Sexual dimorphism and diergism of the mammalian central nitric oxide producing systems

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Nitric oxide-producing neurons, identified by the presence of neuronal nitric oxide synthase (nNOS), are widely distributed within the CNS, including regions involved in the control of reproduction and sexual behavior (like the bed nucleus of the stria terminalis (BST), the amygdala, the medial preoptic area (MPA), the mediobasal hypothalamus, or the magnocellular nuclei), where the distribution of nNOS overlaps that of gonadal hormones' receptors [i.e., estrogen receptors (ERalpha and ERbeta), androgen receptors (AR), and progesterone receptors (PR)]. However, only a few studies have detailed the co-expression of nNOS (or the corresponding NADPH-diaphorase activity) with these receptors, and have revealed species-specific and region-specific differences in the proportion of the colocalization.

Overall, these data suggest the existence of significant neuroendocrine relationships among gonadal hormones and nNOS system. Experimental studies have, therefore, been performed. In mammals, sex steroids control the expression of nNOS in the preoptic-hypothalamic region. In the male, castration decreases the number of nNOS-IR neurons in the rat and the hamster MPA. In the female, estradiol (E2) increases, the NADPH–diaphorase staining in the guinea pig ventrolateral nucleus and in the rat paraventricular nucleus (PVN), and MPA. It increases also the nNOS mRNA in the ventrolateral subdivision of the rat ventromedial nucleus (VMH). However, contrasting data were also collected (i.e. no effects of castration or an increase of mRNA for nNOS in the hypothalamus of male rat). These discrepancies in the effects of gonadal hormones on the nNOS could be due to a combination of several factors: differences between species, methodology used, parameter studied or even a regional specificity. In addition, the presence of Norris-IR cells is not always reflecting the same amount of NADPH-diaphorase positive elements as recently demonstrated for the mouse basal forebrain. Differential expression of specific nNOS inhibitors should also be considered in the future to better clarify these relationships.

Estrogen receptors seem to be important also for the differentiation of the limbic-hypothalamic nNOS system. A first study on the distribution of nNOS in male mice knockout for ERalpha (ERaKO) demonstrated significant changes in the limbic-hypothalamic region, when compared to that observed in wild-type mice. However, what we have observed was a nucleus-specific decrease rather than a total disappearance of the system. In particular, a significant decrease in NOS-IR cell number has been observed in PVN and arcuate nucleus (ARC), as well as a significant decrease in the density of NOS immunostained fibres in MPA. Other regions that are important targets for estrogens in females, as the VMH, do not show significant differences. To confirm the important role of estrogens, we observed a significant decrease of nNOS-IR elements in the MPA of aromatase knockout male mice (ArKO). A moderate decrease in immunoreactivity was also detected in the PVN and VMH. Other studies demonstrated that under E2 treatment, the presence of functional AR increases the number of nNOS-IR cells in the posterior ventral region of MeA and in the PVN. In summary, these data suggest that ERalpha and AR interact to regulate nNOS in male and female brain in a site-specific manner.

In general, all the reported studies were based on medium or long treatments with gonadal hormones (from one to several weeks). Obviously, this is a not physiological condition in adult laboratory rodents, where, in the female, the levels of circulating ovarian hormones change in a very short period (the total duration of estrous cycle is 4-5 days). Based on the presumption that gonadal hormones may influence the expression of nNOS, we wondered if short-term changes might influence the number of nNOS-IR elements and if this fact could also influence the demonstration of sexual dimorphism for this system.

In a study performed in mice we considered four hypothalamic and limbic nuclei that are involved in the control of sexual behavior and targets for gonadal hormones: MPA, BST, ARC, VMH. In some of these nuclei (e.g.: MPA, ARC) we observed statistically significant changes in the population of nNOS-IR elements throughout the estrous cycle, whereas, in the other two nuclei (e.g. BST and VMH) we have not detected any statistically significant variation. Changes in the number of nNOS-IR cells in MPA and ARC do not follow the same pattern. In MPA, the highest number of positive neurons was detected during estrus, whereas in proestrus and diestrus we have the lower values. In ARC, the highest number of nNOS-IR
cells was detected in proestrus, this value is significantly different from metestrus and diestrus.

Sex differences were statistically significant only in the BST, with females showing more nNOS-IR cells than males. For the MPA and ARC, in which nNOS-IR varied with the estrous cycle, significant sex differences depended on the phase of the estrous cycle. Therefore, this indicates the presence of a sexual diergism (i.e. functional sex difference) rather than a real dimorphism.

In the rat, variations in expression of nNOS, or associated NADPH-diaphorase activity, during the estrous cycle were studied in two structures belonging to the vomeronasal system: the bed nucleus of the accessory olfactory tract (BAOT) and the anteroventral subdivision of medial amygdala (MeAV). These two nuclei are implicated in the control of reproductive behaviors. In both structures two types of positive neurons were identified: intensely- and medium-stained elements. The sensitivity to E2 of these two subpopulations of NO producing cells may vary depending on the investigated nucleus. In the BAOT, there was a greater density of medium-stained cells in estrous females then in males or diestrous females. However, in the MeAV nucleus the intensely stained cells were the most sensitive group. Estrous females had significantly more NADPH-diaphorase-positive cells than did male and diestrous female. These hormone-dependent fluctuations in NADPH-diaphorase activity suggest that distinct subpopulations in the BAOT-medial amygdala pathway might regulate the expression of reproductive behaviors in which these two structures are involved. As was the case of MPA and ARC in mice, a functional diergism occurs also in rat BAOT and MeAV. Recent data on the lack of effect of T on quail nNOS-IR system suggest that class-specific and species-specific differences may occur, therefore, particular caution is needed to generalize data obtained from studies in rodents. In addition, present data point also to the importance of establishing the precise stage of female cycle when performing studies on the sex dimorphism of neurochemical markers.