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CEREBELLAR LOCALIZATION AND COLOCALIZATION OF GABA AND CALCIUM BINDING PROTEIN-D28K

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INTRODUCTION

In the last decade, development of new antibodies and specific binding markers for protein messenger RNA and DNA have greatly increased the ways in which we can study the nervous system. The cerebellum has become a target for many immunocytochemical studies because its neurons immunoreact with antibodies raised against substances of different origin and function. Among other substances identified by these methods in the Purkinje cells (PC) (30, 37, 59, 63, 66, 69, 84) are the inhibitory neurotransmitter aminobutiric acid (GABA) and a 28 Kd vitamine D dependent calcium binding protein called calbindin (104). These have been of particular interest since the possibility of a close relationship between calciproteines and GABA release has recently been proposed (23) and also because the neurotoxicity due to the excitatory amino acids appears to be caused by an excessive influx of calcium (91). We will illustrate here all the localisation and colocalisations of these two substances we have found in our studies in the cerebellum of the rat using the immunohistochemical preparations. We will also discuss the possible functions for the calbindin and its usefulness as a specific cerebellar marker.

The immunohistochemical methods used in this work have been described elsewhere (8, 107).

I. GABA LOCALIZATION.

A. Cerebellar cortex.

In the cerebellar cortex, the PC as well as the Golgi, Basket and Stellate interneurons produce and utilise GABA as the inhibitory neurotransmitter (see 47). They have all been shown to contain glutamic acid decarboxylase (GAD), the GABA synthetising enzyme and GABA transaminase, the GABA catalysing enzyme (7, 24, 25, 75). They also bind antibodies for GABA in the rat (42, 76, 88, 98) and cat (93) and we will call them GABAergic. In addition to these classical inhibitory neurons of the cerebellar cortex, the cells of Lugaro (65) have also been found to contain GABA (4, 42). On the contrary, the granular cells, the mossy fibres, the climbing fibres (CF) afferents as well as the glial cells, have

been found not to bind the antibody. The GABA positive elements of the cerebellar cortex have been described in great detail at the light and electron microscope levels by the cited authors and particularly well by Gabbott *et al.* (42).

Binding of the GABA antibody is not uniform, tending to be most dense in particular regions of the cells. This is illustrated in figure 1 showing that the

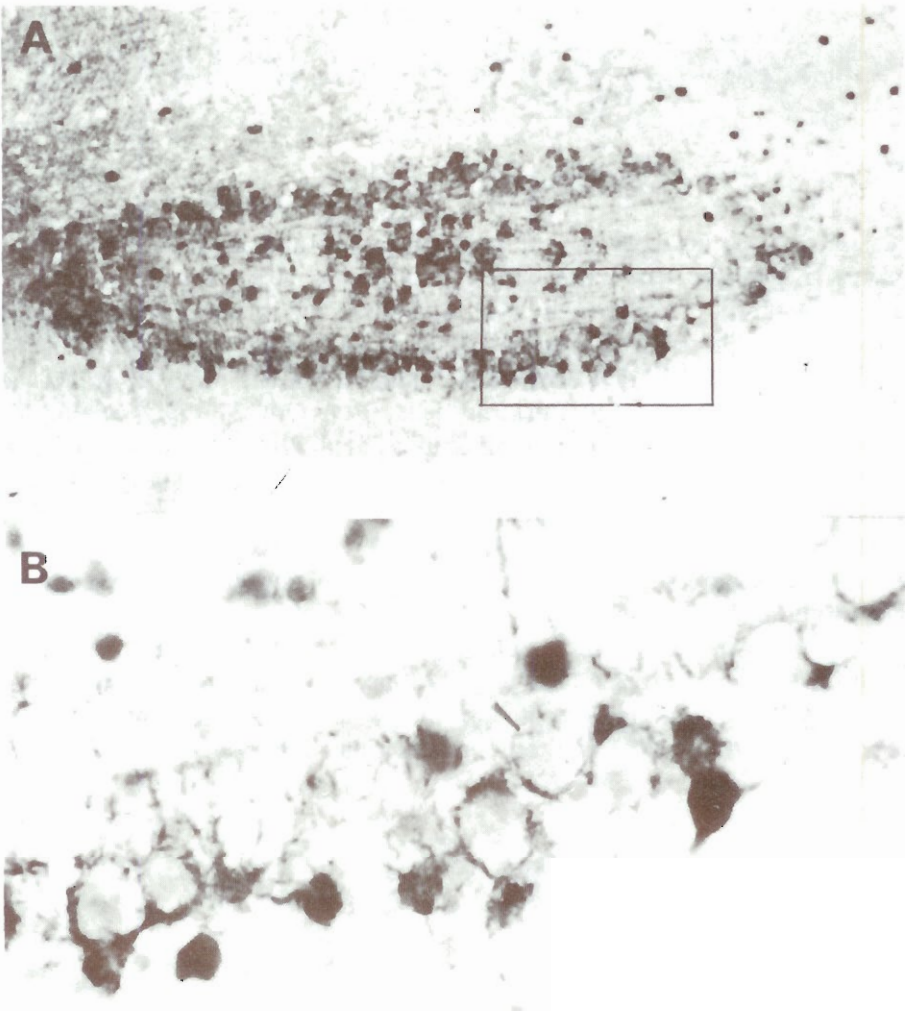

Fig. 1. – *GABAimmunostaining in the cerebellar cortex of the rat.*

A: Photomicrograph of a frontal section of the cerebellum in which one lamella was sectioned tangentially. *B*: Enlargement of the inset of *A* showing the Purkinje cell layer in the central part, the molecular layer in the upper part and the granule cell layer in the lower part. Marked cell bodies in the molecular layer are stellate and basket cells, in the granular layer, they are Golgi cells. The Purkinje cells are seen as empty bodies surrounded by immunopositive boutons and "pinceau". (500 X).

soma of the inhibitory interneurons are heavily stained whereas the soma of PC are not. On the contrary, axon terminals of both interneurons and PC, were always immunopositive. The PC soma were thickly surrounded by heavily stained material, presumably boutons from Basket cells, identified by the "pinceau" shape, and from PC recurrent collaterals (see Fig. 1*B*). In the molecular and granular layers abundantly immunostained material correspond respectively to the stellate and Golgi cell axons and axon terminals.

Interpretation of differential staining must be done with care however, the degree of staining varies following the antisera used and the dilution as discussed extensively by Gabbot *et al.* (42). Care must also be exercised in choosing the source for antibodies: in our hands GABA antibodies from Immuno Nuclear Corporation gave consistent results.

B. Cerebellar nuclei.

The cortex is not the only part of the cerebellum showing GABA immunoreactive neurons. The three pairs of cerebellar nuclei, medialis, interpositus and lateralis, also have neurons staining for GAD (70, 72) and for GABA (8, 22, 58, 71). These are shown in Fig. 2 illustrating the nucleus medialis in a frontal section of a rat treated with GABA antibody. It will be noticed that in addition to immunopositive cell bodies, other immunostained material corresponding to axons and terminals are also present in the cerebellar nuclei. Most of these are obviously PC axons and axon terminals, but processes of other origins cannot be excluded. While axons from GABAergic extracerebellar parent neurons have not been described, some of these processes might belong to intranuclear GABAergic neurons. Using PC degenerated mutant mice, Wassef *et al.* (103) evaluated that 15% of the total number of boutons in the cerebellar nuclei belong to local circuits.

GABAergic neurons are described to be scattered in the cerebellar nuclei (8, 71). They have a large spectrum of sizes, from 5 μm to more than 30 μm (8) and are unimodally distributed with a peak at about 12 μm. While the possibility that these are GABAergic local interneurons has been raised and evaluated (58, 103), some direct evidence exists that many are extrinsic projection neurons. Their target structures have been investigated using double labelling techniques and appear to be the inferior olive (22, 72, 73) and the cerebellar cortex (8). The bulbar reticular formation has been described to be involved but has not been extensively studied (22) and other structures not yet looked for might also be involved. Those neurons projecting to the inferior olive have been shown to be distinct in size and localisation from those projecting to the reticular formation (22) and from those projecting to the cerebellar cortex (8). These latter neurons are medium to large in size with a peak at about 17 μm. Therefore identification of these intracerebellar GABAergic neurons by their size and localization may also be correlated with their projection sites.

Since the early detailed descriptions by Brodal (14) and Jansen and Brodal (50,

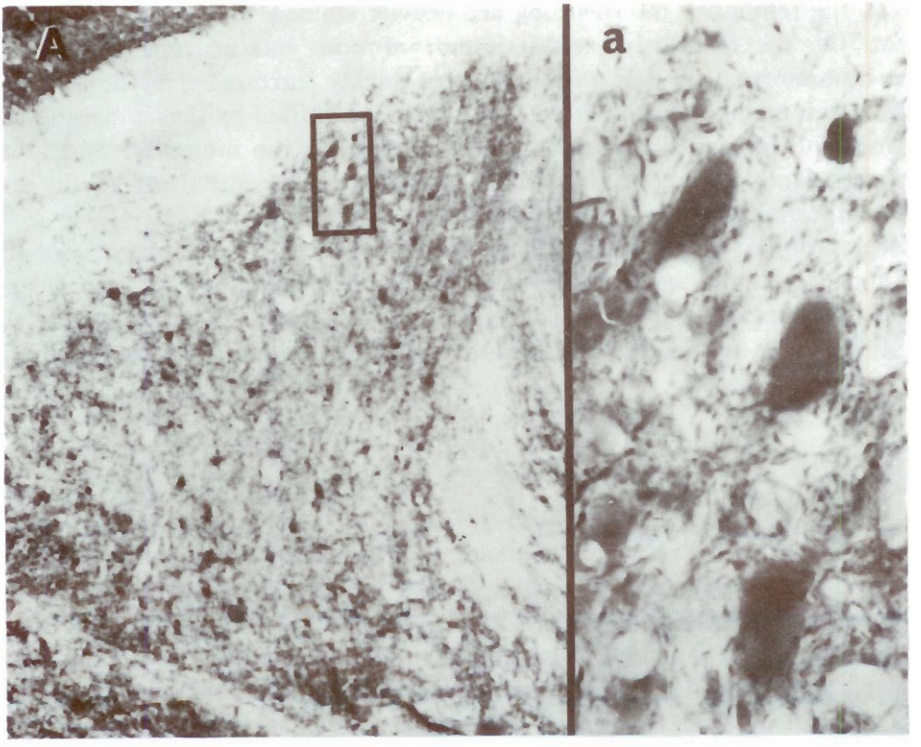

Fig. 2. – *GABAimmunostaining in the cerebellar nuclei of the rat.*

A: Photomicrograph of the nucleus medialis in a frontal section of the cerebellum showing the scattered distribution and the variable size of the GABAergic neurons.
a: Enlarged photomicrograph of the inset of *A*, showing three large sized GABAergic neurons of the nucleus medialis. (600 X).

see 51) numerous subsequent works have shown the sagittal organisation of the projections of the inferior olive to the cerebellar cortex and from the cerebellar cortex to the cerebellar nuclei (see 15). Sagittal zones A, B, C1, C2, C3, D0, D1, D2 have been retained throughout the system in the rat (17, 18, 20). The nucleo-olivary pathway, while projecting to the contralateral inferior olive is also highly organised into sagittal zones (2, 9, 37). The connections from the same sagittal zones in the inferior olive cross over to project back to the original zones in the cerebellar cortex. The striking coincidence and high selectivity of the sagittal zones of these two pathways has recently been stressed (9) therefore showing the existence of an olivo-cerebello-olivary pathway. In addition, it is now demonstrated; *i*) that at least part of the neurons of the nucleo olivary pathway contains GAD (72, 73) and GABA (22); *ii*) that their axons are very likely to terminate mostly on the dendrites, close to the gap junctions of the olivary neurons (3, 94), and *iii*) that the pathway is inhibitory (1).

The nucleo-cortical pathway has also been shown to be essentially organised

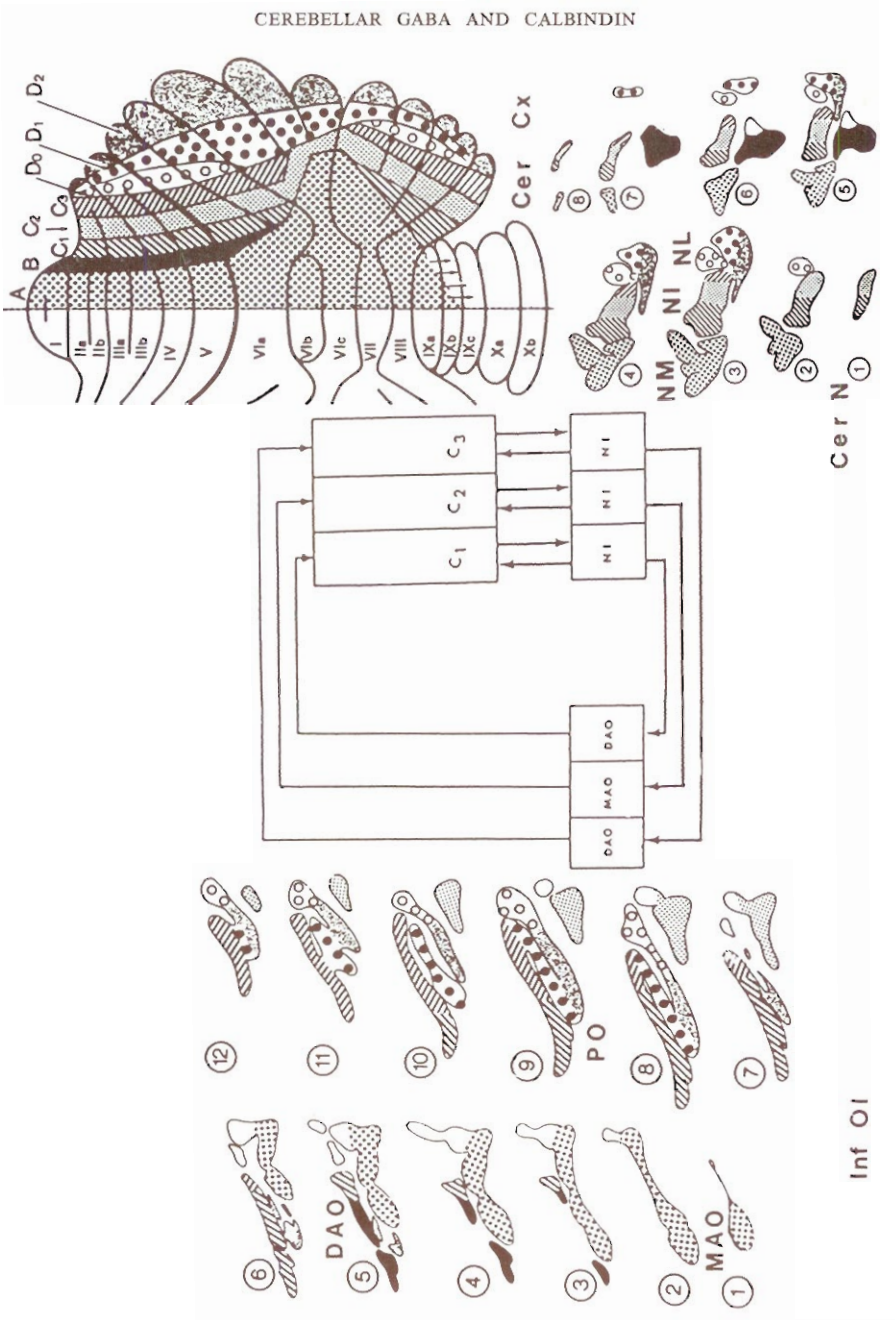

Fig. 3. – *Schematic representation of the GABAergic nucleocortical and nucleoolivary pathways in the rat.*

Somatotopic arrangement in longitudinal zones (A, B, C1, C2, C3, D0, D1, D2) of che cerebellar cortex. (Cer. Cx.), of the cerebellar nuclei (Cer. N.) and of the Inferior Olive (Inf. Ol.), with the same symbols for the same zones (modified from 20). In the central part of the figure the inhibitory feed back loops are schematically represented for the zones C1, C2 and C3, the thin lines representing the excitatory projections and the thick lines the inhibitory projections.

I to X: Larsell's lobules in the cerebellar cortex; NM: nucleus medialis; NI: nucleus interpositus; NL: nucleus lateralis; DAO: dorsal accessory olive; MAO: medial accessory olive; PO: principal olive.

in sagittal zones reciprocal to the sagittal zones of the cortico-nuclear projection, although, minor non reciprocal and symmetrical projections, are also present (19, 21). We have demonstrated that all the projections of the nucleo-cortical pathways originate from GABAergic neurons and are presumably inhibitory (8). We do not know however the function of this pathway nor on which elements of the cortex the axons terminates. This last information is important in view of the fact that the overall activity finally acting on the PCs will be; *i*) an inhibition if the postsynaptic element is a PC itself; *ii*) a disfacilitation if the postsynaptic element is the excitatory granule cells; *iii*) a disinhibition if the postsynaptic element is an inhibitory interneuron of the kind of the stellate and basket cells; *iiii*) a facilitation if the postsynaptic element is an inhibitory neuron of the Golgi cell kind.

It is of interest to note that both the nucleo-olivary and the nucleo-cortical pathways are GABAergic. We therefore admit that the cerebellar nuclei give rise to two inhibitory feedback control systems, one short and intracerebellar, the other long and olivocerebellar. They are schematically represented in Fig. 3 showing that both are somatotopically highly organized in the sagittal plane. The Brodal sagittal organization of the cerebellum is here again confirmed for the nuclear inhibitory pathways.

Although it has been shown that these two pathways are mostly inhibitory (8, 73), an important point, still under study, is to know whether a part of them, even small, may not be excitatory. Another important question to elucidate is whether other target structures exist for the axons of the GABAergic neurons of the cerebellar nuclei and whether collaterals of the already known GABAergic pathways from the cerebellar nuclei have other targets as well. Finally we would like to point out that the feed back loops described above, suppose that the GABAergic neurons of the cerebellar nuclei are innervated by the PCs. While we know (see 47) that PC axons massively terminate upon the neurons of the cerebellar nuclei, a direct demonstration that they innervate the GABAergic neurons projecting to the inferior olive and to the cerebellar cortex do not exist as yet.

II. Calbindin localization.

A. Experiments in vivo.

Several calcium binding proteins have been isolated, some of which, like calmodulin (55, 61), are presumably present in all neurons, whereas others, like parvalbumine or calbindin, have neuron and regional specific localizations. Calbindin is one of the high affinity calcium binding proteins (44), first described in chick intestinal mucosa (105). This protein is found in very large quantity in the cerebellum and kidney but exists also in other tissus (48, 74, 86, 101, 102). Calbindin synthesis is dependent on vitamine D, being triggered by a vitamin D receptor (95). In these cells the calbindin content falls during vitamin D deprivation, but this can be prevented by 1,25- dihydroxyvitamin D (1,25(0H)2D3) administration (102).

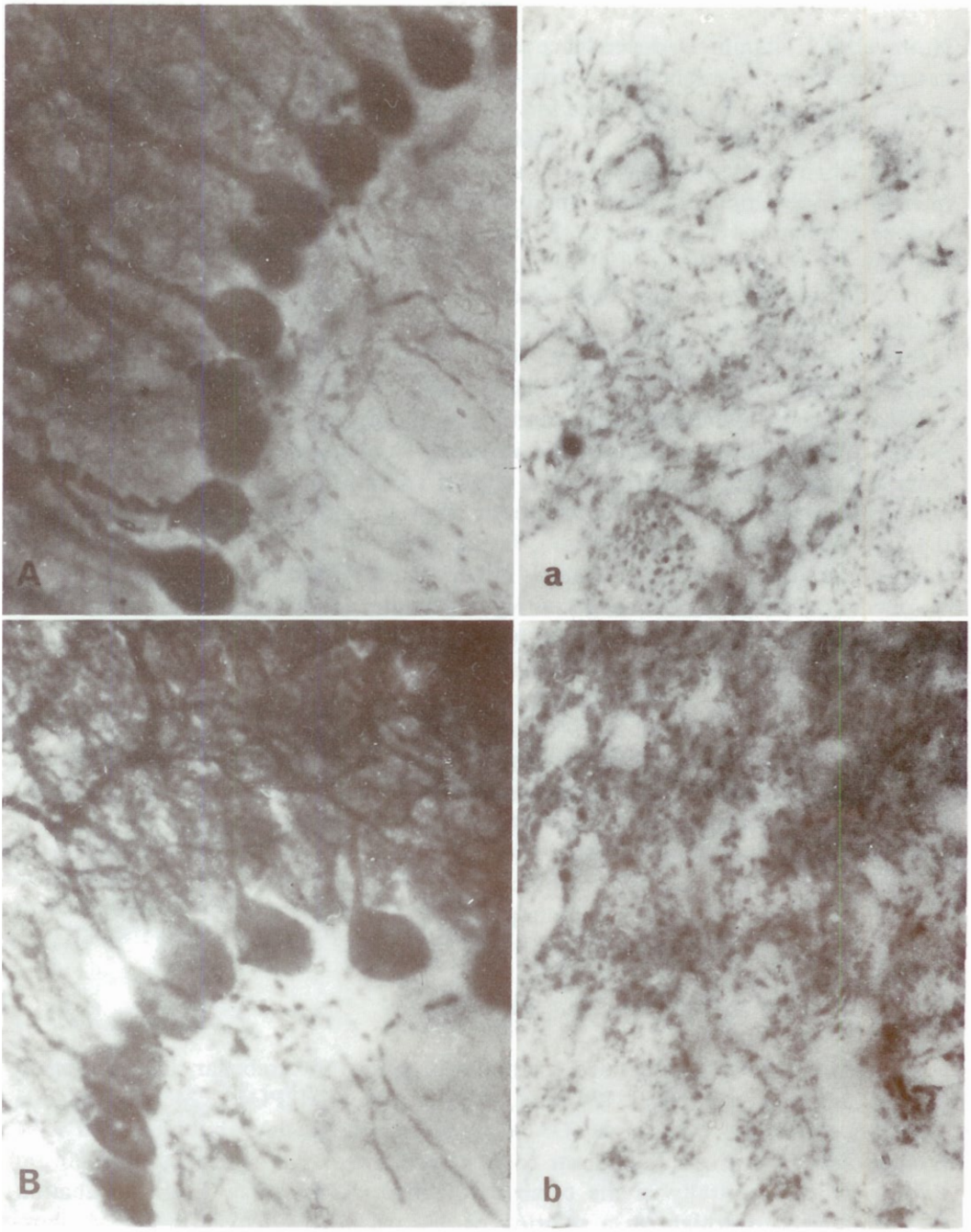

Fig. 4. – *Calbindin immunostaining in the cerebellum of the rat.*

A: Purkinje cells in a sagittal section through the cerebellar cortex in a normal animal. Note the immunostaining of the soma, axon and dendrites. (500 X). *a*: immunostaining of the Purkinje Cell fibers and terminals in a sagittal section through the cerebellar nuclei of the same animal.

B and *b*: same as in *A* and *a* but in a rat in which the inferior olive had been neurotoxically destroyed 24 hours earlier.

However the vitamin D dependence described for kidney and duodenum (95) is not observed in the cerebellum which also lacks the 1,25(OH)2 D3 receptors found in the kidney (28). This difference in synthesis pathways might indicate differences in function for calbindin in the different organs.

Since its first description by Jande *et al.* (49) using specific antibody, increasing interest has developped for the immunohistochemical localization of calbindin in various populations of neurons in the nervous system from fish to mammals including man (5, 6, 16, 32-34, 38-41, 43, 52, 56, 60, 78, 80-83, 85, 87, 96, 99, 108). Most attention however has been given to the cerebellum which has the highest content of this protein with 17.9 ng/mg soluble protein as compared to the second highest 8.6 in the kidney of the rat (107). Furthermore, only the PCs have been found immunoreactive in the cerebellum (48).

Fig. 4*Aa* was obtained from the cerebellum of a normal rat perfused intracardially and treated with antibodies raised in rabbit immunized with purified calbindin from rat kidney (46). It shows in *A* a sagittal section through the cerebellar cortex with PCs intensely marked: the cell body as well as the dendrites, the axon and the axon terminals contain calbindin. It also shows in *a*, a sagittal section through the fastigial nucleus with PC terminals either scattered, and presumably terminating on dendrites, or localized around empty cell bodies. It is worth noting that using counterstaining for Nissl substance, we have observed that all the PCs, are immunopositive since we have not found extra PCs staining for Nissl only. Heterogeneity of labelling in different zones or from one PC to another has been observed but can be attributed to a non homogeneous penetration of the antibody.

Among the brainstem nuclei giving mossy fibre afferents to the cerebellum, many have scattered neurons immunopositive for calbindin but we do not know if they are the neurons projecting to the cerebellum. The neurons of the inferior olive whose axons all terminate in the molecular layer of the cerebellar cortex as CF, are also immunopositive for calbindin (5) but it is not yet clear whether the CF themselves are also immunopositives.

B. The Purkinje cells of the in vitro slices.

The same immunohistochemical method was applied to cerebellar slices incubated in a standard Krebs (NaCl 124 mM, KCl 5 mM, KH2PO4 1.24 mM, MgS04 1.3 mM, CaCl2 2.4 mM, NaHCO3 26 mM, glucose 10 mM) bubbled with 95% Oxygen and 5% carbon dioxide at room temperature. The slices, 300 to 400 μm thick were cut sagittally in the cerebellar vermis. After a few hours incubation they were fixed overnight in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer pH 7,4 with 10% sucrose. They were then frozen and cut again in the same sagittal plane at 20 to 40 μm before being treated immunohistochemically.

The PCs were strongly immunopositive for calbindin with the soma, dendrites, axons and axon terminals very well marked as shown in figure 5. Surprisingly and contrary to our results with the intact perfused animal, the PCs of the in

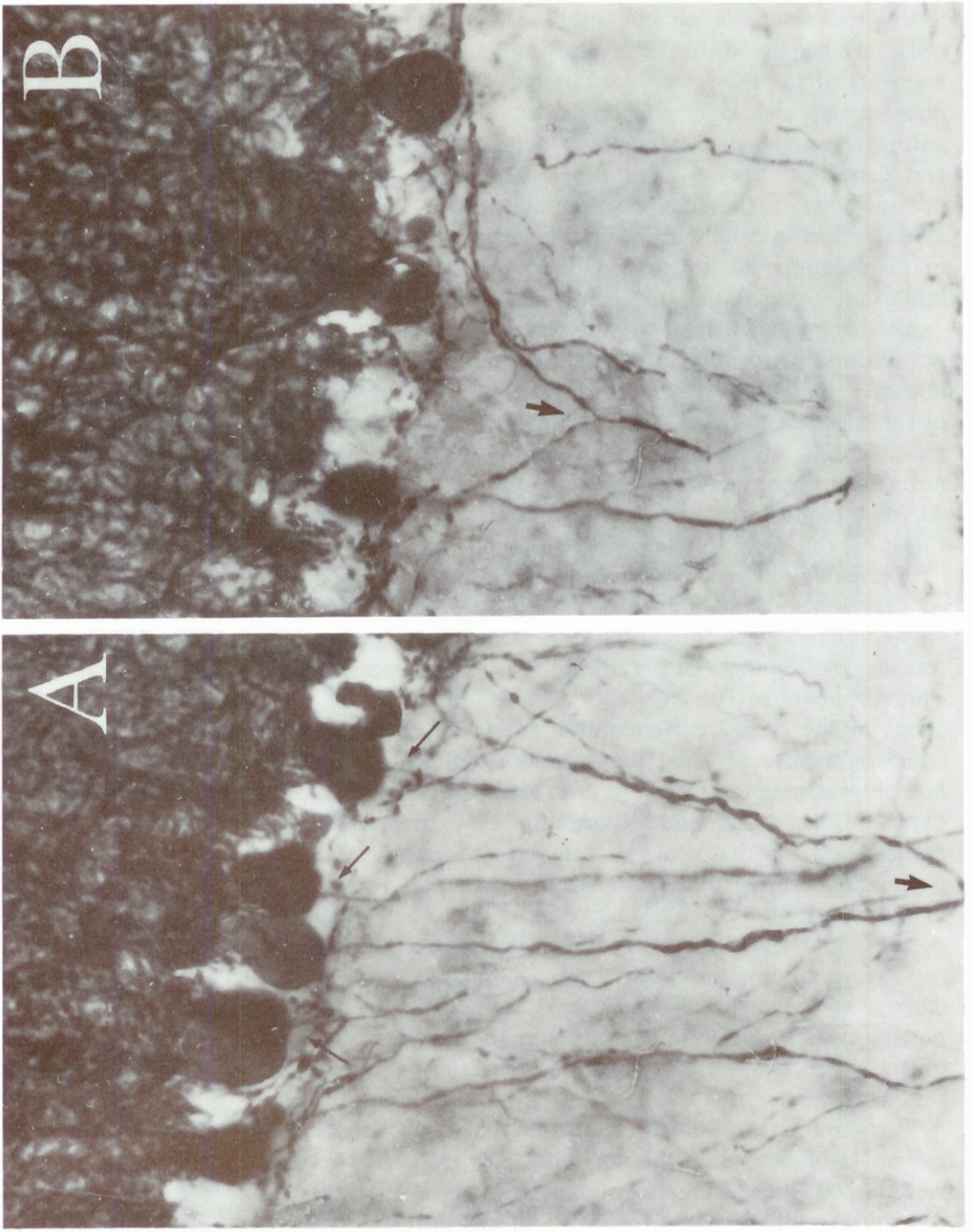

Fig. 5. – *Calbindin immunostaining of the Purkinje cells in a cerebellar slice of rat maintained in vitro.*

Note the intense immunoreaction in the somata, dendrites, axons (thin arrows in *A*) and recurrent collaterals (thick arrows in *A* and *B*). Note also the lack of staining of some of the Purkinje cells in *B*. The slices had been kept in vitro for three hours (see text for details). (600 X).

vitro slices were not all immunopositive. In some lamellae only a few scattered cells were negative (Fig. 5*B* and 6*B* and compare with Figure 4*A*), in others entire segments of the PC rows were missing. Fig. 6*A* shows one such segment in which many axons and axon collaterals, as well as the corresponding supraganglionic and infraganglionic plexus appear clearly marked against the negative PC somata and dendrites. We do not know whether the plexus comes from positive PCs not included in the same section or whether the antibodies were bound to the axons and axon collaterals but not on the soma and dendrites. In fact heterogeneity of the labelling in the soma and dendrites of different PCs was definitely more important then in the preparation from in vivo perfused animals.

An unexpected benefit of this peculiar reduction of marked cells was that it has allowed us to observe isolated individual marked PCs. One of them is shown in fig. 7: it will be appreciated that the entire dendritic tree up to the surface of the lamella and including dendritic tertiary branches are strongly immunopositive for calbindin. This result is important since the presence of Ca^{2+} conductances has recently been demonstrated up to the tertiary dendrites of the PCs (100). At least part of the local need for buffering Ca^{2+} influxes could be easily fulfilled by the local presence of calbindin.

Counterstaining for Nissl substance of these same slice preparations demonstrated that some of the immunonegative PCs for calbindin were also negative for Nissl staining. Therefore absence of immunoreactivity might indicate that damage was incurred by the PCs because of the slicing process. Slicing cuts CF and parallel fibre afferents to the PCs and also will occasionally cut the dendrites and the axons of the PCs. Deafferentation from the cut CF occurs in every PC in slices and would have affected the immunoreactivity of all of them or of none, and not only some of them. It is not likely that a partial deafferentation from the parallel fibres only would change the immunoreactivity of the PC. We cannot exclude however that some PCs undergoing total deafferentation of both climbing and parallel fibres would not survive or would loose immunoreactivity. Absence of the PC immunoreactivity however seems to be peculiar of the in vitro cerebellar slices since it has not been observed in the in vivo perfused cerebella (see under).

Absence of immunoreactivity to calbindin, more likely indicate severe direct damage to the dendrites and the axons of the PCs. Although not excluded, this possibility is reduced to a minimum by cutting the slices in the sagittal plane of the cerebellar vermis where the dendrites and the axons of the PCs lie in the same plane.

We therefore provisionally conclude that "dead injured" PCs are immuno negative to calbindin while the surviving are positive. This is an important point in view of the increasing use of calbindin as a specific marker for the PCs and for other populations of neurons during development (13, 26, 39, 60) and in culture (11, 45, 106). In addition it has been demonstrated that the amount of calbindin is reduced in the cerebellum of the Purkinje cell deficient and nervous mutant mice (77) because the number of PCs is reduced. In these animals the surviving PCs remain immunoreactives for calbindin even if they are morphologi-

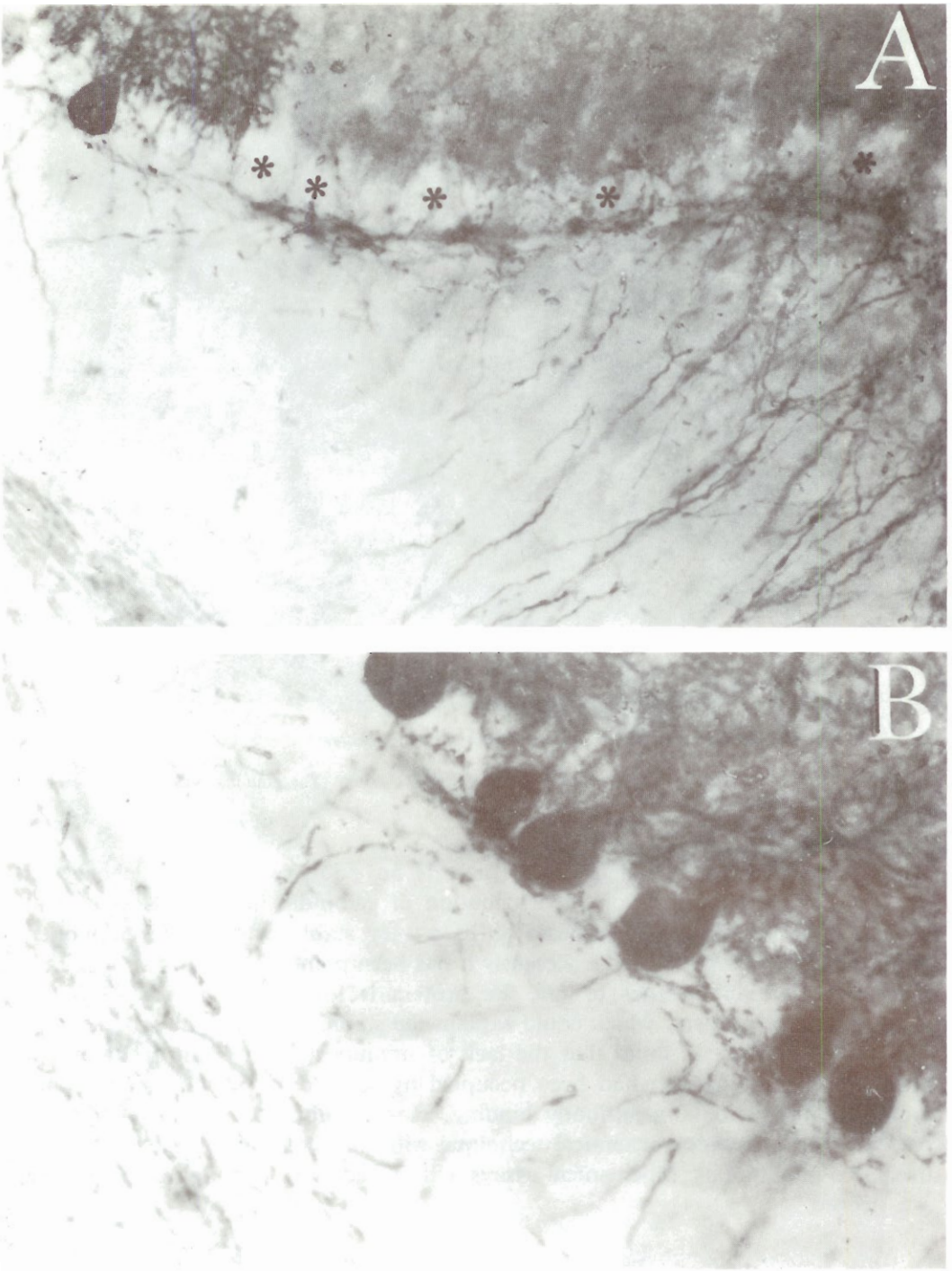

Fig. 6. – *Calbindin non immunoreactive Purkinje cells in cerebellar slices of rat maintained in vitro.*

A: Stars indicate the locations of non-staining Purkinje cell somata. Note the infraganglionic and supraganglionic plexus of the recurrent collaterals. (350 X).

B: Example of isolated non-staining PC. Note the immuno positive boutons of recurrent collaterals against the stained PC soma. (600 X).

Both sections were from a sagittal slice maintained in vitro for three hours.

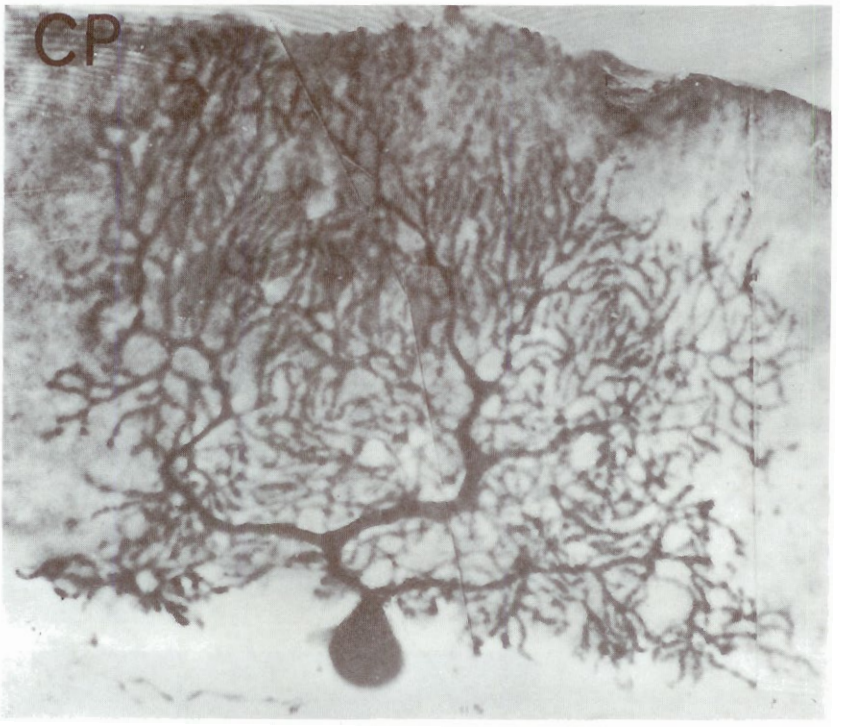

Fig. 7. – *An isolated Purkinje cell of rat calbindin immunopositive.*

Section from a sagittal slice maintained in vitro for three hours. The staining was complete even to the level of the dendritic spines. (500 X).

cally abnormal (77). In other words, there is no evidence as yet for changes in the intracellular concentration or distribution of calbindin in the PCs as a result of manipulation. Calbindin appears to be a very stable cytoplasmic component of the neurons which contain it. Recently it has been pointed out that the antiserum binds to calbindin whether or not the protein is loaded with Ca^{2+} (79). This is an important property which could explain the stability of the immunoreactivity.

It should be kept in mind that the lack of immunoreactivity can occur by two means, either the antigen had been occupied by some other equally high affinity molecule thus preventing antibody binding, or the calbindin is indeed no longer present. The immunocytochemical technique will not allow distinguishing between these two possibilities. Biochemical assays will be necessary if quantitative analyses are needed.

C. Can calbindin content be experimentally modified?

Calbindin is thought to be a cytoplasmic calcium regulating protein because of its high binding affinity for this ion. We have carried out preliminary experi-

ments on rat cerebella deafferented from the CF. Electrophysiological experiments in this preparation in slices have indicated that a consequence of the deafferentation may be a modification of cytoplasmic calcium regulation (53). Figure 4*Bb* shows a sagittal section of the cerebellum of a rat in which CF deafferentation was obtained one day before by systemic injection of 3-acetylpyrydine thus destroying the neurons of the inferior olive (35). It can be seen that the PC soma, dendrites, axons (Fig. 4*B*) and axon terminals (Fig. 4*b*) remained immunoreactive to calbindin. Counterstaining for Nissl substance has demonstrated that all the PCs are immunopositive for calbindin just as they are in the cerebellar cortex of the intact animals (compare Fig. 4*B* with Fig. 4*A*). Parkes *et al.* (77) also did not find discernable changes in the amount of calbindin in the cerebellum of the rat CF deafferented one month before. Nevertheless more work is in progress to detect possible discrete changes not visible with the present technique.

D. Is calbindin immunoreactivity in the cerebellum confined to PCs?

How specific is calbindin as a marker for the PCs in the cerebellar cortex? In our material, a few atypical PCs were occasionally found to be strongly marked (Fig. 8), some of which were reminiscent of the cells of Lugaro (75). However they were very few and mostly localized in lobule X, while at least in the baboon there is one fusiforme neuron, believed to be the cell of Lugaro, for every four PCs (27).

Calbindin immunoreactive glial cells have never been described in the cerebellum, although some ependymal cells facing the ventricule were found stained in the adult rat, and many during early development (39). In hippocampal tissue cultures a significant number of glial cells were found to express calbindin (36). In addition, studies carried out on glial cultures from different parts of the nervous system, including the cerebellum of new born rats, show that astrocytes synthesize calbindin (36). The possibility that glial cells may express calbindin under special conditions has to be taken into consideration indeed, but this should not prevent the use of calbindin as a good marker for PCs. In fact they can easily be distinguished from glial cells by their peculiar morphology, whether they are in intact animals or even in cultures.

Other markers specific for the PCs have been described. Among them the calcitonin gene related peptide (54) and cerebellin (69) are PC specific but differ from calbindin in that they do not invade the entire cytoplasm of the PC and particularly not the axon nor terminals. On the contrary, cGK (3-:5′-phospate-dependent protein kinase) (30) and PEP-19 (68) another calcium binding protein are, like calbindin, found throughout the PC. In addition, cGK has been found only in the PCs and may therefore be a more specific marker for the PC. In conclusion, although cGK remains so far the best marker for the PCs, nevertheless we think that both calbindin and PEP-19 are just as good markers of the PC when dealing with the cerebellum only.

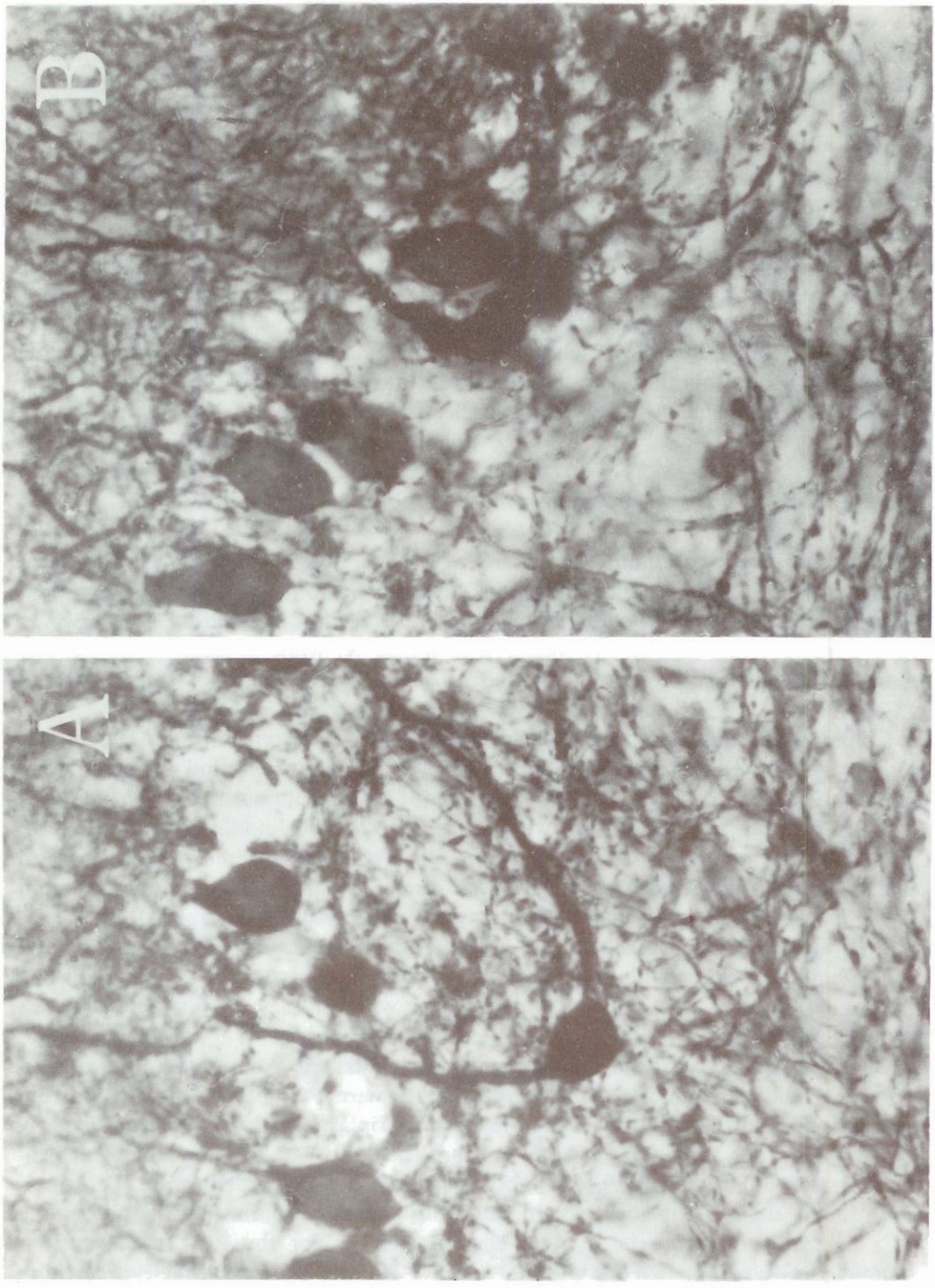

Fig. 8. – *Calbindin immunostaining of atipycal Purkinje cells in a cerebellar slice of rat.*

A and *B* from sagittal slices of cerebellum kept in vitro for three hours. (600 X).

III. Colocalizations.

A. GABA and calbindin.

Among the GABAergic interneurons of the cerebellar cortex, Golgi, Basket and Stellate cells have not been shown to express calbindin at any time from embryo to adult. Our studies revealed that while cells of Lugaro are also GABAergic interneurons, they do not synthesize calbindin. We can therefore conclude that in the cerebellar cortex, colocalization of the two substances only exists in the PC. In fact we have shown that all the intact PCs are immunopositive for both GABA and calbindin. It is interesting to stress that PCs are different from other cortical inhibitory neurons in that they are extrinsic inhibitory neurons. We have reported another example of extrinsic inhibitory neurons with colocalization. They are the neurons of the medial nucleus of the trapezoide body which project to the lateral superior olive and are, nearly all, immunopositive for both GABA and calbindin (107).

In the cerebellar nuclei we have found numerous neurons staining for GABA, but not for calbindin. Nevertheless it is interesting to note that nuclear cells express calbindin transiently during late embryonic development, between E 15 and E 20 (39). Whether these cells will become the extrinsic GABAergic neurons described above, is not known. Colocalization of GAD and calbindin have been found, on the other hand, in the interneurons of the visual cortex in cat (32).

B. GABA and other calcium binding proteins.

A class of calcium binding proteins not considered here are those thought to be directly involved in triggering exocytosis particularly in neurotransmitter release. Such proteins called calectrins, appear to have unique calcium binding domains different from those found in the soluble calcium binding proteins (92).

Colocalization in the PCs of GAD and calcitonin has recently been reported (54). Cerebellin and PEP-19 also specifically mark the PCs and the Cartwheel neurons in the dorsal coclear nucleus and colocalization of both substances with GABA in these two kinds of cells has been also recently reported (68, 69). A calcium binding protein known for a very long time is parvalbumin. In the monkey thalamic nuclei Jones and Hendry (52) have shown that Parvalbumin, but not calbindin, when found is mostly in GABAergic cells. While it has been stated that parvalbumin is almost only present in GABAergic neurons of the cortex (23), of the thalamic nuclei (52) and of the lateral geniculate nucleus (96), this certainly does not appear to be an absolute rule. Broun *et al.* (13) working in the visual system of birds reported no reliable colocalization of GABA with either calbindin or with parvalbumin. In all cerebella studied, parvalbumin is found in the PC as well as in the intrinsic inhibitory neurons of the cerebellar cortex with the exception of the Golgi neurons (12).

C. Functions for the calcium binding proteins.

A large number of possible cytoplasmic calcium buffering proteins has been identified as mentioned previously. Their roles in cell function are thought to be in regulating cytoplasmic calcium because they generally have high binding affinities for calcium (see 10), and most often to regulate transmitter release at the terminals by buffering its calcium level. In a recent extensive work, Pfyffer *et al.* (79) in hypothalamic neurons in culture, show that immunoreactivity of parvalbumin is decreased by depolarization of the cells. In vitro they also show that the binding affinity for antisera is decreased by calcium depletion and increased by calcium loading. This indicates that antiparvalbumin binding is dependent upon the parvalbumin having already bound calcium and could be a useful tool for studying calcium regulation. The same kinds of experiments were not able to show that calbindin antisera binding was also calcium dependent (79). Calbindin has also been proposed to regulate neurotransmitter release (16, 87) but in this case, it seems to have at least one other role since it is expressed during the embryonic stages of development before neurotransmitters appear (39). The same authors propose a role for calbindin in the regulation of calcium during development of the cytoskeleton. A possible role for a calcium binding protein is the maintenance of low cytoplasmic free calcium levels in the nanomols.

CONCLUSIONS

Our experimental work has been with only one of these calcium binding proteins. Our finding that calbindin is very abundantly distributed throughout the cytoplasm of the PC lead us to give it a role in the regulation of intracellular free calcium since the PC are known to have large calcium influxes (29, 53, 62, 67). The PC probably depends also on the other calcium binding proteins in this regulation but it will not be possible to study the combined roles of all at once, we plan to continue to focus our attention only on calbindin for the time. We would like only to recall that messenger RNA for parvalbumine were found to be evenly distributed in the soma and the dendrites of the PC (90), whereas messenger RNA for calbindin were found in the soma only of the PC and of other neurons (31, 64, 89).

The published results cited above seem to indicate that non inhibitory neurons also expressing calbindin, or any of the other putative calcium binding proteins, will necessarily be colocalized with one or the other of the neurotransmitters as well. The fact that the PC, a extrinsic inhibitory neuron colocalize GABA and calbindin offers a tempting opportunity to speculate on the neurophysiological significance of the colocalization. However, the significance of this colocalization probably lies more in the particular physiological properties of these neurons and not with a general property of all neurons.

SUMMARY

Immunocytochemical studies using antibodies raised against the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) and against the 28 Kd vitamin D dependent calcium binding protein (calbindin) in the cerebellum, are reviewed.

The GABA immunoreactive neurones found in the cerebellar cortex were the Purkinje cell (PC), the three classes of intrinsic inhibitory interneurones, stellate, basket and Golgi cells and the cells of Lugaro.

Some of the neurons of the cerebellar nuclei were also found to be GABA immunoreactive. A part of these could be identified as extrinsic neurones projecting either back to the cerebellar cortex, or to the inferior olive, both these pathways being topographically highly organized but arising from independent parent neurons. The presumed inhibitory function of these two pathways are discussed.

Calbindin immunoreactivity in the cerebellum was confined to the PCs, staining concerned the whole cell including soma, branching dendrites, axons and axons terminals. The antibody, which appears to be tightly bound to the PC in vivo, failed to stain some of the PC when cerebellar slices maintained in vitro were studied. The stability of the antigen-antibody binding and the use of calbindin as a marker specific for the PC in the cerebellum, is discussed.

Co-localization of GABA with calbindin as well as with other calcium binding proteins are reported to be found in the PCs. While these co-localizations have led to much speculation, conclusive functional roles for them have not been identified at present.

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