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The Role of Gonadal Steroids in Determining Sexual Differences in Expression of C-Fos-Related Antigens in the Rat Striatum

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Expression the the proto-oncogene product, c-fos protein was studied in the dorsal striatum of male and female rats during postnatal development at 20 and 60 days of age. Morphometric analysis revealed sexual dimorphism in the density of c-fos-immunoreactive neurons in 20-day-old prepubertal and 60-day-old pubertal rats. Females showed higher amounts of c-fos-positive neurons than males. The present results suggest that sex differences in the number of c-fos-positive neurons in the rat dorsal striatum may be related to epigenetic effects of gonadal hormones in pre- and pubertal periods of postnatal development.

Key words: c-fos, dorsal striatum, prepubertal and pubertal age, sex differences, rat.

Introduction

Both age and gender were recognized as factors influencing CNS structure and function [10]. Puberty is the attainment of fertility, a process encompassing morphological, physiological and behavioural development [2]. This dynamic period is characterized by marked changes in the activity and connectivity of the brain [9]. On the other hand, the ability of the variety of physiological [3] and pharmacological stimuli to increase neuronal expression of protooncogene c-fos has led to the suggestion that it might serve as a marker of neuronal reactivity [6]. In the light of the above data, the purpose of the present study was to examine the density of c-fos-immunoreactive neurons in dorsal striatum during the postnatal development in male and female rats.

Material and Methods

We used 6 female and 6 male Sprague-Dawley rats to study the c-fos immunoreactivity in the striatum. Male and female rats were sampled over two nested periods, 20 and 60 days of age, representing prepubertal and pubertal age groups, respectively. Animals were anaesthetized with Thiopental (40 mg/kg b. w.). Transcardial perfusion was done with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. Coronal

sections were cut on a freezing microtome (Reichert-Jung) at 40 μm. Sections were incubated in polyclonal primary antibody to c-fos (1:1000 in PBS with 0.3% Triton X-100; Santa Cruz Biotechnology, Santa Cruz, CA) for 24 hs. The sections were then incubated for 1h at room temperature in biotinylated anti-rabbit IgG secondary antibody (1:400 in 0.4% TX-PBS; Vector), washed in PBS, and incubated in ABC solution (1:100 in 0.4% TX-PBS; Vectastain Elite, Vector) for 1h. The sections were finally washed in Tris buffer 0.05 M, pH 7.6, and then immersed in 0.03% 3,3'-diaminobenzidine tetrahydrochloride and 0.003% H_2O_2 to visualize the reaction product.

Morphometric analysis was performed using a microanalysis system (primary magnification 40 ´ objective). Data of the entire drawings were entered in the computer program (Olympus CUE-2), recorded automatically and calculated. Data from males and females were compared by Student's t-test. All values are presented as means ± standard error of the mean (S.E.M.).

Results and Discussion

The principal findings in the present study were as follows. First, c-fos-immunohistochemistry allowed us to determine the specific distribution pattern of reactive neurons (positive cells were identified as those expressing a black nuclear reaction product). The outlines of measures for the density of c-fos-immunoreactive neurons and contours were performing using the computer-assisted program. The average number of c-fos-immunoreactive neurons of 20 days old male and female rats was measured and compared with the same measures of the 60 day old rats (Fig. 1).

Second, our data provide the evidence that there are sex differences in the density of c-fos-immunoreactive neurons of the rat striatum at 20 and 60 days of age. Females have greater density of c-fos-immunoreactive neurons than males in all parts in the dorsal striatum (Fig. 1). There is the greater but not significant expression of c-fos protein in the striatum of pubertal female and male than of that of prepubertal males and female (Fig. 1).

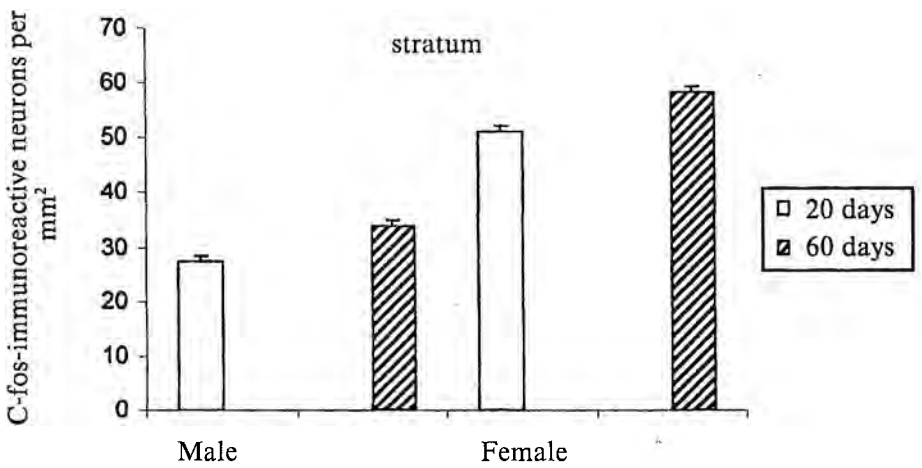

Fig. 1. The number of c-fos-immunoreactive neurons in the dorsal striatum of 20 days and 60 days old male and female rats, $p<0.01$. Values are presented as means ± S.E.M.

These results suggest that sex differences in the density of c-fos-immunoreactive neurons in the dorsal striatum can be related to the epigenetic action of gonadal hormones during the pre-pubertal and pubertal stages of the development. This conclusion corresponds to results that reported such correlation between androgens and expression of different neuroactive substances in various brain regions [1, 2, 4, 5, 7, 8]. However, the exact mechanism, by which sex differences in c-fos-immunoreactivity are settled during the pre- and pubertal development, remains an intriguing question. Our new data emphasize the need to examine the c-fos-immunoreactivity in sectors of the striatum at different days of ages and after experimental manipulations of the hormonal environment.

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