DEVELOPMENTAL CHANGES IN TELENCEPHALIC ENKEPHALINERGIC NEURONES

Enrique ECHEVARRIA, Oscar CASIS Juana Maria CLEMENTE, Margarita SILIO and Roberto SERRANO
Department of Physiology, Medical School. University of the Basque Country. Bilbao. Spain.

Endogenous opioid peptides have been shown to be implicated in modulating cardiorespiratory functions as well as other autonomic functions (1) and are believed to play a role in a large number of CNS functions, including pain modulation, endocrine and immune function, ingestive and reproductive activities, learning and motor behavior (2) (3) (4). On the other hand, development of the nervous system is governed in part by these peptides (5). It has been demonstrated that prenatal exposure to opiates affects the development of opiate receptors in the rat brain (6), thus suggesting that initial differentiation of the enkephalin neuron system occurs during the prenatal stage (7). This is the case for the brainstem (8), the hippocampus (9), or the hypothalamus (10). Increasing leu-enkephalin levels are detectable in the cortex of the rat brain from the late embryonic period to P25 (11) and enkephalinergic cells are present in frontal, parietal, occipital and enthorinal cortices and are particularly dense in the cingulate cortex in the adult rat brain (12).

Male Sprague-Dawley rats, in the second (P2) and 20 day of life (P20), bred in our colony and mantained under conditions of controled light (12 h) and temperature (24 ºC), with food and water "ad libitum", were used in this investigation. The animals were anesthesized with Equithensin (0.2 ml/Kg) intraperitoneally and perfused transcardially under deep anesthesia with saline followed by 4 % paraformaldehyde. The brains were removed, cut into smaller pieces and then immersed in the same fixative medium overnight. 60 micrometer sections were cut using a criostatic microtome (Cryocut 3.000, Leica) with an stereotaxic atlas guide (13) and immunostained for leu-enkephalin with polyclonal antisera raised in rabbits.

The antigens were detected by the avidin-peroxidase technique, using 3,3`-diaminobenzidine as chromogen. Following reduction of endogenous peroxidases with 1 % hydrogen peroxide and blocking nonspecific background staining with 5 % normal goat serum (NGS) the sections were incubated with the following immunoreagents: 1-primary antiserum (rabbit anti leu-enkephalin, Chemicon, dilution 1:2.000); 2-goat anti rabbit immunoglobulin (goat antirabbit biotinilated, Chemicon, dilution 1:200); 3-avidin-peroxidase complex (strept ABC complex HRP, Dakopatts); 4-chromogen (3,3`-diaminobencidine, Sigma). Each step was followed by an appropiate wash per triplicate in phosphate buffer saline and 0.3% Triton X100 was used. Sections were carefully extended and mounted (DPX, Fluka), examined with a Olympus BX50 optic microscope and further analyzed with a Leica image analysis system (Quantimet 500 MC), a high speed digital image processing system, for the automatic measurement of densitometric and morphometric parameters.

The microscope image was taken by a black and white video camera and digitized. Measurements for an particular area are performed by tracing contours with a cursor. Light intensity for the microscope was continously controlled, and measurements of perimeter, convex perimeter, area, convex area, equivalent circle diameter, roundness and fullness ratio were made on every slice analyzed, in frontoparietal and cingulate cortex.

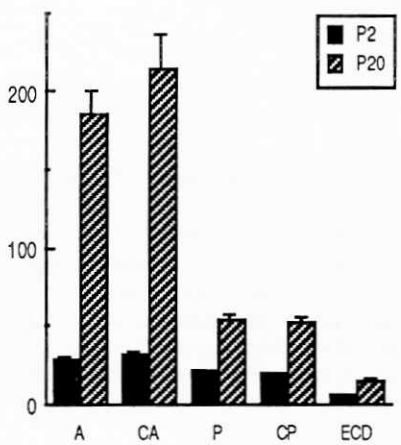

Figure 1

Previous calibration in micrometers was made, considering a pixel as the smallest resolvable area or the element in which the picture is divides for analysis. The parameter "area" is defined as the total number of detected pixels within the selected feature (expressed in micrometers). The parameter "convex area" is the area of the polygon

circumscribing the feature, formed by tangents to its boundary. The variable "perimeter" represents the total length of the boundary of the feature; this is calculed from the horizontal and vertical projections with an allowance for the number of corners.

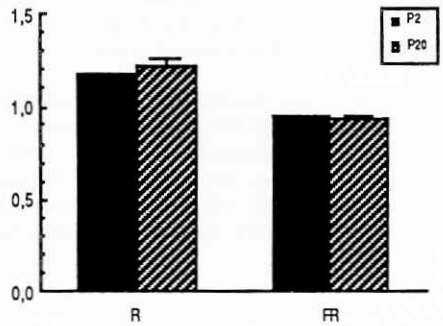

Figure 2

The parameter "convex perimeter" is the length of the polygon circumscribing the feature, formed by tangents to its boundary; this is similar to the length of a piece of string stretched around the feature. "Equivalent circle diameter" represents the diameter of a circle having the same area as the feature. "Roundness" represents a shape factor which gives a minimum value of unity for a circle; this is calculed from the ratio of perimeter squared
to area. The parameter "fullness ratio" is a shape factor equal to the square root of the ratio of area to circumscribed area. Values are represented as the mean ± S.E.M. (Fig. 1, 2)

Leu-enkephalin immunostaining in the cerebral cortex of the rat brain in the second day of life (P2, considering P0 the day of parturition) shows abundant stained cells in the deep layers of the frontoparietal and cingulate cortex. On the other hand, leu-enkephalin immunostaining in the cerebral cortex of the rat in the day of life 20 (P20) reveals the presence of scattered and weakly stained perykaria in more superficial layers of the frontoparietal and cingulate cortex, showing a higher perimeter and area. This distribution pattern is similar to the adult rat.

Values of measured parameters in P2 were the following: area 29.091 ± 1.775, convex area 31.913 ± 2.063, perimeter 21.220 ± 0.681, convex perimeter 20.183 ± 0.633, equivalent circle diameter 6.059 ± 0.182, roundness 1.167 ± 0.017, fullness ratio 0.955 ± 0.004. On the other hand, values of measured parameters in P20 were the following: area 185.464 ± 14.748, convex area 214.813 ± 20.786, perimeter 54.820 ± 2.801, convex perimeter 52.816 ± 2.610, equivalent circle diameter 15.281 ± 0.614, roundness 1.222 ± 0.042, fullness ratio 0.934 ± 0.013. Figures 1 and 2 represent the values of morphometric parameters measured in neuronal perykaria in the frontoparietal and cingulate cortex of the rat brain in P2 and P20. The key for the representation is as follows: A area, CA convex area, P perimeter, CP convex perimeter, ECD equivalent circle diameter, R roundness, FR fullness ratio.

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