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Calbindin D28k in the olivocerebellar projection. A light and electron microscope study

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ABSTRACT

The distribution of the calcium binding protein calbindin D28k (CaBP) in the rat and gerbil olivocerebellar system was compared and an ultrastructural analysis of the inferior olive and cerebellum was performed in the gerbil. CaBP is present in the perikarya and dendritic arborisations of inferior olivary neurons. The olivocerebellar projection in the inferior cerebellar peduncle stains for CaBP. CaBP is also contained in the terminal branches of the olivocerebellar projection: the climbing fibres in the inner molecular layer of the cerebellum. Thus CaBP is a reliable marker for the whole olivocerebellar projection in adult rodents. Presence of the calcium binding protein CaBP in inferior olivary neurons may influence the different calcium conductances of their dendritic, somatic and terminal compartments responsible for the physiological properties of these nerve cells.

Key words: Rat; gerbil; calcium-binding proteins; inferior olivary nucleus; cerebellar climbing fibres.

INTRODUCTION

The vitamin D dependent calcium binding protein calbindin D28k (CaBP) belongs to a superfamily of intracellular proteins with high affinities for calcium. Other members include calmodulin, parvalbumin and troponin C. Common feature of all these proteins is the amino acid sequence responsible for calcium chelation, a helix–loop–helix domain referred to as the EF hand (Heizmann, 1988). CaBP contains 6 helix–loop–helix repeats in its sequence (Hunziker, 1986). Measurements of the calcium binding activity report stoichiometries ranging from 3 or 4 calcium ions per molecule (Bredderman & Wasserman, 1974; Cheung et al. 1993) to 6 ions per molecule (Leathers et al. 1990). CaBP affinity for calcium is about 0.5 μM (Bredderman & Wasserman, 1974; Cheung et al. 1993) and within the physiological range of free cytosolic calcium (0.05–1 μM). Dissociation constants of 10 nM (Leathers et al. 1990) and 2 μM (Pansini & Christakos, 1984) have also been measured.

CaBP occurs in the intestine, in the kidney—where it seems to mediate vitamin D dependent calcium transport across membranes—and many other vertebrate tissues, including the brain (Norman et al. 1982; Christakos et al. 1989). In the central nervous system (CNS) CaBP is widely distributed in both excitatory and inhibitory neurons (for review, see Celio, 1990). CaBP lacks vitamin D dependency in nerve cells where it functions as a major intracellular calcium buffer, also affecting calcium conductances and other membrane properties (Köhr & Mody, 1991; Lledo et al. 1992; Kawaguchi & Kubota, 1993). In pathological conditions presence of CaBP can protect neurons from excitotoxic, calcium mediated injury (Mattson et al. 1991).

The presence of CaBP in inferior olivary neurons has been mentioned and/or shown in previous work mostly on the general distribution of the protein in the rodent and chick CNS (Jande et al. 1981; Garcia-Segura et al. 1984; Celio, 1990; Sequier et al. 1991; Wassef et al. 1992; Frantz & Tobin, 1994) and in the rodent and chick cerebellum (Rogers, 1989). Most of these authors reported that the distal part of the inferior olivary axons in the inferior peduncle (Jande et al. 1981; Wassef et al. 1992) and the climbing fibres in the cerebellar cortex were devoid of CaBP immunoreactivity (Jande et al. 1981; Garcia-Segura et al. 1984; Rogers, 1989; Celio, 1990).

To answer the question whether the protein shows

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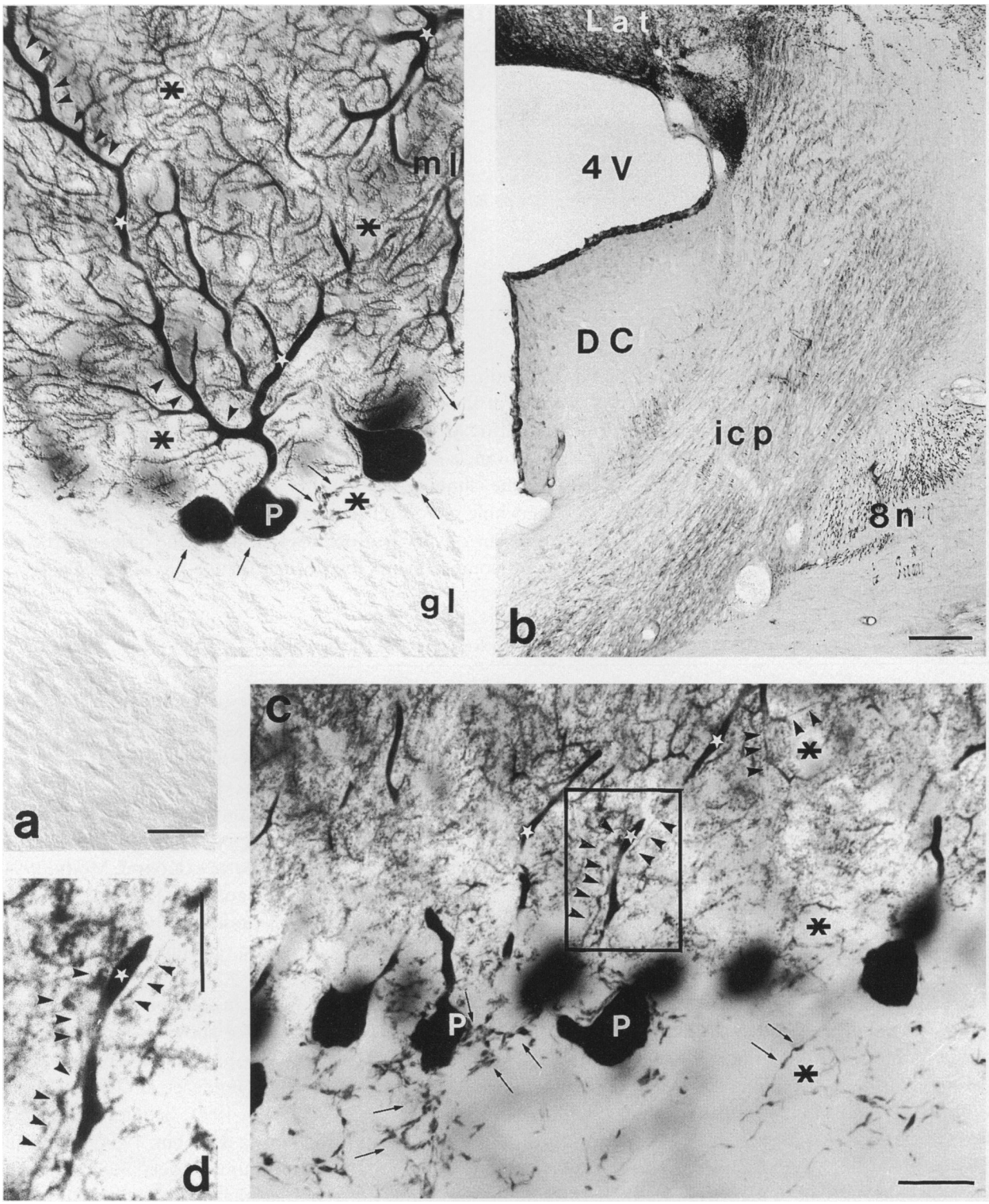

Fig. 1. CaBP in the olivocerebellar projection of the rat. (*a*) Parasagittal section through the cerebellar cortex, interference contrast. Purkinje cell somata (P) and the whole dendritic arborisation within the molecular layer are CaBP-stained. The numerous Purkinje spines appear as small dots lining on both sides the distal dendritic branches. Asterisks mark unstained somata of stellate and basket interneurons. Thin CaBP immunoreactive profiles that may belong to the climbing fibre collaterals (arrowheads) can sometimes be detected within the molecular layer where they follow the main branches of the Purkinje dendrites (white stars). Recurrent collaterals of Purkinje axons forming the supraganglionic and infraganglionic plexuses are stained (arrows). No CaBP immunoreactive perikarya can be seen within the granular layer. Bar, 10 μm. (*b*) Parasagittal section through the brainstem. The fibres travelling to the cerebellum within the inferior peduncle are CaBP immunoreactive. They represent for the greatest part axons of inferior olivary neurons. Bar, 100 μm. (*c*) Parasagittal section through the cerebellar cortex, bright field. The inner molecular layer and outer granular layer are shown in more detail. Fibres of the supraganglionic and infraganglionic plexuses (arrows) are more numerous; some of them surround the unstained soma (asterisk) of a granular layer

the ubiquitous location characteristic of calcium binding proteins in most neurons, or whether it is really limited to the soma and dendritic compartments, we looked for CaBP-immunoreactive climbing fibres in the cerebellum and characterised in a more comprehensive way CaBP-containing neurons of the inferior olivary nucleus. Part of this work has been published in abstract form (Scotti & Nitsch, 1991*a*).

MATERIAL AND METHODS

Control male Wistar rats and gerbils (*Meriones unguiculatus*) were deeply anesthetised and perfusion fixed with 0.2% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Serial sagittal sections, 40 μm thick, were cut with a Vibratome and collected in wells. Every 7th section was mounted on gelatine-coated slides for cresyl violet staining.

Sections were pretreated with 1% sodium borohydride for 10 min, then with 0.4% Triton-X-100 for 2 h. The monoclonal antibody 300 against chicken gut CaBP D28k (Celio et al. 1990) was diluted 1:2500 or 1:5000 in 5% normal horse serum and incubated for 24 h at room temperature and another 36 h at 4 °C. Biotinylated horse antimouse IgG (Vector) diluted 1:200 and avidin biotin-peroxidase complex (ABC, DAKO) diluted 1:100 followed with an incubation time of 90 min each. On control sections either incubation of the CaBP antibody or of the secondary antibody or of the ABC was omitted. Immunoreaction was visualised with 0.05% diaminobenzidine and 0.01% hydrogen peroxide. Enhancement with nickel was routinely adopted (Adams, 1981). Incubation steps were performed under constant agitation. Between each step sections were extensively rinsed. For dilution and rinsing 0.05 M TRIS buffered saline pH 7.4 was used. For light microscopy sections were dehydrated and coverslipped with Eukitt.

Only the brainstem of gerbils was processed for electron microscopy. Sections destined for electron microscopy were not subjected to the Triton-X-100 treatment and nickel enhancement. The inferior olivary nucleus was dissected from the floating sagittal sections at 2 lateralities: ~ 0.6 mm and 1.2 mm (Paxinos & Watson, 1986). The cerebellum at different lateralities was also dissected. The tissue was postfixed with 0.1% aqueous osmium tetroxide (1 h at 4 °C). Partially dehydrated sections were blockstained with 1% uranyl acetate in 70% ethanol (1 h at 4 °C), followed by further dehydration in graded ethanol, propylene oxide and overnight immersion in a propylene oxide Epon mixture with gentle shaking. The next day the tissue was flat embedded in Epon and the resin was allowed to polymerise at 60 °C. Ultrathin sections (~ 40 nm) of the caudal part of the principal olive and dorsal accessory olive, and of the cerebellar cortex were obtained from the superficial 1 μm of the slice, mounted on uncoated nickel grids, counterstained with lead citrate and viewed on a Zeiss EM 10 transmission electron microscope.

RESULTS

Light microscopy

The cerebellar cortex is intensely CaBP immunoreactive. CaBP is contained in the somata of the Purkinje cells and in the whole dendritic compartment, including the spines (Figs 1*a*, *c*, *d*, 2*a*). The axons of the Purkinje neurons also contain CaBP. The main axon can sometimes be seen as it leaves the cell body (Fig. 2*a*). Purkinje axon collaterals originating in the granular layer can be followed as they travel back to the Purkinje cell layer where they divide to form the large infraganglionic and the sparse supraganglionic plexuses (Figs 1*a*, *d*, 2*a*). The interneurons of the molecular layer, the basket and stellate cells are not CaBP immunoreactive (Figs 1*a*, *d*, 2*a*). The majority of Golgi interneurons in the granular layer do not contain CaBP. No differences in CaBP staining can be detected between rats and gerbils, confirming the homogeneity of CaBP expression in the avian, rodent, and human cerebellum (Jande et al. 1981; Fournet et al. 1986; Rogers, 1989; Scotti & Nitsch, 1992).

CaBP stained axons can be seen travelling in the white matter of the cerebellum. A CaBP immunoreactive axonal plexus spreads within the boundaries of the deep cerebellar nuclei (Figs 1*b*, 2*b*). These axons, however, do not exclusively belong to the Purkinje cells. In fact CaBP is also contained in 2 important afferent pathways to the cerebellum, part of the spinocerebellar and the whole olivocerebellar projection. Both pathways travel in the inferior cerebellar peduncle (Figs 1*b*, 2*b*). Olivocerebellar axons leaving the different CaBP immunoreactive

interneuron. Smooth thin CaBP immunoreactive profile bearing varicosities (arrowheads within the box; see also enlargement in (*d*)) run parallel to a Purkinje dendrite (white stars), on alternating sides or traverse the molecular layer for short distances (arrowheads). Bar, 10 μm. (*d*) Higher magnification of inset in (*c*). Arrowheads indicate CaBP-containing climbing fibres. Bar, 5 μm. DC, dorsal cochlear nucleus; gl, granular layer; icp, inferior cerebellar peduncle; Lat, lateral cerebellar nucleus; ml, molecular layer; 4V, 4th ventricle; 8n, vestibulocochlear nerve.

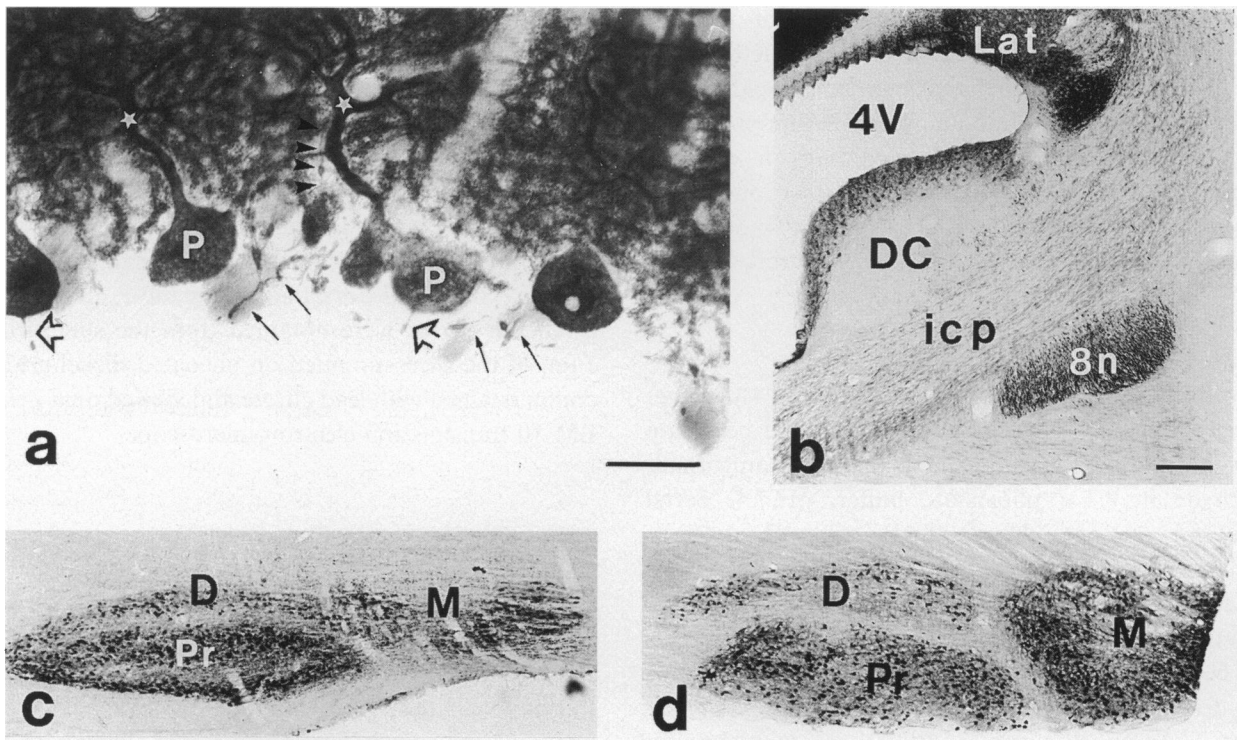

Fig. 2. CaBP in the olivocerebellar system of the gerbil. (*a*) The cerebellar cortex of the gerbil shows no differences in CaBP staining pattern as compared with the rat. Perikarya of Purkinje (P) cells and their whole dendritic tree are intensely CaBP immunoreactive. White stars mark the principal dendrites. Open arrows indicate the main Purkinje axon as it leaves the cell body. Arrows point to the mesh of recurrent Purkinje axon collaterals of the infraganglionic plexus. Arrowheads point to the varicose CaBP stained fibre approaching the main dendrite of a Purkinje cell. This axon, penetrating into the molecular layer, may represent a climbing fibre collateral. Bar, 10 μm. (*b*) Olivary axons coursing through the inferior cerebellar peduncle (icp) are CaBP stained. Bar, 100 μm; also applies for (*c*) and (*d*). (*c*, *d*) Parasagittal sections of different lateralities cut through the inferior olivary nucleus (*c*, ~ 1.2 mm; *d*, ~ 0.6 mm). All neurons in all subdivisions of the inferior olive are CaBP-immunoreactive in the gerbil. D, inferior olive, dorsal nucleus; M, inferior olive, medial nucleus; Pr, inferior olive, principal nucleus. For abbreviations in (*b*), see Fig. 1.

subnuclei of the inferior olive are therefore mixed with the efferent Purkinje axons in the deep cerebellar nuclei, the white matter of the cerebellar cortex and in the granular layer. Since their terminals, the climbing fibres, typically arborise within the inner molecular layer, CaBP immunoreactive fibres detected in this layer may represent climbing fibre branches. With adequate focusing, thin beaded CaBP-stained axons resembling climbing fibre collaterals can be seen to follow the secondary ramifications of the Purkinje dendritic tree or traverse the molecular layer for a small distance, on the way to neighbouring dendrites (Figs 1*a*, *c*, *d*, 2*a*). Some varicosities are closely apposed to the Purkinje dendrite (Fig. 1*c*, *d*).

In the inferior olivary nucleus most cell somata in all subnuclei are CaBP immunoreactive. The neuropil stains homogeneously for CaBP (Fig. 2*c*, *d*).

Ultrastructure of the cerebellar cortex

Ultrastructural analysis of the inner molecular layer confirmed that the CaBP immunoreactive beaded profiles lining Purkinje dendrites on alternate sides are climbing fibre axons (Fig. 3). Diaminobenzidine reaction product is present in synaptic terminals densely packed with round vesicles (Fig. 3*b*, *c*). The boutons originate en passant from an axon of small calibre, filled with microtubules (Fig. 3*a*). Two successive synaptic varicosities are connected by a short thin tendril also packed with microtubules (Fig. 3*c*). The boutons appose to the shaft of intensely CaBP immunoreactive Purkinje dendrites and are enwrapped by glial processes. They make synaptic contacts on thorns budding from the Purkinje dendrite (Fig. 3*c*). These ultrastructural features correspond in fact to the climbing fibre terminals. CaBP immunoreactivity was absent from the smaller, round synaptic boutons originating from the parallel fibres and terminating on CaBP positive Purkinje spines that spread all over the molecular layer (not shown). The terminals of the molecular layer interneurons were also devoid of CaBP immunoreactivity. They made synaptic contacts with the shaft of Purkinje dendrites and contained pleomorphic vesicles (Fig. 3).

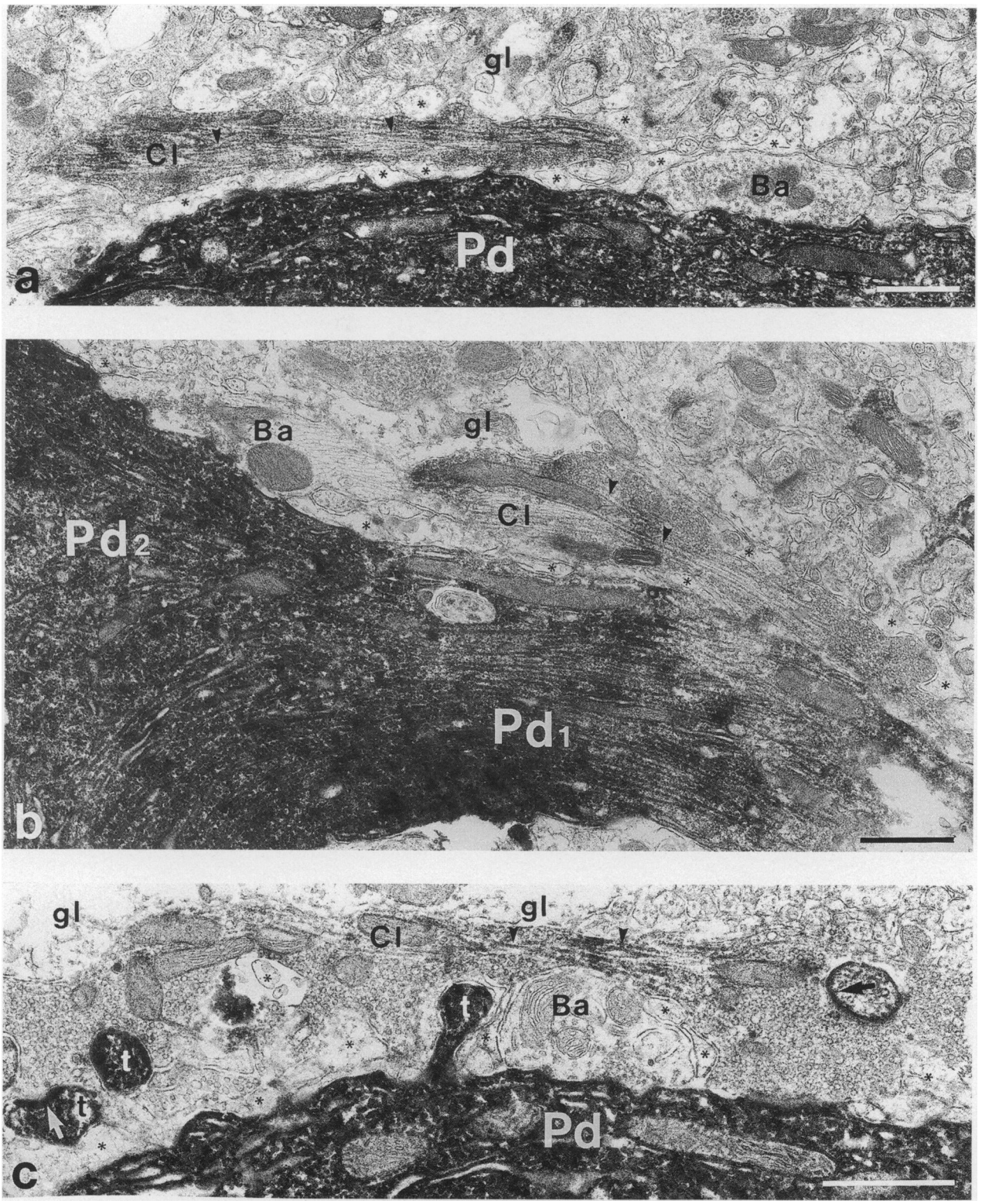

Fig. 3. Cerebellar cortex, molecular layer. (*a*) A CaBP-labelled climbing fibre, typically rich in microtubules, aligns with a CaBP-stained Purkinje dendrite for a short distance. A basket cell bouton terminates on the shaft of the Purkinje dendrite. Glial processes slip between the 2 CaBP immunoreactive profiles and cover the surface of both climbing fibre and basket cell bouton. (*b*) A CaBP immunoreactive climbing fibre varicosity characteristically filled with round vesicles and rich in microtubules approaches a branching Purkinje dendrite. Glial sheets enwrap the climbing fibre from both sides and also cover a basket axon apposed to the Purkinje dendrite. (*c*) Two climbing fibre varicosities, connected by a short thin tendril filled with microtubules, make synaptic contacts on thorns of Purkinje dendrite. The climbing fibre overrides the bouton of a basket cell axon. Asterisks, thin glial processes; arrowheads, microtubules; arrows, postsynaptic densities. Ba, basket cell bouton; Cl, climbing fibre; gl, glia; Pd, Purkinje dendrite; t, thorns of Purkinje dendrite. Bars, 1 μm.

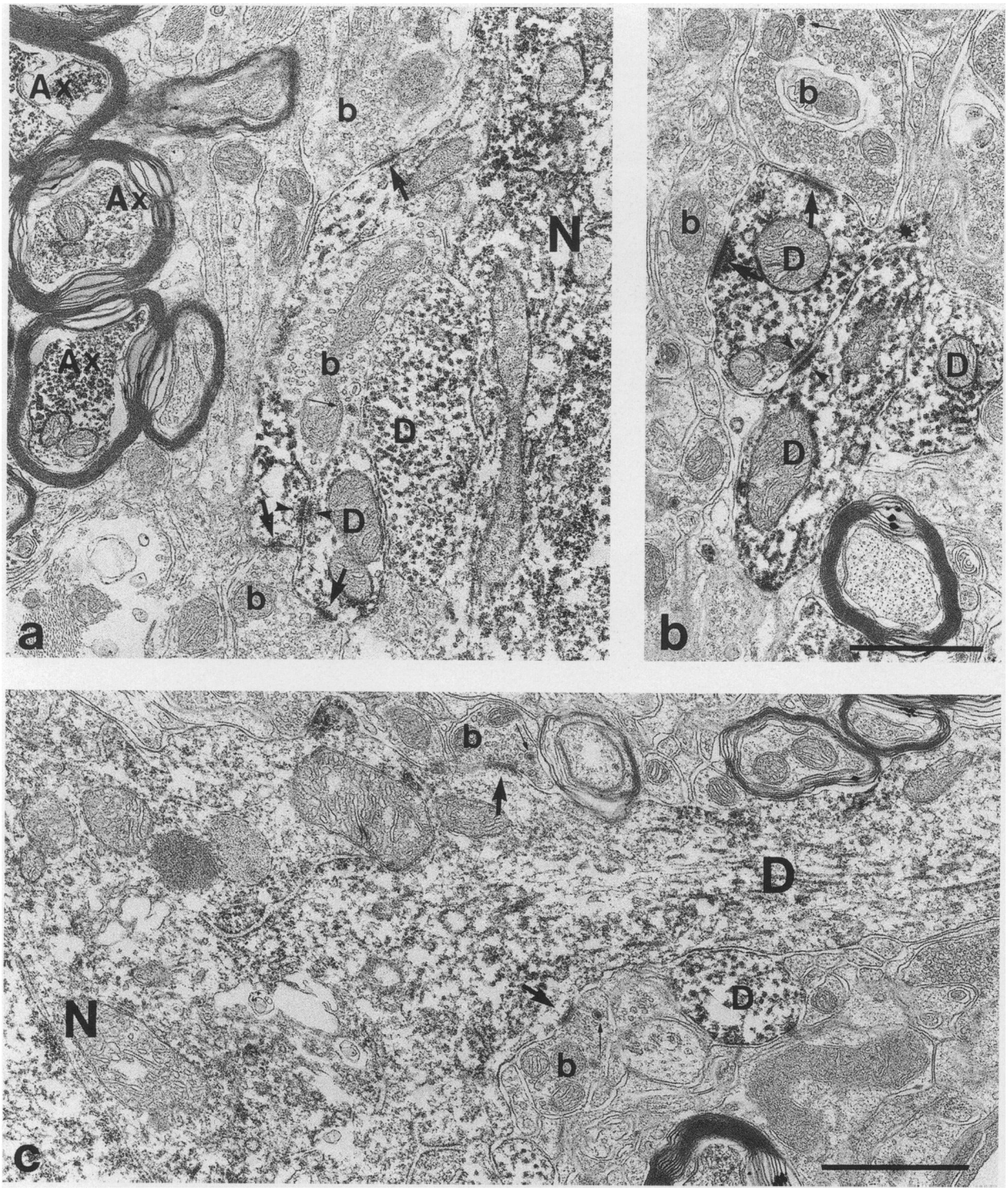

Fig. 4. Inferior olivary nucleus. (*a*) From a CaBP immunoreactive perikaryon a thin appendage arises and participates in the formation of a dendritic thicket apposed to the same neuronal soma. A bouton containing pleomorphic vesicles makes synapses on the stem of the appendage. The bulbous apex of the appendage receives synaptic contact within the thicket. CaBP immunoreactive myelinated axons are also present. (*b*) Two boutons make synaptic contact on a CaBP immunoreactive distal dendrite. The same dendrite is flanked by 2 dendrites to form a thicket. It also gives rise to a spine whose head is out of the plane of section. (*c*) Two small boutons synapse on a CaBP immunoreactive main dendrite as it leaves the soma. A distal dendrite also apposes to it. Arrowheads, zonula adherens; arrows, postsynaptic densities; small arrows, dense core vesicles; star, spine stem; Ax, myelinated axon; b, bouton; D, dendrite; N, neuron. Bars, 1 μm. Bar in (*c*) also applies for (*a*).

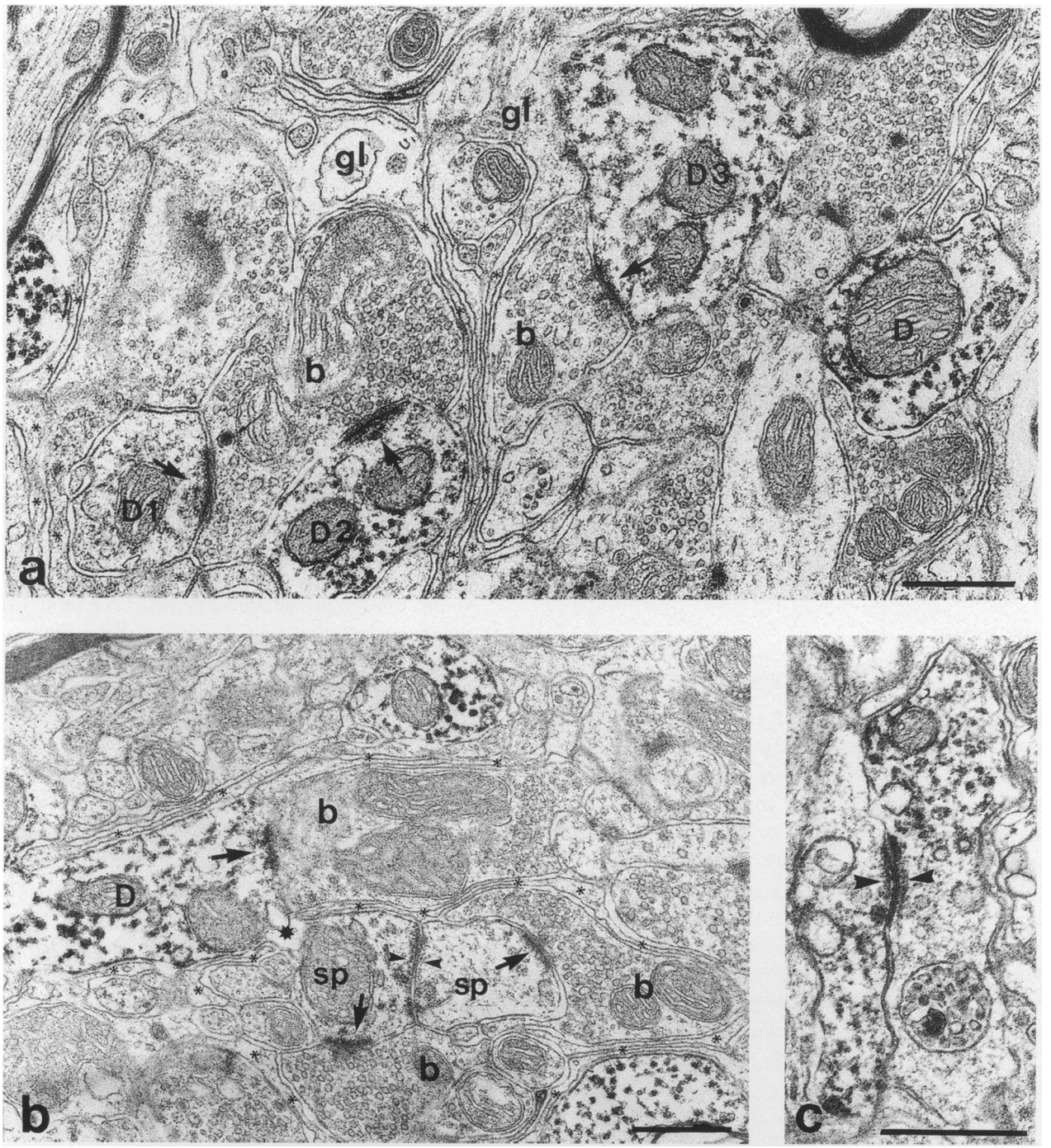

Fig. 5. Inferior olivary nucleus. (*a*) Two neighbouring glomeruli are each enwrapped by thin glial lamellae. The 2 CaBP immunoreactive distal dendrites D1 and D2 are contacted by a bouton filled with round vesicles and also containing a dense core vesicle. The CaBP-labelled distal dendrite D3 is contacted by a bouton containing pleomorphic vesicles. (*b*) A CaBP-labelled distal dendrite gives origin to a spine that contacts an adjacent spine via a gap junction. Both spines are contacted by boutons containing pleomorphic vesicles. (*c*) Detail of 2 CaBP immunoreactive spines in a glomerulus communicating via gap junction. Arrowheads, gap junction; arrows, postsynaptic densities; asterisks, glial lamellae; small arrow, dense core vesicle; star, spine stem; b, bouton; D, dendrite; gl, glia; sp, spine. Bars, 0.5 μm.

Ultrastructure of the inferior olive

Myelinated axons containing electron-dense immunoprecipitate are present in the inferior olivary nucleus (Fig. 4*a*). Most neuronal somata are CaBP immunoreactive (Fig. 4*a*, *c*). Dendrites originating from different neurons ramify extensively and form an intricately interwoven network (Figs 4, 5). Dendrites of different size appose each other and often exhibit a membrane specialisation in the form of zonulae adherentes at the site of contact (Fig. 4*a*, *b*). These complexes of dendrites are often contacted by the

same synaptic terminal (Fig. 4*a*). Dendrites exhibiting the above characteristics have been termed dendritic thickets (Sotelo et al. 1974). Numerous dendritic branches flank the somata or the principal dendrites in proximity to the perikaryon (Fig. 4*a*, *c*). Distal dendrites give origin to spines that cluster in the characteristic glomeruli (Fig. 5). The spines often communicate via gap junctions (Fig. 5*b*, *c*) and are less intensely stained, probably due to difficulties in penetration of immunoreagents into the non-permeabilised tissue. Spines are contacted by numerous boutons containing round or pleomorphic vesicles and occasionally dense core vesicles. These boutons are always devoid of CaBP-immunoreactivity. The type of the synaptic contacts on immunoreactive profiles cannot be differentiated exactly because of the predilection of diaminobenzidine to precipitate nonspecifically on membranous and filamentous materials within the cell. Synaptic profiles of a glomerulus are surrounded by glial lamellae (Fig. 5*a*, *b*).

DISCUSSION

CaBP in the olivocerebellar projection

Here it is shown that CaBP is transported into the axon of the inferior olivary neurons all the way through to its target. CaBP is present in its terminal branches, the climbing fibre collaterals, which can be detected as varicose fibres following the secondary ramifications of the Purkinje dendritic tree. Ultrastructural analysis demonstrated that the immunoprecipitate was contained in the thin, microtubule-filled tendrils coursing parallel to the Purkinje dendrites. Their characteristic terminal varicosities were densely packed with round vesicles and made synaptic contacts on the thorns of Purkinje dendrites (Larramendi & Victor, 1967; Sotelo, 1969; Palay & Chan-Palay, 1974).

The arborisation pattern of the climbing fibres within the molecular layer of the cerebellum has been thoroughly studied in Golgi impregnated material (Scheibel & Scheibel, 1954). Inferior olivary axons run along the Purkinje cell bodies and start to divide approximately at the level of the first bifurcation of the Purkinje cell dendrite. The axon branches follow with further subdivisions the secondary ramifications of the Purkinje dendrites. Light microscopic analysis of CaBP immunostained cerebellar folia does not permit the distinction of the climbing fibre branches in the inner molecular layer because the Purkinje cell somata and their dendritic arborisation, including the spines, are also intensely CaBP immunoreactive. Characteristically, climbing fibre branches give origin to fine beaded collaterals running approximately parallel to them. These collaterals have been observed to twine like tendrils around the Purkinje dendrites (Palay & Chan Palay, 1974). Some of them, however, remain parallel to the Purkinje dendrites and eventually leave them to reach smaller Purkinje branches or the dendrites of interneurons in the molecular layer (Palay & Chan Palay, 1974). The few CaBP-labelled axons recognised in this layer in the present study corresponded roughly to this description: they were extremely thin and beaded, located parallel to the Purkinje dendrite or extending into the molecular layer. In most cases, detection was facilitated by the fact that the branching Purkinje dendrites and the adjacent climbing fibre tendril were situated in slightly different levels of focus. Although more modest in number, the fine CaBP-positive axons, bearing fusiform enlargements, resembled anterogradely traced climbing fibre collaterals visualised with an immunohistochemical method (Rossi et al. 1993).

At the border of the Purkinje cell layer and the granular layer, numerous beaded CaBP immunoreactive axons surrounded the perikarya of Purkinje cells and coursed in part parallel to the sagittal plane. This appearance corresponds to the descriptions of climbing fibres in the granular layer based on Golgi and Nauta silver impregnation methods (Scheibel & Scheibel, 1954; Szentagothai & Rajkovits, 1959; O'Leary et al. 1968). Nevertheless, most of the CaBP immunoreactive axons observed at the light microscopic level belong to the Purkinje neurons: they are Purkinje recurrent collaterals that originate from the middle and upper granular layers and ascend to the layer of the Purkinje cell bodies. Here they give rise to 2 plexuses of myelinated fibres, a more profuse infraganglionic plexus and a sparse supraganglionic one. The terminal ramifications of Purkinje axon collaterals travel long distances in the parasagittal plane and bear large varicosities (Chan-Palay, 1971). Thus, in general, CaBP labelled climbing fibres cannot be traced into the Purkinje cell layer because the numerous branches of the Purkinje axon collaterals present there are also CaBP immunoreactive.

Our results are in contrast with previous work by Jande et al. (1981), Garcia-Segura et al. (1984), Rogers (1989) and Celio (1990), who could not identify CaBP-stained climbing fibres within the cerebellar cortex. This divergence may depend on differences in the fixation and processing of the tissue and/or in sensitivity between the immunocytochemical methods used. The tissue was Bouin or Carnoy fixed and paraffin embedded in the case of

Jande et al. (1981), Garcia-Segura et al. (1984) and Celio (1990). Celio (1990) used the same monoclonal antibody 300 against CaBP, though highly diluted (up to 1:20000). Jande et al. (1981), Garcia-Segura et al. (1984) and Rogers (1989) employed an antiserum and different detection systems. In contrast, the CaBP staining of axons within the inferior cerebellar peduncles is in agreement with the findings of some authors. These axons belong for the major part to the olivocerebellar projection but can also belong to the spinocerebellar tract, since neurons from which these fibres originate also contain CaBP (Garcia-Segura et al. 1984; Celio, 1990). Wassef et al. (1992) reported the absence of CaBP labelling from the myelinated olivocerebellar bundle in the inferior peduncle. Differences in antibody sensitivity and reduced antibody penetration, probably depending on a different permeabilisation protocol, may be responsible for this divergence in results.

Because of their prevalent cytosolic distribution, calcium binding proteins such as CaBP or parvalbumin are well-known for their ability to confer a Golgi-like labelling to the nerve cells in which they are contained when visualised by immunocytochemistry. Only in few cases has the isolated presence of the calcium binding protein in the axons and synaptic terminals of neurons giving rise to long projections been observed (Celio, 1990; Kosaka et al. 1993). The reverse situation, i.e. of predominant somatic and dendritic compartmentation of calcium binding proteins, has not yet been described for CaBP.

The consequences of the selective expression of CaBP in some neurons, in addition to the basic ubiquitous machinery responsible for the temporal and spatial control of calcium transients across membranes and inside the cytoplasm, are still largely unclear. In particular, the role CaBP may play within the synaptic terminals of these nerve cells has rarely been discussed. CaBP in climbing fibre boutons may well influence calcium-dependent release of excitatory neurotransmitters, or at least affect secondarily connected events, as has been speculated for a related neuronal calcium buffering protein (Scotti & Nitsch, 1991; Scotti et al. 1993). The terminals of the climbing fibres have been indicated as a source of the membrane-permeable second messenger molecule nitric oxide (Southam & Garthwaite, 1993). Nitric oxide is required for the induction of long-term depression of parallel fibre–Purkinje cell synaptic transmission (Shibuki & Okada, 1991). Nitric oxide synthesis is calcium dependent and therefore probably coupled to the influx of calcium into the presynaptic terminal that eventually triggers neurotransmitter release (Garthwaite, 1991). Nitric oxide can inhibit key enzymes and cause free radical formation in ischaemia and epilepsy. The excessive calcium entry into nerve cells, characteristic of these pathological conditions, probably leads to inappropriately increased production of the molecule (Meldrum & Garthwaite, 1990). The calcium buffer CaBP may help to control the synthesis rate of this potentially toxic molecule within synaptic terminals of climbing fibres.

CaBP in inferior olivary neurons

At the light microscopic level CaBP immunoreactive neurons could be demonstrated in all subdivisions of the inferior olivary nucleus of both adult rats and gerbils in accordance with previous work on the distribution of CaBP in the rodent brain (Celio, 1990; Sequier et al. 1991) and on the development of CaBP immunoreactivity in the rat inferior olive (Wassef et al. 1992). This confirms the homogeneity of CaBP expression in the olivocerebellar system throughout species (Jande et al. 1981; Fournet et al. 1986; Rogers, 1989). The contrast to Garcia-Segura et al. (1984) describing only few stained neurons in the inferior olive may again be due to the differences in tissue preparation and the antibody used. Negative structures were encountered under the electron microscope when less superficial ultrathin sections were viewed. In fact, pre-embedding immunocytochemistry in non-solubilised tissue results in labelling restricted to the superficial 1 μm of the section.

Olivary neurons are thoroughly characterised functionally. They exhibit a pacemaker activity which can result in rhythmic firing. The oscillatory behaviour depends on the interplay of different calcium conductances: (1) a high-threshold calcium conductance which does not inactivate for several tens of milliseconds and is responsible for the broad complex spike-like depolarisation, followed by (2) a calcium-dependent potassium conductance which in turn activates (3) a low-threshold calcium conductance which causes a rebound spike to trigger the next sequence (Llinas & Yarom, 1981, 1986). A close overlap between the distribution of CaBP in the CNS and the autoradiographic mapping of the dihydropyridine-sensitive calcium channel has been reported (Celio, 1990). This type of calcium channel is responsible for the high threshold calcium conductance that is the same as the L-type current (Tsien et al. 1988). CaBP seems to modulate the calcium-dependent inactivation of the high threshold calcium conductance (Köhr & Mody, 1991; Lledo et al. 1992). In hippocampal granule cells—excitatory neurons homogeneously ex-

pressing CaBP—the protein is probably responsible for the slow inactivation rate of this current (Köhr & Mody, 1991). A similar action on calcium currents can be speculated for CaBP in the inferior olivary nucleus.

Olivary neurons tend to fire synchronously (Llinas et al. 1974; Llinas & Yarom, 1981) because they are electrically coupled via gap junctions (Sotelo et al. 1974). Like ion channels, gap junctions are gated by different mechanisms including ions, second messengers (Spray & Bennett, 1985) and neurotransmitters (Llinas et al. 1974; Sasaki & Llinas, 1985; Sotelo et al. 1986). A rise in cytosolic calcium results in a decrease in gap junction conductance while phosphorylation of channel proteins by calcium/calmodulin-dependent kinases or protein kinase C can increase or decrease cell coupling (Rose & Lowenstein, 1975; Weiner & Lowenstein, 1983). The presence of CaBP in inferior olivary neurons could therefore be linked to the control of gap junction permeability and thus to functional changes in their coupling state.

In conclusion, we consider CaBP as a reliable marker for the whole olivocerebellar projection in adult rodents that will be useful for further morphological and functional characterisation of the inferior olivary nucleus and its remarkable influence on the cerebellum (Llinas, 1989). Such studies would also help to assess the possibly different roles of CaBP in dendrites and synaptic terminals of neurons whose physiological properties depend on calcium conductances.

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