IJAE
Vol. 115, n. 1/2: 103-108, 2010
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Effects of estrous cycle and xenoestrogens expositions on mice nitric oxide producing system

Stefano Gotti[1,2], Mariangela Martini[1], Carla Viglietti-Panzica[1,3], Desiree Miceli[1,2], GianCarlo Panzica[*, 1,2,3]

[1] Laboratory of Neuroendocrinology, Department of Anatomy, Pharmacology, and Forensic Medicine, University of Turin, Italy. [*]Corresponding author, Email: giancarlo.panzica@unito.it
[2] Neuroscience Institute of Turin (NIT) – Cavalieri Ottolenghi Foundation
[3] National Institute of Neuroscience-Italy (INN)

Presented at a meeting in honour of Prof. G. Orlandini, Florence, February 15, 2010

Summary

Nitric oxide (NO)-containing neurons are widely distributed within the central nervous system, including regions involved in the control of reproduction and sexual behavior. Nitrergic neurons may co-localize with gonadal hormone receptors and gonadal hormones may influence neuronal NO synthase expression in adulhood as well as during development. In rodents, the female, in physiological conditions, is exposed to short-term changes of gonadal hormones levels (estrous cycle). Our studies, performed in mouse hypothalamic and limbic systems, reveal that the expression of neuronal NO synthase may vary according to the rapid variations of hormonal levels that take place during the estrous cycle. This is in accordance with the hypothesis that gonadal hormone activation of NO-cGMP pathway is important for mating behavior. NO-producing system appears particularly sensitive to alterations of endocrine balance during development, as demonstrated by our experiments utilizing perinatal exposure to bisphenol A, an endocrine disrupting chemical. In fact, significant effects were detected in adulthood in the medial preoptic nucleus and in the ventromedial subdivision of the bed nucleus of the stria terminalis. Therefore, alteration of the neuronal NO synthase expression may be one of the causes of the important behavioral alterations observed in bisphenol-exposed animals.

Key words

NO system, gonadal hormones, estrous cycle, xenoestrogens,

Nitric oxide

Nitric oxide (NO), an inorganic free radical gas synthesized from L-arginine by the enzyme nitric oxide synthase (NOS), is considered a gaseous neuronal messenger (Bredt et al., 1990). Its action takes place primarily by inducing an increase of soluble cyclic guanosine monophosphate (cGMP) in target cells (Miki et al., 1977). NO has been implicated in the regulation of several physiological and behavioral functions (for reviews see Prast and Philippu, 2001; Hull and Dominguez, 2006; Panzica et al., 2006). There are at least three isoforms of NOS: (a) neuronal NOS (nNOS or NOS type I), (b) endothelial NOS (eNOS or NOS type III), both $Ca2^{+}$ dependent, and (c) macrophage NOS (mNOS or NOS type II), $Ca2^{+}$ independent (Alderton et al., 2001). All these isoforms are present within the brain in different cellular compartments, but the neuronal isoform (nNOS) is largely predominant (Bredt et al., 1990).

NO-producing neurons have been localized in several parts of the mammalian and non-mammalian central nervous system (for review see Gotti et al., 2005). In rodents, nNOS-immunoreactive (nNOS-IR) neurons and fibers were described in several hypothalamic and limbic nuclei belonging to neural circuits implicated in the control of reproductive behavior, e.g., medial preoptic nucleus (MPOM), paraventricular nucleus (PVN), supraoptic nucleus, arcuate nucleus (ARC), ventromedial nucleus (VMH), bed nucleus of the stria terminalis (BST), and amygdaloid complex (Hadeishi and Wood, 1996; Collado et al., 2003; Gotti et al., 2005; Carrillo et al., 2007; Sica et al., 2009). Several reports indicate that the involvement of NO in the control of reproductive behavior is modulated by gonadal hormones and probably mediated by interactions with other neurotransmitter systems as dopamine and glutamate (for reviews see: Etgen, 2003; Hull and Dominguez, 2006; Panzica et al., 2006).

Neuronal nitric oxide synthase and gonadal hormones

In several brain regions (like the BST, the amygdala, the preoptic region, the mediobasal hypothalamus, or the magnocellular nuclei) the distribution of nNOS overlaps that of gonadal hormone receptors: estrogen receptor (ER)alpha and ERbeta (Merchenthaler et al., 2004), androgen receptors (AR), (Simerly et al., 1990), and progesterone receptors (PR), (Lauber et al., 1991).

The percentage of co-localization is highly variable, ranging from 90% cells double positive for nNOS and ERalpha in MPOM to 10% in PVN. AR co-localizes with nNOS in a more limited number of cells in the same nuclei (ranging from 20% in MPOM, to 6% in BST) (Scordalakes et al., 2002).

Some studies demonstrated that long-term exposure to estrogens or androgen may affect the expression of nNOS (Ceccatelli et al., 1996; Du and Hull, 1999; Putnam et al., 2005). However, in adult female rodents the levels of circulating ovarian hormones change in a very short period (the total duration of estrous cycle is 4-5 days). Therefore, 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.

Thus, in different studies performed in mice (Gotti et al., 2009; Sica et al., 2009) we considered several hypothalamic and limbic nuclei that are involved in the control of sexual behavior and express gonadal hormone receptors. In some of these nuclei (e.g.: MPOM, ARC, hippocampus) we observed statistically significant changes in the population of nNOS-IR elements throughout the estrous cycle, whereas in other nuclei (e.g. BST and VMH) we have not detected any statistically significant variation. Changes in the number of nNOS-IR cells in MPOM, ARC, and hippocampus do not follow the same pattern with each other. In MPOM, the highest number of positive neurons was detected during estrus, whereas in proestrus and diestrus we observed the lowest values (Fig. 1). On the contrary, in ARC and hippocampus, the highest number of nNOS-IR cells was detected in proestrus and metestrus.

Sex differences were statistically significant only in the BST, with females showing more nNOS-IR cells than males. In MPOM and ARC, where nNOS-IR varied with the estrous cycle, significant sex differences were observed only in relation to specific phases of the estrous cycle. Therefore, this indicates the presence of a sexual dier-

gism (i.e. functional sex difference, Rhodes and Rubin, 1999) rather than of a real sex dimorphism. Similar changes, when comparing estrus and diestrus, were observed also in rat bed nucleus of the accessory olfactory tract (Collado et al., 2003) and medial amygdala (Carrillo et al., 2007).

Effects of bisphenol A administration

Several observations have shown that early exposure to some industrial pollutants (endocrine disrupting chemicals, EDCs) can induce adverse effects on endocrine structures development, and therefore may affect humans as well as farm and wildlife animals (Colborn et al., 1993). Many EDCs are able to bind estrogen receptors and have been therefore classified as environmental estrogens or xenoestrogens (XEs) (Singleton and Khan, 2003).

One worldwide diffused EDC is bisphenol A (BPA), a monomer used in the manufacturing of polycarbonate, epoxy and polyester-styrene resins largely used in food containers (Brotons et al., 1995). BPA can bind to both isoforms of the estrogen receptor (ERalpha and ERbeta) (Kuiper et al., 1997), in addition it shows antiandrogenic activity (Kruger et al., 2008).

Given the well-known ability of gonadal hormones to affect sexual differentiation of the brain during a critical period, i.e., perinatal life in rodents (McCarthy, 2008), it is not surprising that some behaviors, like mating behavior, can be affected by perinatal exposure to EDCs, in particular to BPA. In our laboratory, we applied a procedure that allowed easy oral administration of controlled amount of the EDCs to the pregnant/lactating female from gestation day 11 to postpartum day 8 (perinatal exposure) (Palanza et al., 2002). Their offsprings were tested, when adults, for different behaviors, and we investigated some behaviorally relevant neural circuits in adulthood (Panzica et al., 2007; Panzica et al., 2009).

In particular, BPA selectively alters the NO producing system at the level of the MPOM and of the BST (Martini et al., 2010), two key nuclei for the control of reproductive behaviors. These data confirm that the NO system is a target for estrogens during development and, therefore, a potential and important target for the destructive action of EDCs in mammals. In addition, the alterations of nNOS system may be the basis of the less efficient socio-sexual behavior that has been reported for BPA-exposed male rats (Farabollini et al., 2002).

Conclusions

Data reported in this review indicate that the nNOS system is regulated both during development and in adulthood by gonadal hormones. Previous studies have only pointed to the medium- or long-term effects of gonadal hormones in experimental conditions (i.e. gonadectomized animals), our results indicate that changes in the expression of nNOS may take place also in physiological conditions (i.e. intact animals) following changes in hormonal levels during the estrous cycle. Noticeably, short-term effects may differ from long-term ones (VMH nucleus is not affected by estrus cycle, but is affected by long term exposure to estradiol, Ceccatelli et al., 1996;

Sica et al., 2009). In addition, the knowledge of these changes in physiological conditions is important to differentiate the presence of a real sex dimorphism in nNOS circuits in opposition to transient activational effects. Finally, the extreme sensitivity of this system to gonadal hormones and the large amount of functions that are modulated by NO determine the high vulnerability of these circuits to the exposure to EDCs during the perinatal period and the destructive effects of this exposure demonstrated in laboratory animals (Panzica et al., 2007; Panzica et al., 2009).

Acknowledgements

These studies have been supported by grants of the University of Torino, Regione Piemonte-Ricerca sanitaria finalizzata, and Fondazione San Paolo (Progetto Neuroscienze).

References

Alderton W.K., Cooper C.E., Knowles R.G. (2001) Nitric oxide synthases: structure, function and inhibition. Biochem. J. 357: 593-615.

Bredt D.S., Hwang P.M., Snyder S.H. (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347: 768-770.

Brotons J.A., Olea-Serrano F.F., Villalobos M., Pedraza V., Olea N. (1995) Xenoestrogens released from lacquer coatings in food cans. Environ. Health Perspect. 103: 608-612.

Carrillo B., Pinos H., Guillamon A., Panzica G.C., Collado P. (2007) Morphometrical and neurochemical changes in the anteroventral subdivision of the rat medial amygdala during estrus cycle. Brain Res. 1150: 83-93.

Ceccatelli S., Grandison L., Scott R.E.M., Pfaff D.W., Kow L.M. (1996) Estradiol regulation of nitric oxide synthase mRNAs in rat hypothalamus. Neuroendocrinology 64: 357-363.

Colborn T., vom Saal F.S., Soto A.M. (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101: 378-384.

Collado P., Guillamon A., Pinos H., Perez-Izquierdo M.A., Garcıa-Falgueras A., Carrillo B., Rodrıguez C., Panzica G.C. (2003) NADPH-diaphorase activity increases during estrous phase in the bed nucleus of the accessory olfactory tract in the female rat. Brain Res. 983: 223–229.

Du J., Hull E.M. (1999) Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydroxylase. Brain Res. 836: 90-98.

Etgen A.M. (2003) Ovarian steroid and growth factor regulation of female reproductive function involves modification of hypothalamic alpha 1-adrenoceptor signaling. Ann. N. Y. Acad. Sci. 1007: 153-161.

Farabollini F., Porrini S., Della Seta D., Bianchi F., Dessi-Fulgheri F. (2002) Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. Environ. Health Perspect. 110 Suppl 3: 409-414.

Gotti S., Sica M., Viglietti Panzica C., Panzica G.C. (2005) The distribution of nitric oxide sythase immunoreactivity in the mouse brain. Microsc. Res. Techn. 68: 13-35.

Gotti S., Martini M., Pradotto M., Viglietti-Panzica C., Panzica G.C. (2009) Rapid changes on nitrinergic system in female mouse hippocampus during the ovarian cycle. J. Chem. Neuroanat. 38: 117-123.

Hadeishi Y., Wood R.I. (1996) Nitric oxide synthase in mating behavior circuitry of male Syrian hamster brain. J. Neurobiol. 30: 480-492.

Hull E.M., Dominguez J.M. (2006) Getting his act together: Roles of glutamate, nitric oxide, and dopamine in the medial preoptic area. Brain Res. 1126: 66-75.

Kruger T., Long M., Bonefeld-Jorgensen E.C. (2008) Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology 246: 112-123.

Kuiper G.G., Carlsson B., Grandien K., Enmark E., Haggblad J., Nilsson S., Gustafsson J.A. (1997) Comparison of ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138: 863-870.

Lauber A.H., Romano G.J., Pfaff D.W. (1991) Gene expression for estrogen and progesterone receptor mRNAs in rat brain and possible relations to sexually dimorphic functions. J. Steroid Biochem. Mol. Biol. 40: 53-62.

Martini M., Miceli D., Gotti S., Viglietti-Panzica C., Fissore E., Palanza P. Panzica G.C. (2010) Effects of perinatal administration of bisphenol A on the neuronal nitric oxide synthase expressing system in the hypothalamus and limbic system of CD1 mouse. J. Neuroendocrinol., in press.

McCarthy M.M. (2008) Estradiol and the developing brain. Physiol. Rev. 88: 91-134.

Merchenthaler I., Lane M.V., Numan S., Dellovade T.L. (2004) Distribution of estrogen receptor alpha and beta in the mouse central nervous system: in vivo autoradiographic and immunocytochemical analyses. J. Comp. Neurol. 473: 270-291.

Miki N., Kawabe Y., Kuriyama K. (1977) Activation of cerebral guanylate cyclase by nitric oxide. Biochem. Biophys. Res. Commun. 75: 851-856.

Palanza P.L., Howdeshell K.L., Parmigiani S., vom Saal F.S. (2002) Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ. Health Perspect. 110 Suppl 3: 415-422.

Panzica G.C., Viglietti-Panzica C., Sica M., Gotti S., Martini M., Pinos H., Carrillo B., Collado P. (2006) Effects of gonadal hormones on central nitric oxide producing systems. Neuroscience 138: 987-995.

Panzica G.C., Viglietti-Panzica C., Mura E., Quinn M.J.Jr., Palanza P., Ottinger M.A. (2007) Effects of xenoestrogens on the differentiation of behaviorally relevant neural circuits. Front. Neuroendocrinol. 28: 179-200.

Panzica G.C., Mura E., Miceli D., Martini M., Gotti S., Viglietti Panzica C. (2009) Effects of xenoestrogens on the differentiation of behaviorally-relevant neural circuits in higher vertebrates. In: Vaudry H., Rubois E., Coast G.M., Vallarino M., editors. Trends in Comparative Endocrinology and Neurobiology. New York, NY: New York Academy of Sciences. p 271-278.

Prast H., Philippu A. (2001) Nitric oxide as modulator of neuronal function. Progr. Neurobiol. 64: 51–68.

Putnam S.K., Sato S., Riolo J.V., Hull E.M. (2005) Effects of testosterone metabolites on copulation, medial preoptic dopamine, and NOS-immunoreactivity in castrated male rats. Horm. Behav. 47: 513-522.

Rhodes M.E., Rubin R.T. (1999) Functional sex differences ('sexual diergism') of central nervous system cholinergic systems, vasopressin, and hypothalamic-pituitary-adrenal axis activity in mammals: a selective review. Brain Res. Rev. 30: 135-152.

Scordalakes E.M., Shetty S.J., Rissman E.F. (2002) Roles of estrogen receptor alpha and androgen receptor in the regulation of neuronal nitric oxide synthase. J. Comp. Neurol. 453: 336-344.

Sica M., Martini M., Viglietti-Panzica C., Panzica G.C. (2009) Estrous cycle influences the expression of neuronal nitric oxide synthase in the hypothalamus and limbic system of female mice. BMC Neurosci. 10: 78 (01-14).

Simerly R.B., Chang C., Muramatsu M., Swanson L.W. (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294: 76-95.

Singleton D.W., Khan S.A. (2003) Xenoestrogen exposure and mechanisms of endocrine disruption. Front. Biosci. 8: s110-s118.

Figures

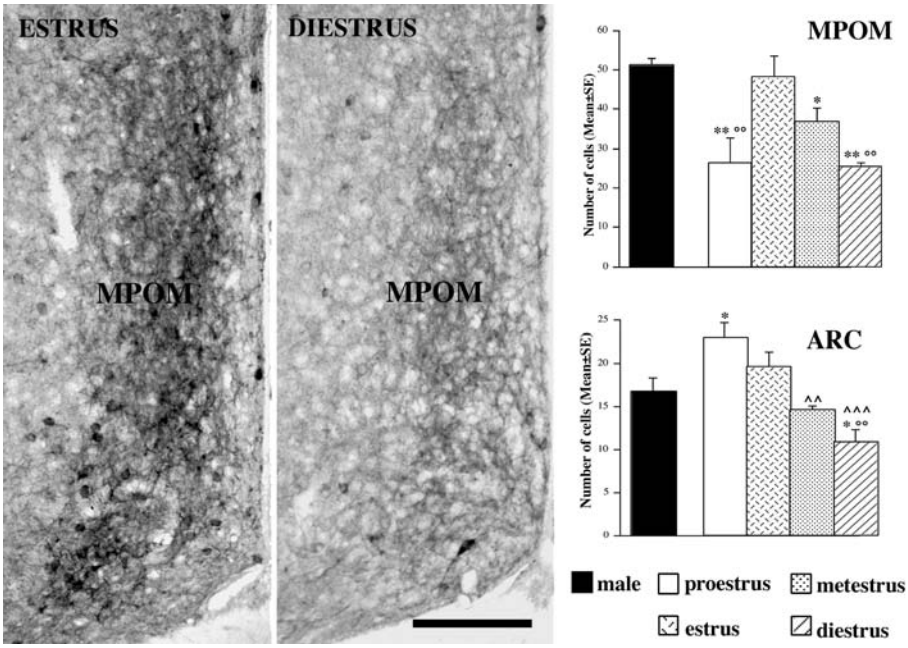

Fig.1 Left panels: micrographs illustrating the nNOS-immunoreactive population within the medial preoptic nucleus (MPOM) of female in different stages of the cycle (estrus and diestrus). Scale bar: 150 μm. Right panels, histograms reporting the differences in nNOS-immunoreactive cell number (mean ± standard error) during female estrous cycle in the MPOM and the arcuate nucleus (ARC). Black histograms represent male mice as comparison.

Asterisks indicate significant differences (Fisher PLSD test). * $p<0.05$, ** $p<0.01$ in comparison to male, °° $p<0.01$ in comparison to estrus female, ^^ $p<0.01$, ^^^ $p<0.001$ in comparison to proestrous female.