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Research Report

Morphometrical and neurochemical changes in the anteroventral subdivision of the rat medial amygdala during estrous cycle

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ABSTRACT

The anteroventral subdivision of the medial amygdala (MeAV) is one of the vomeronasal structures involved in the control of hormonally dependent behaviors such as sexual and agonistic behaviors in rats. The present study investigates some anatomical and neurochemical parameters of this nucleus (volume, number of neurons, number of glial elements, and of NADPH-diaphorase-positive neurons) in females in two estrous cycle phases (diestrous and estrous) and in males. We also investigate the possible existence of adult neurogenesis in this nucleus in the females. Results showed that volume and estimated number of Nissl-stained neurons in the MeAV vary with the estrous cycle phase: estrous females have greater values than diestrous females. As a consequence of these variations, there is a transient sex difference between males and diestrous females. Two subpopulations of NADPH-diaphorase-positive neurons were detected: intensely stained and medium stained. The intensely stained neurons were more numerous in the estrous than the diestrous females. Neither BrdU nor GFAP inmunostaining revealed significant differences between the two groups, suggesting that adult cell generation, i.e., increases in the number of glial elements, has no significant role in the changes detected in the number of Nissl-stained sections. In conclusion, the MeAV shows functional diergism, due to plastic changes in the female rat brain probably linked to the increase of estradiol during estrous. Finally, these changes are probably functionally related to changes in the behaviors that are controlled through this nucleus.

1. Introduction

The present study is focused on the MeAV subdivision of the amygdala, a nucleus belonging to the vomeronasal system (VNS) and implicated in the control of reproductive and agonistic behaviors (De Olmos et al., 2004; Scalia and Winans, 1975, Canteras and Swanson, 1992; Canteras et al., 1995). This nucleus is characterized by the presence of aromatase activity (Roselli et al., 1984) and of cells expressing mRNA for androgen receptors (Simerly et al., 1990). In

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addition to vomeronasal input, the MeAV is also reached by olfactory input (Licht and Meredith, 1987; Scalia and Winans, 1975) and is therefore considered an integration area (Halpern and Martínez-Marcos, 2003; Licht and Meredith, 1987; Scalia and Winans, 1975).

Several sex differences organized by gonadal steroid levels during early development have been reported in the whole medial amygdaloid nucleus (Me): nuclear volume (Mizukami et al., 1983), intra-amygdaloid synaptic input (Nishizuka and Arai, 1983), and dendritic spine density (Nishizuka and Arai, 1981a,b); and all these parameters are greater in males than in females. Moreover, other studies investigated the presence of similar dimorphisms in the posterior part of Me (Cooke, 2006; Cooke and Woolley, 2005; Hines et al., 1992; Kerchner et al., 1995). However, until now no structural sexual dimorphism has been reported in any part of the Me anterior subdivision.

Many experimental studies have demonstrated that sexually dimorphic structures and/or systems, including Me, can also be influenced by circulating gonadal hormone levels in adulthood (for reviews, see Cooke et al., 1999; Kawata, 1995; Panzica et al., 1995). However, in physiological conditions in rodents, gonadal hormone levels can change sharply within a short time, for example, during the estrous cycle. During diestrous circulating estradiol levels are low, but increase in proestrous and estrous, stimulating some reproduction-related behaviors. Variations in the levels of estrogens during the estrous cycle may induce changes in cell morphology of some brain structures, i.e., the ventromedial hypothalamic nucleus (VMH) (Madeira et al., 2001), or even increase the number of cells, as in the dentate gyrus during proestrous (Tanapat et al., 1999). In the MePD, CCK-I levels fluctuate with the changes in estradiol levels during the estrous cycle, with the highest CCK-I levels occurring during proestrous, when estradiol levels are also increased (Oro et al., 1988).

In a recent study, dealing with estrous cycle-linked changes in the NADPH-d (Nicotinamide adenine dinucleotide phosphate-diaphorase) population of the BAOT (Collado et al., 2003), we observed a large population of nitrergic elements within the MeAV (Collado et al., 2003, see Fig. 1). Therefore, in the present study, we have further investigated their morphological organization, as well as their sensitivity to gonadal hormone fluctuations. The nitrergic system is identified by the presence of the enzyme neuronal nitric oxide synthase (nNOS) or its associated histochemical activity (NADPH-d). In rodents, nNOS immunoreactivity or NADPH-d is widely diffused within the brain (for a recent summary of immuno- and histochemical studies, see Gotti et al., 2004) and is present in nuclei belonging to circuits controlling reproductive behavior (McDonald et al., 1993; Vincent and Kimura, 1992). Gonadal steroids may affect both nNOS expression and NADPH-d activity after both medium- and long-term administration (Ceccatelli et al., 1996; Okamura et al., 1994; Rachman et al., 1998), as well as in physiological situations such as during the estrous cycle (see for a review Panzica et al., 2006). In addition, data collected in estrogen receptor-alpha knockout or in aromatase knockout mice suggest a primary role for estradiol in regulating and/or differentiating the nNOS system (Panzica et al., 2000; Sica et al., 2002).

Here we investigate if MeAV might show sexual dimorphism in some anatomical parameters such as volume and number of neurons and glial cells. In addition, we have also investigated whether the NADPH-d-positive neurons would show sexual dimorphism and/or changes linked to the estrous cycle, in order to find out if there are some functional sex differences (sexual diergism) in the MeAV. Lastly, we investigated the possibility of adult neurogenesis in this nucleus.

2. Results

2.1. *Distribution of NO-producing neurons*

As previously reported (Collado et al., 2003), nNOS- and NADPH-d-positive elements are both abundant in the MeAV (Figs. 1A and B) and the distribution of the two nitrergic markers is strongly similar: an intense staining in both MeAV and BAOT but a very low presence of positive elements in the surrounding areas. Cells in the MeAV show a high affinity for both markers and, at a high power enlargement, many cells can be distinguished. Since NADPH-d staining allowed better identification of the different types of cell populations (see also Collado et al., 2003), we used histochemical staining to investigate quantitative differences between intensely and medium-stained cells in the MeAV.

2.2. *Experiment 1*

2.2.1. *MeAV volume (as detected with Nissl-stained sections)*

No interaction was found between laterality and group [$F(2,15)=2.415$; $P>0.05$] and no differences between hemispheres were detected [$F(1,15)=0.742$; $P>0.05$]. However, a statistically significant effect between the groups was found [$F(2,15)=22.498$; $P<0.05$]. Two by two comparisons between groups revealed the existence of a sex difference between control males and diestrous females, with males having a significantly higher volume than diestrous females (CM vs. DF; $P<0.001$), however, this sex difference was not present when control males and estrous females were compared (CM vs. EF; $P>0.05$) (Fig. 2A). Comparison between the two groups of females indicates the presence of a statistically significant difference, and estrous females have a larger MeAV volume than diestrous females (EF vs. DF; $P<0.001$) (Figs. 1D–E).

2.2.2. *Number of neurons in the MeAV (Nissl-stained sections)*

Analysis revealed no interaction between laterality and group [$F(2,15)=0.108$; $P>0.05$], and no differences between right and left hemispheres in the MeAV [$F(1,15)=0.226$; $P>0.05$]. However, statistically significant differences between groups were found [$F(2,15)=18.322$; $P<0.05$]. When comparisons were carried out among the groups, the results showed that males presented a significantly greater number of neurons than diestrous females (CM vs. DF; $P<0.001$), however, when control males and estrous females were