PERINATAL DEVELOPMENT OF RAT CEREBELLUM FROM ADRENALECTOMIZED MOTHERS

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It is well known that glucocorticoids (GCs) play an important role in the control of brain development (1,2) It has been also demostrated their role as proliferation inhibitors (3). The ontogeny of the Gcs receptors (type I and type II) has been established by immunocytochemistry, being the day 15/16 the first when they are detected in the postnatal cerebellum (4).

Because the fetal serum corticosterone (CORT) levels become detectable by day 18 of gestation (5), the rat brain is sensitive to maternal CORT across the placenta during time windows crucial for ontogenetic events. The aim of the present work was to study the effects of lack of maternal adrenal hormones during gestation in the cerebellum of the perinatal stages (E20-P3), by immunohistochemical, electron microscopy technics.

For this purpose, pregnant rats were divided into two groups, one of them was subjected to bilateral adrenalectomy (ADX), while the other received laparatomy only (C). Surgery was performed on the first gestational day under ether anaesthesia. Rats had free access to standard food and tap water supplemented with 0,9% NaCl to ensure optimal survival after ADX. On day 20 of gestation, delivery of all animals was accomplisshed by cesarean section. Each fetus was removed one by one, crio-anaesthetized during 5 minutes and perfused through the left ventricule with a solution of paraformaldehyde 4% for immunohistochemical technic, and paraformaldehyde 4% and glutaraldehyde 2% for electron microscopy method, both of them dissolved in 0,1 M phosphate buffered saline (PBS), ph 7,4. Newborn (P0), P1, P2 and P3 pups were anaesthetized with Nembutal (35ml/Kg) and then perfused with the same fixers.

For the electron microscopic study the cerebellar vermis were processed to obtain blocks, which were trimmed and cut 1μm thick for semithin sections. Therefore, the selected blocks were trimmed again and cut in sections of 40 nm thick.

Calbindin D28K was chosen for the immunohistochemical study, an intracitosolic calcium binding protein, specific for Purkinje cells in the cerebellum. Sagital floatting sections were processed following the PAP and the avidin-biotin complex (ABC) methods. Dilution of the monoclonal primary antibody Calbindin D28K (SIGMA) was 1:2000. All dilutions were made in 0,1 M PBS, pH 7,4. Reactions were revealed using DAB 0,003%. Controls, made by omitting the primary antibody, produced no immunostaining.

Serum corticosterone levels of both mothers and E20 fetuses were determined by radioimmunoassay (RIA) commercial kit. Statistical analysis was performedd using a multifactorial ANOVA, followed by Duncan´s multiple range test. Significant differences were tested with Student´s test.

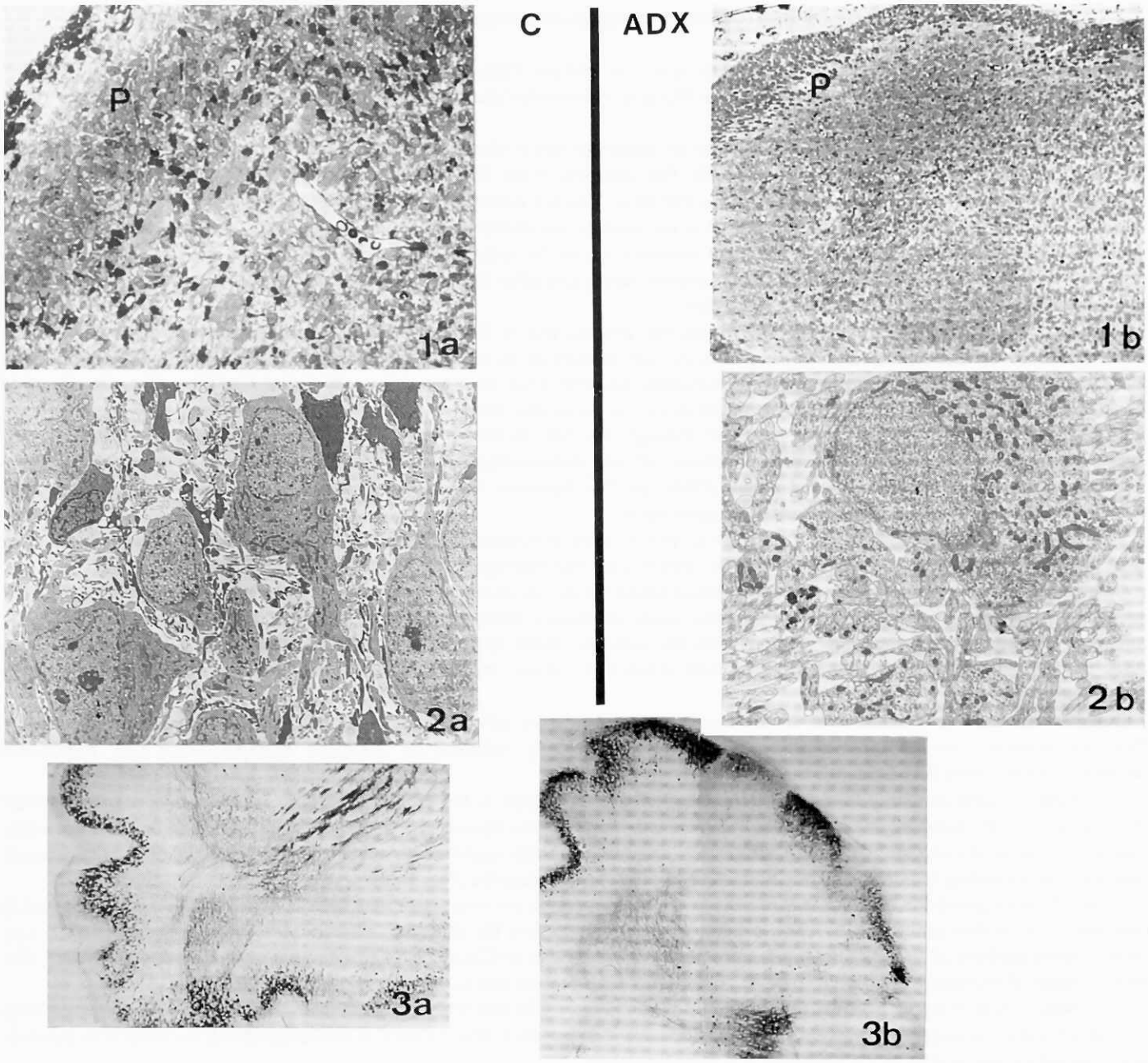
The maternal adrenalectomy leads to a stimulation of mitotic activity in the cerebellar cortex which shows a wide external granular layer, with abundant mitosis, a very thin molecular layer and an appreciable delay in the maturation of the Purkinje cells. Between the layer of irregularly spaced Purkinje cells and the cerebellar nuclei some scattered medium sized-cells, a composite population of migrating Purkinje cells and some Golgi cells, can be observed (Fig. 1a,b).

In the E20 ADX group the total number of cells in the Purkinje layer per cross-sectional area is significantly lower (195±16,83) than that in the control group (248+17,74), but only in its medial portion. No statistical differences were found between ADX and control lateral portions of the Purkinje layer. But due to the cerebellum of E20 ADX fetuses show a marked reduction in size; the mean number of Purkinje cells per 10^3 μm^2 is higher (12,11±1,05) than that in the control group (11,05±1,02).

The precursors of Purkinje neurons are generated between days E13 and E16 with generation peaks at E14-15 and according to a lateromedial neurogenetic gradient (6). It is noticeable that the ADX effect shows a similar gradient, pointing to a putative neuroregulatory role of GCs in the determination of cell numbers.

Electron microscopy confirms the widely immaturity of the cerebellar cortex, being greater in neonates from adrenalectomyzed mothers than in control groups. The cerebellar cortex displays a leaky nervous parenchima without precise lamination. The Purkinje cytoplasm shows an ultrastructure characteristic of intense growth, large accumulation of mitochondria, Golgi apparatus, coated vesicles and multivesicular bodies. The cytoplasm of the mygratory cells has a few organulles, except for a rich accumulation of clusters of free ribosomes (Fig. 2a,b).

These results are in agreement with the immunohistochemical study using Calbindin D28 K. Purkinje cells grow and move to their folia in waves, therefore the folia show patches of calbindin positive Purkinje neurons. This immunoheterogeneity is noticeable during all the perinatal studied period. Positive Purkinje migrating cells reach their layer later in ADX animals in relation to controls. Cerebellum of 1 day postnatal is foliated and the multilayered stratum of Purkinje cells shows a conspicuous stain in the areas, around the primary and secondary fissures, which lie in the presumptively 4-5, 8 and 9 lobules; until P3 C, the same immunoheterogeneity appears in the stratum of Purkinje neurons in the lobules. ADX posnatal group presents a more wide stratum immunostained and also many migrating cells of Purkinje in the white matter displacing toward the cerebellar cortex can be seen (Fig. 3a,b).



1) Semithin sections. Azul Toluidine-Borax.*a*) E20C (X80) *b*) E20 ADX (X50). Note the greatter indiferentiation generalized in the ADX group than that in the Control group.

2) Electron micrographs. *a*) P1C (X1500) *b*) P1ADX (X4400). ADX cerebellum shows a leaky nervous parenchima and Purkinje cells quite immatured.

3) Calbindin D28K immunopositivity in Purkinje cells. *a*) P1C (X20) *b*) P1ADX (X15). Cerebellum from ADX group shows a marked immunoheterogeneity, with many Purkinje migrating cells.

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