or aspartatergic parent cell bodies.

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