Prenatal Development of Calbindin D-28K in Human Visual Cortex

The distribution of the calcium-binding protein calbindin D-28K (CB) was investigated in human fetal primary visual cortex. CB is present in Cajal–Retzius cells of layer I, in sparse neurons of the ventricular and intermediate zones (VZ, IZ), and in tangential fibres in IZ by 15 weeks (W) of gestation. Cajal–Retzius cells lose their staining by 30W. CB appears in layers II–VI mainly from 26W, following an inside–outside sequence. Until 34W, CB labelling is in somata and neuropil located primarily in layers IVA, IVC and V. Then reactive perikarya and puncta increase in layers II–IVA and deep IB and C, but are reduced in infragranular layers from 34W to term. From 30W positive somata form clusters in the cell-rich bands in layers IV and V and labelled neuropil in layers III and IV has a periodic pattern from 34W. Also from 34W, numerous lightly reactive pyramidal cells are present in layers II to IVA in primary, but not secondary, visual cortex. Our results show precocious expression of CB before full laminar differentiation of the cortex and that some of this expression is transient.

Introduction

The calcium-binding proteins calbindin D-28K (CB) and parvalbumin (PV) have been extensively studied in the mammalian cerebral cortex. In the visual system of primates, including man, they are found in neurons associated with separate signal channels from retina to primary visual cortex (area 17 of Brodmann, 1909), and reveal discrete subpopulations of cortical GABAergic interneurons and structural and functional cortical compartmentation (Celio et al., 1986; Blümcke et al., 1990, 1994; DeFelipe and Jones, 1991; Blümcke and Celio, 1992; Mize et al., 1992; Glezler et al., 1993; Hendry and Carder, 1993; Casagrande, 1993; Gutierrez and Cusick, 1994; Spatz et al., 1994; Johnson and Casagrande, 1995; Yan et al., 1995; Yoshioka and Hendry, 1995; Cao et al., 1996; Yan et al., 1996).

The development of expression of PV and CB has been studied in monkey visual cortex (Hendrickson et al., 1991; Spatz et al., 1994). In macaque, PV-immunoreactive cell bodies appear mainly postnatally and seem to coincide with the onset of visually evoked activity. The expression of CB in neurons of area 17 is earlier than that of PV, but correlates with the time of arrival of thalamocortical afferents (Hendrickson et al., 1991). We have shown that in human visual cortex PV-positive somata begin to emerge in the cortical plate around mid-gestation, and develop mostly during the last trimester, there being a maturer pattern of expression of PV in visual cortex at birth than in monkey (Cao et al., 1996). We now report the prenatal development of CB.

Materials and Methods

Five fetal and two neonatal human brains were used, estimated by maternal history, body and brain weight, crown–rump length and histological features at 15, 20, 26, 30, 34, 38 and 40 weeks (W) of gestation. Four fetuses (15–30W) were obtained at therapeutic termination of pregnancy, and one (34W) from an accident-induced abortion. One neonate (38W) died after a normal but slightly premature delivery, and another full-term neonate (40W) died from respiratory distress. The three youngest fetuses were cases of elective termination and the fourth was performed because of maternal malignant disease. The 38W case was born with a thoracogastroschisis and died on the second day after birth, but the brain had normal macroscopical and microscopic parameters (brain weight, gyral and sulcal pattern, and histology).

All cases were perfused transcardially with 500–1000 ml of saline solution within 4 h of death, followed by 2500–4000 ml of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4, 4°C), and the brains removed and postfixed in the same solution for 2–4 h at 4°C. The occipital lobes were removed and immersed in 30% sucrose in 0.05 M Tris-PBS (TBS, pH 7.6, 4°C) until they sank. Coronal 50 µm thick sections were cut on a cryostat and collected in cold TBS. Selected sections were stained immunocytochemically for CB using a standard avidin–biotin (Vector) protocol. Monoclonal mouse anti-CB antiserum (Sigma) was used diluted at 1:5000. Adjacent sections were stained with cresyl violet for laminar determination, and some immunocytochemically reacted sections were also lightly counterstained.

In order to sample from comparable areas at all ages, two regions were selected for close study: these were the middle of the upper bank of the calcareous sulcus, and the area 17/18 border more anteriorly. Controls were run for non-specific labelling by substituting primary antibody with PBS or normal mouse serum. To control for interindividual variation, some cortex from each case was saved in buffer at 4°C and sections from different cases processed in one session under the same conditions.

Results

We have described the lamination of immature human area 17 elsewhere (Yan et al., 1992; Cao et al., 1996). CB-immunoreactive cells were already present in the cortical primordium in our youngest case (15W). The reactive perikarya were small, lightly to moderately reactive, and scattered in the ventricular, intermediate and marginal zones (VZ, IZ, MZ), but not in the cortical plate (CP) or subplate (SP) (Fig. 1). They were unipolar or bipolar in shape, without particular orientation. Reactive perikarya were densest in MZ (layer I). Distinctly stained tangential fibres were observed in IZ (Fig. 1C) but not other layers, and a few were connected to local positive somata.

By 20W, layers V and VI were differentiated from the deep part of CP (Fig. 2A). CB-reactive somata were mainly distributed in two strata, in MZ and IZ (Fig. 2B,C), though faint staining was found in layers V and VI, representing the beginning of CB expression in CP. Positive somata in MZ and IZ were darkly labelled and of relatively mature morphology: they were mainly multipolar with distinct processes (Fig. 2B,C). The perikarya observed in VZ at 15W had largely disappeared at this time, and tangential fibres in IZ were fewer and lighter.

CB-containing neurons were much commoner in CP by 26W, by when layers IV–VI were identifiable. Positive perikarya were bipolar or multipolar with varying soma sizes and staining

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Cerebral Cortex Jan/Feb 1997;7:57–62; 1047–3211/97/$4.00
Figure 1. Microphotographs of 15 week (W) human fetal area 17 showing (A) laminar organization of the primitive cortical Anlage (Nissl stain). Sparse calbindin (CB)-positive perikarya are found in layer I (or marginal zone) (B) and in intermediate and ventricular zones, but not in the cortical plate and subplate. A few labelled fibres run tangentially in IZ (C). Abbreviations: I, layer I; CP, cortical plate; SP, subplate; IZ, intermediate zone; VZ, ventricular zone. Bars = 500 µm in A; 150 µm in B,C.

Figure 2. (A) Nissl section of area 17 at 20W. Layers VI and V are differentiated. CB-reactive neurons in IZ are darkly stained and mature-looking (B), as are positive Cajal–Retzius cells in layer I (C). (D) CB-positive perikarya with varying soma size and shape, and staining intensity, in layers IV–VI at 26W. Reactive fibres are present in these layers and the subcortical white matter (W). Bars = 150 µm in A, 50 µm in B–D.
intensities, localized in the three deep cortical layers, densest in layer IV, with a few in the underlying white matter (Fig. 2D). Reactive fibres were distributed mainly in infragranular layers and often originated from CB-labelled somata. CB-reactive neurons in layer I were still dark and mature-looking, but their number and the density of their processes had declined. CB-reactive somata and processes were no longer detectable in IZ.

By 30W, all layers and sublayers of adult cortex were differentiated (Fig. 3A). CB-reactive neurons were still more numerous than at 26W, particularly in layers IV–VI (Fig. 3A,B). The somata were distributed in bands. The densest was in layer V and contained large, strongly reactive multipolar somata. The next densest band was in layer IVA, containing CB perikarya of varying size and staining intensity. Between the two, layers IVB and C contained lightly to moderately reactive, relatively small neuronal somata. The positive somata in these bands, especially in IVA and V, formed more or less marked clusters. Immunoreactivity in the neuropil was impressive at this age. The staining of cells and neuropil in layers IVA and V are comparable, and that new somata are scattered in layers II and III compared with 30W. Bars = 100 µm.

Figure 3. (A,B) Laminar distribution of CB-reactive neurons at 30W. All layers of area 17 are identifiable. Cajal–Retzius neurons are hardly seen at this age. CB-reactive neurons are distributed in layers IVA–VI, mostly in three bands. In descending order of numerical density they are in layers V, IVA and IVB/C. Somata and neuropil in these bands present tangential clustering (B). (C) Distribution of CB labelling at 34W. (A) Bands of CB somata and puncta. Note that the staining of cells and neuropil in layers IVA and V are comparable, and that new somata are scattered in layers II and III compared with 30W. Bars = 100 µm.

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CB-reactive somata further had increased in packing density at 34W, with a banding pattern peaking equally in layers IVA and V (Fig. 3D). Both bands also contained many large dark somata and some small to medium-sized lightly reactive cells. Reactive processes, some forming distinct puncta, were also comparable in richness in layers IVA and V. Some large, dark somata were scattered in layers II/III (Fig. 3D). Positive puncta and processes in IVB/C and VI were scarce. Perikarya in IVB/C were mostly small and lightly reactive. Labelled somata in layer VI and subcortical white matter were mostly large and multipolar, and strongly reactive. Somata and puncta in layers IV and V were clustered.

At 38W little change in somata and neuropil reactivity had occurred (Fig. 4A), but by 40W the densest neuropil band occupied layers III and IVA, with a moderately intense one in V (Fig. 4B). Moreover, the neuropil in III/IVA formed distinct reactive patches interspersed with lighter zones. The patches contained more large, dark CB somata than interpatch areas (Fig. 4B). The packing density of CB-reactive neurons was much less in area 18 (Fig. 4C).

At term most CB-reactive neurons were multipolar or bipolar (Fig. 5). However, many lightly stained cells in layers II–IVA were apparently pyramidal neurons (Fig. 5B). A few CB-reactive pyramidal neurons were seen by 34W, increased in number by 38W and were numerous at term. However, they were rarely encountered deep to layer IVA and, although common in area 17, were not found in area 18.
Discussion

Transient Expression in Early Neuronal Elements
We have detected transient expression of CB in early neuronal elements in the cortical primordum before laminar differentiation of CP. These include Cajal–Retzius cells in MZ (layer I), neurons in IZ and VZ, and tangential fibres in IZ. The finding of early expression of calcium-binding proteins in primate layer I has been reported previously (Hendrickson et al., 1991; Spatz et al., 1994; Cao et al., 1996). The later loss of CB reactivity in IZ and VZ may be due to apoptosis, or upward neuronal migration (Huntley and Jones, 1990; Hendrickson et al., 1991; Cao et al., 1996). The tangential fibres in IZ may not be derived from intracortical neurons, but may instead have subcortical origins. Subcortical fibres invade the cortical Anlage before CP differentiates, and ‘wait’ deep to CP, where their interaction with local neurons is important for subsequent cortical connectivity (Shatz and Luskin, 1986; Shatz et al., 1988).

Whether CB-reactive fibres in IZ are related to such waiting axons remains to be determined.

Laminar Pattern and Differentiation
CB expression in human area 17 follows an inside–outside sequence, as does the laminar differentiation of CP (Sauer et al., 1983; Yan et al., 1992; Cao et al., 1996). By term, CB immunoreactivity is localized mainly in three bands, in layers III/IVA, IVC and V. In adult human area 17 CB-labelled somata and neuropil occur in layers II–IVA and in IVC (Hendry and Carder, 1993; Yoshioka and Hendry, 1995). Thus, postnatally, CB reactivity in man is downregulated in infragranular layers and upregulated in supragranular layers. In the monkey there is also reduction of CB reactivity in IV/V after birth (Hendrickson et al., 1991).

Clusters and Patches
Clusters of CB somata and CB and PV neuropil patches are seen...
in primate areas 17 and 18 (Celio et al., 1986; Blümcke and Celio, 1992; Hendry and Carder, 1993; Johnson and Casagrande, 1995).

We detected an uneven distribution of PV somata and puncta in layers IV–VI of fetal human area 17 (Cao et al., 1996). The CB neuropil pattern in III–IVA in human area 17 at term is particularly striking in our study. This non-uniform organization of CB reactivity in prenatal human visual cortex may represent differences in distribution of subpopulations of cortical interneurons and geniculocortical terminals (Cao et al., 1996). In fact, periodicity of PV and CO reactivities are visible in developing visual areas before functionally segregated activity emerges (Hendrickson et al., 1991; Wong-Riley et al., 1993).

Expression in Pyramidal Neurons

CB-reactive pyramidal cells are not a feature of adult human cortex (Hendry and Carder, 1993; Yoshioka and Hendry, 1995). Transient CB expression in cortical pyramidal neurons during development was reported in experimental animals (Stichel et al., 1987; Hendrickson et al., 1991; Alcantara et al., 1993; Hogan and Berman, 1993), and is confirmed here in man. CB expression in pyramidal neurons of fetal human cortex emerges in late gestation (after 30W), is especially pronounced by birth, is present in layers II–IVA, and appears widely in area 17 but not 18. These features suggest that expression of CB in pyramidal cells is not artefactual.

Notes

This study was supported by grants from the Stanley Foundation (European Research Center) (L.J.G.), Human Medical University (Q.L.C.) and the National Educational Committee of China (X.X.Y.). We are grateful to Mr. T.B. Bull for expert technical help.

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