Nitric Oxide at the cross-road of immunoneuroendocrine interactions

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| Complete List of Authors: | Rettori, Valeria; School of Medicine, University of Buenos Aires, CEFYBO
Fernandez-Solari, Javier; School of Medicine, University of Buenos Aires, CEFYBO
Mohn, Claudia E.; School of Medicine, University of Buenos Aires, CEFYBO
Zorrilla Zubilete, Maria A.; School of Medicine, University of Buenos Aires, Neuropharmacology
Prestifilippo, Juan Pablo; School of Medicine, University of Buenos Aires, CEFYBO
de la Cal, Carolina; School of Medicine University of Buenos Aires, CEFYBO
De Laurentiis, Andrea; School of Medicine University of Buenos Aires, CEFYBO |
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Authors: Valeria Rettori\textsuperscript{a}, Javier Fernandez-Solari\textsuperscript{a}, Claudia Mohn\textsuperscript{a}, María A Zorrilla Zubilete\textsuperscript{b}, Carolina de la Cal\textsuperscript{a}, Juan Pablo Prestifilippo\textsuperscript{a}, Andrea De Laurentiis\textsuperscript{a}

Affiliations: Center of Pharmacological and Botanical Studies, School of Medicine, University of Buenos Aires, Paraguay 2155 16 floor, Buenos Aires, Argentina\textsuperscript{a}. Department of Neuropharmacology, School of Medicine, University of Buenos Aires, Paraguay 2155 15 floor; Buenos Aires, Argentina\textsuperscript{b}.

Corresponding author: Dr. Andrea De Laurentiis, Centro de Estudios Farmacológicos y Botánicos, Facultad de Medicina, Paraguay 2155 piso 16, Ciudad Autónoma de Buenos Aires, AR 1121ABG, Buenos Aires, Argentina. Phone: 54-11-4508-3680 int 112. Fax: 54-11-4508-3680 int 106. andredelaurentiis@yahoo.com.

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ABSTRACT

Nitric Oxide (NO) was initially described as a mediator of endothelial relaxation, now its participation is recognized in numerous physiological and pathological processes. It was demonstrated that LPS-stimulated CRH release involves NO production. Furthermore, it has been shown that IL-1, TNF-α, IL-6 and IL-2 can stimulate ACTH release from anterior pituitary via NO. Also, we found that NO released from hypothalamic NOergic neurons in response to NE diffuses to LHRH neurons where activates COX and GC with the consequent increase of PGE2 and cGMP, respectively, that lead to the exocytosis of LHRH granules. During pathological conditions, such as manganese intoxication, NO production is increased leading to increase in LHRH secretion that can advance puberty. In another study we demonstrated that NO reduces oxytocin, as well as vasopressin secretion from the posterior pituitary suggesting its modulatory role during dehydration. There was found an increase in NOS activity and protein in hippocampus and cerebellum in offspring of rats that were submitted to prenatal stress that correlated with behavioral changes in adults. Also NO participates in signal transduction pathways in peripheral tissue in physiological conditions such as in corticosterone release from the adrenal gland. Pathological conditions, such as tumors of head and neck that are treated with radiation are followed by xerostomy. In a rat model, radiation diminished NOS activity in the submandibular gland followed by inhibition in salivary secretion.

In summary, this review describes the wide participation of NO in the cross-talk between neuroendocrine and neuroimmune systems in physiological and pathological processes.

INTRODUCTION

At the end of the 1980s, it was clearly demonstrated that cells can produce nitric oxide (NO) and that this gaseous molecule is a highly reactive free radical with multiple and complex roles within many biological systems. In the present review our particular aim is to describe the role of NO in the field known as neuroimmunomodulation in which, central nervous system (CNS) activity modulates immune system and in turn, the immune system modulates the activity of the nervous system. In fact, during the past years it has been recognized that NO is a key player in the cross talk between both systems.
The first example of neuroimmunomodulation has been known since 1936, from the pioneer work of Selye, in which a noxious stimulus called stress induced the release of adrenocorticotrophic hormone (ACTH) from the pituitary, which in turn released adrenal cortical steroids. The introduction of bacteria into the body causes the release of toxic soluble products of their cell wall such as lipopolisaccharide (LPS), that induces fever and a concomitant increases in plasma cortisol, as well as the synthesis and release of various cytokines, such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), IL-6, IL-2, interferon gamma (IFN-γ), between others. These cytokines can release corticotrophin-releasing hormone (CRH) that activates ACTH followed by cortisol release. The cytokines can be produced not only by immune cells, particularly monocytes and macrophages, but also within the brain by glial elements and neurons. We have shown an IL-1 immunoreactive neuronal system with cells bodies in the dorsal preoptic area and anterior hypothalamus, and relatively short axons that could not be traced to the median eminence (ME). In addition, these substances are synthesized within the pituitary itself.

The research of the last decade indicates that cytokines induce NO production and that NO has a powerful influence on the secretion of hypothalamic peptides and classic synaptic transmitters, such as catecholamines and gamma aminobutyric acid (GABA); and also can suppress or stimulate the release of pituitary hormones directly.

One of the main physiological effects of NO is due to its binding to heme moiety of guanylate cyclase (GC), by altering its conformation and increasing its activity, causing the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). NO also reacts with several other metalloproteins, such as cytocrome P-450 side chain cleavage, which is essential for steroidogenic reactions. Another target for NO action is the heme group of the enzyme cyclooxygenase (COX). COX metabolizes free arachidonic acid (AA) to prostaglandins (PG) and thromboxanes. There are two known COX forms; COX-1 is constitutively expressed, whereas COX-2 is strongly induced during inflammation, but it also has been shown to be expressed constitutively in the brain.

NO is synthesized by NO synthase (NOS), an enzyme that converts arginine, in the presence of oxygen and several cofactors, into equimolar quantities of citrulline and NO. There are three variants of the enzyme; two of these are constitutively expressed while the other must be induced. The inducible NOS (iNOS), is formed mainly in immune cells, such as macrophages. LPS combines with its receptors on the surface of
macrophages and other cells inducing the synthesis of iNOS mRNA. LPS also induces mRNA expression of various cytokines, such as IL-1, IL-6 and TNF-α.8

One of the constitutive forms was originally characterized in endothelial cells and was therefore known as endothelial NOS (eNOS), while the other constitutive form, originally characterized in neurons was known as neuronal NOS (nNOS). These two isoforms have been found to be distributed more widely than originally thought. The eNOS is found in the caveoli of endothelial cells and is activated following cholinergic stimulation and the consequent increase of intracellular calcium, producing NO, which diffuses to overlying smooth muscle, and activates GC converting GTP to cGMP, which produces vasodilatation9. nNOS is found in the cerebellum and various regions of the cerebral cortex and also in various ganglion cells of the autonomic nervous system. Large number of nNOS-containing neurons were also found in the hypothalamus, particularly in the paraventricular nucleus (PVN) and supraoptic nuclei (SON) with axons projecting to the ME and neural lobe, which also contains large amount of nNOS.10 Because of this distribution in the hypothalamus in regions that contain peptidergic neurons that control pituitary hormone secretion, we studied the role of NO in the hypothalamic-pituitary axis.

**Role of NO in the hypothalamic-pituitary axis during infection**

As was previously described, the hypothalamic-pituitary response to infection can be mimicked by the injection of bacterial LPS iv or ip. There is a rapid increase in plasma ACTH and prolactin (PRL) within few minutes accompanied by a rapid inhibition of luteinizing hormone (LH) and thyroid-stimulating hormone (TSH).11 Growth hormone (GH) secretion is stimulated in humans but not in rats.4 Also, LPS causes the induction of cytokines synthesis and release from cells of the immune system. It has been found that the first cytokine to be released in the rat and in large quantities is TNF-α, that apparently causes the induction of IL-1 synthesis and release that in turn induces secretion of IL-6.12 Since the response of the pituitary hormones occurs within few minutes, it is obvious that the secretion of cytokines from immune cells in the periphery cannot be responsible for the immediate alterations in pituitary hormone secretion triggered by LPS. Our research demonstrated that ip injection of LPS induced IL-1 β and iNOS mRNA in the brain, anterior pituitary and pineal glands. The induction of both mRNAs occurred in the meninges, the choroids plexus, the circumventricular organs, such as the subfornical organ and the ME and in the
parvocellular neurons of the PVN and arcuate nucleus (AN), areas of particular interest since they contain the hypothalamic releasing and inhibiting hormone producing neurons and also other neurotransmitters controlled by NO. The greatest induction of iNOS occurred in the anterior lobe of the pituitary. This massive increased in NO production should further increase the effects of NO to maintain the pattern of hypothalamic hormone secretion already induced by LPS.

The initial response to LPS is mediated in the brain by nNOS. There is no participation of iNOS in this initial response. Indeed, the initial response must be due on LPS receptors in areas where the blood-brain barrier is not present, such as the choroid plexus, ME, organum vasculosum laminae terminalis (OVLT) and other circumventricular organs. LPS induced-input to the hypothalamus occurs at least in part by activation of the locus ceruleus that sends noradrenergic axons to the hypothalamus that synapse on cholinergic interneurons in the PVN and activate CRH release. LPS-stimulated CRH release involves NO production, because it can be blocked by inhibitors of all forms of NOS. Also it has been shown that NO activates COX I leading to generation of prostaglandin E2 (PGE2), that activates adenylyl cyclase (AC) and therefore increase cAMP. This cyclic nucleotide activates protein kinase A (PKA) which induces exocitosis of CRH secretory granules into hypophyseal portal vessels, activating ACTH release from the corticotrophs of the anterior pituitary gland. NO activates not only COX, but also lipoxygenase (LOX) and GC, which produces cGMP, that increases intracellular calcium that converts membrane phospholipids into AA, the substrate for COX and LOX, generating PG’s and leucotrienes, respectively. Activation of CRH by cytokines can be blocked by cyclosporine, probably by blockade of dephosphorilation of NOS by calcineurin rendering NOS inactive. Furthermore, it has been shown that IL-1, TNF-α, IL-6 and IL-2 can directly stimulate ACTH release from anterior pituitary via NO.

Role of NO in the control of luteinizing hormone releasing hormone release

The control of gonadotropin secretion is extremely complex as revealed by the research since the discovery of LHRH, now commonly called gonadotropin-releasing hormone (GnRH). LHRH controls the release of LH and follicle stimulating (FSH) from the pituitary and also induces mating behavior and penile erection in rats. We demonstrated that intracerebroventricular microinjections of NOS inhibitors inhibited pulsatile LH release and mating behavior which indicates that NO controls the pulsatile
release of LHRH and mating behavior.\textsuperscript{23,24} In \textit{in vitro} experiments, we showed that NO, released from sodium nitropruside (NP), promoted LHRH release from medial basal hypothalamus (MBH), and that this action was blocked by hemoglobin, a scavenger of NO. NP also increases the release of PGE\textsubscript{2} and LOX products that have been shown to play a role in LHRH release.\textsuperscript{25} Moreover, inhibitors of COX blocked the release of LHRH induced by norepinephrine, providing further evidence for the role of NO in the control of LHRH release via the activation of COX, as we mentioned above.\textsuperscript{26,27} We postulate that the NO released from the NOergic neurons, near the LHRH neuronal terminals, increases the intracellular free calcium required to activate phospholipase A that converts membrane phospholipids to AA, which then can be converted to PGE\textsubscript{2} via COX. The released PGE\textsubscript{2} activates AC increasing cAMP which activates PKA leading to exocytosis of LHRH secretory granules into the hypophyseal portal vessels for transport to the anterior pituitary gland.\textsuperscript{28}

Norepinephrine (NE) plays a controlling role in LHRH release by acting on hypothalamic NOergic neurons.\textsuperscript{26-28} We determined that the release of NO from the NOergic neurons has a tonic inhibitory action to decrease the release of NE from hypothalamic explants. Presumably, the NO produced by NOergic neurons diffuses to the terminals of the catecholaminergic neurons, where it acts on GC within the terminals to activate the enzyme and cause the production of cGMP. This cGMP may cause a decrease in intracellular free calcium in the cell, which may block the depolarization of the catecholaminergic terminals, inhibiting NE release from storage vesicles.\textsuperscript{29} We have shown that NO is increased by NE by $\alpha_1$ receptor stimulation, since it can be blocked by prazosine, an $\alpha_1$ receptor blocker.\textsuperscript{28} The NO diffuses to the noradrenergic terminals and inhibits them, generating a negative feedback to terminate the release of NE and consequently contributing to finalize LHRH surge.\textsuperscript{29}

The principal excitatory transmitter in the central nervous system is glutamic acid (GA). We demonstrated that the mechanism by which GA stimulates LHRH release involves NE release from noradrenergic neurons, since the alpha receptor blocker, phentolamine, blocked the GA-induced LHRH release.\textsuperscript{30-32} Therefore, GA neurons synapses with the noradrenergic terminal which, in turn, synapses with the NOergic neuron, which then generates NO that diffuses to LHRH terminal to stimulates LHRH release.

Gamma-aminobutyric acid (GABA) plays a dual role in the control of LHRH release in rats.\textsuperscript{33,34} In female rats it inhibits LHRH secretion by acting on LHRH
neurons in the medial preoptic area and it has a stimulatory effect on LHRH secretion from the arcuate nucleus-median eminence region. NO stimulates GABA release from the hypothalamus of adult male rats, and GABA inhibits LHRH release. We have shown that this inhibition is mediated by NO since the inhibitory effect was prevented by hemoglobin or by NG-monomethyl-L-arginine (NMMA), a competitive inhibitor of NOS. Therefore, NO is involved not only in the stimulation of LHRH release induced by NE, but also in its inhibition by inducing GABA release.

Dopamine (DA) is another catecholamine that, as well as NE, stimulates LHRH release, however, the role of dopamine in pulsatile LHRH release is less clear. There are tuberoinfundibular dopaminergic neurons in the hypothalamus which have been shown to stimulate LHRH release from male rat hypothalami in vitro; however, other studies indicate that at certain concentrations and in different hormonal states such as in the castrate rat, both DA and NE can inhibit LHRH release. The release of NO from the NOergic neurons in the hypothalamus has a tonic inhibitory action to decrease the release of DA from the tissue. Whether DA is released prior to each LH pulse to stimulate NO and LHRH release has yet to be determined, but this catecholamine may contribute to release of NO and augment pulsatile LHRH release, since DA receptor blockers can block LH release.

The presence of an ultra-short negative feedback that controls the episodic secretion of LHRH was suggested for the first time by Hyppa et al. studying FSH secretion. Morphological and molecular evidences show that LHRH receptors are expressed in LHRH hypothalamic neurons. However, there was not much evidence about the signal transduction pathways triggered by the activation of these receptors in vivo. Krzmanovic et al. reported a signal transduction mechanism in GT1-7 neurons, driven by inositol trisphosphate-induced Ca\(^{2+}\) mobilization, responding to LHRH receptors activation. We demonstrated that LHRH produces differential effects on the mediators of its own release, in MBH incubated in vitro, depending on its concentration in the inter-synaptic space. LHRH 10\(^{-11}\)M produced an increase in GABA release but at 10\(^{-7}\)M inhibited GA release, in both cases leading to the inhibition of LHRH release. Based on our results, we propose the existence of different populations of LHRH receptors that respond with distinct threshold, activating different signal transduction pathways. Also, it is possible that LHRH affects its own release by activating an additional neurotransmitters- independent mechanism. Moreover, it was shown that the inhibitory effect of buserelin on LHRH release started before changes on
neurotransmitters release were observed. These findings led us to study the effect of LHRH on cellular messengers involved in LHRH release such as NO and PGE$_2$. LHRH at $10^{-7}$ and $10^{-11}$M decreased PGE content, after the incubation of MBH explants during 30 min. However, LHRH $10^{-7}$M stimulated NOS activity but at $10^{-11}$M had no effect. Although NO is a known promoter of LHRH release, the stimulatory effect of the higher concentration of LHRH on NOS activity is not contradictory to the ultra-short negative feedback theory, since, as was described above it was proposed that NO has a dual function inducing LHRH exocytosis during the normal surge, but also, increasing GABA release and decreasing NE and DA release to promote the termination of LHRH pulse. Additionally, LHRH $10^{-7}$M but not $10^{-11}$M increased phosphatidylinositol breakdowns, suggesting that LHRH receptors coupled to PLC are involved in this NO-dependent mechanism. Although the mechanism of episodic release of LHRH is not clear to date, the NO-dependent pathway described could be one of the different existing mechanisms that provide redundancy for this important cycle that controls the physiology of reproduction.

These data together with those described above indicate that the NO released from NOergic neurons stimulates LHRH release and initiates the pulse, but also acts back on the norepinephrine and dopaminergic neurons to terminate the pulse. This is coupled with the negative feedforward of GABA released by NO to bring about the termination of the pulse.

**Participation of NO on the effect of Manganese on LHRH release**

Manganese (Mn$^{2+}$) is an essential metal which acts as a cofactor for many enzymes and therefore plays important biological functions. Nevertheless, high doses of Mn$^{2+}$ can cause reproductive dysfunction. It was reported that chronic administration of low doses of Mn$^{2+}$ to female rats resulted in increased serum levels of puberty-related hormones such as LH, FSH and estradiol, and advanced the time of vaginal opening indicating an advance in puberty. We demonstrate that manganese chloride (MnCl$_2$) is capable of stimulating LHRH secretion from MBH and this effect is accompanied by an increase in plasma LH levels. Also, MnCl$_2$ is capable of stimulating DA secretion and NOS activity in the MBH. Since it was shown that DA can induce NOS/NO, and based on the important role of NO in the control of LHRH this suggests that DA/NO activation may mediate the MnCl$_2$-stimulated secretion of LHRH. In addition, we have also found an action of this metal stimulating the inhibitory
transmitter, GABA. Furthermore, when GABA-A receptors were blocked by bicuculline the release of LHRH elicited by MnCl₂ was doubled. We suggest that the action of MnCl₂ to induce GABA secretion may represent a neurotoxic effect to ultimately inhibit LHRH secretion. Also, it was shown that MnCl₂ stimulates LHRH release from prepubertal female ⁴⁵ and male ⁴⁷ rats via the activation of guanylyl cyclase and subsequent stimulation of the cGMP/PKG pathway. ⁴⁸ In concordance with that, our recent experiments demonstrate that MnCl₂ is capable of stimulating LHRH secretion through the activation of the hypothalamic NO/cGMP/protein kinase G pathway. ⁴⁹

**The role of NO in control of Posterior Pituitary function**

The neural lobe of the pituitary is one of the regions richest in NOS in the rat, with a predominance of the nNOS isoform, suggesting that NO may play a role in controlling the release of neuropeptides and neurotransmitters from the posterior pituitary. ¹⁰, ⁵⁰ NADPH-diaphorase, used as a marker of NOS, was co-localized with vasopressin (VP) and oxytocin (OT) in the hypothalamic nuclei that synthesized these hormones. ⁵¹, ⁵² The synthesis of NO by oxytocinergic or vasopressinergic neurons suggests that it may participate in auto- and/or cross-regulation of OT and VP secretion. Our studies indicate that NO donors reduce OT secretion from the neural pituitary lobe and we postulated that released NO may suppress OT secretion through an ultrashort negative feedback mechanism. ⁵³ It has been reported that intracerebroventricular administration of L-NAME enhanced plasma levels of both OT and VP. ⁵⁴ Since NOS activity increases following salt loading and dehydration, it has been suggested that this increase may provide a negative feedback to prevent overstimulation of OT and VP release. ⁵⁵ In conclusion, NO may intervene in the control of OT response to osmotic stimuli. Also, chronic saline ingestion increases hypothalamic tachykinins concentration in SON. ⁵⁶ Tachykinins belong to a family of peptides that include substance P and neurokinin A (NKA). They are contained in hypothalamic neurons and nerve fibers and secretory cells of the posterior and anterior pituitary lobes, suggesting that these peptides may have a physiological role in the control of pituitary function. ⁵⁷ Some actions of tachykinins are known to be exerted through NO release. ⁵⁸ Although we observed an inhibitory effect of NKA on OT release by activation of NOergic neurons, thus suggesting that NKA may have a dual effect on OT release, decreasing it through NO and increasing OT release by an NO-independent mechanism. ⁵⁹ Nevertheless, the net effect of NKA on OT release from posterior pituitary seems to be inhibitory. As we
mentioned above, NO has been linked to the release of several neurotransmitters, including glutamate, acetylcholine, dopamine and GABA. Results on the effect of NO on GABA release are contradictory. Our study showed that endogenous NO has an inhibitory effect on GABAergic activity in posterior pituitary contrary to the effect that we have reported at hypothalamic level. GABAergic terminals in the neural and intermediate lobes participate in the control of OT and VP release raising the possibility that NO modulation of GABAergic activity in the posterior pituitary may be involved in the regulation of the secretory function of this pituitary lobe. However, since NO decreased both, OT and GABA release from the posterior pituitary and GABA was reported to inhibit OT release from nerve terminals of the neural lobe, it is possible that NO might influence the release of OT at the level of the cell bodies and on nerve terminals of the neural lobe by different mechanisms. NO is involved, at least partially, in the reduction of the GABAergic activity induced by NKA. The reduction in GABA release may contribute to the stimulatory effect of NKA on lactotroph function. This effect will increase the release of PRL, considered a proinflammatory factor that could participate in the regulation of the immune response during the acute phase of endotoxemia.

**Role of NO on the corticosterone release from the adrenal gland**

ACTH is the major regulator of steroid secretion and synthesis from the adrenal cortex, inducing an acute secretory response within few minutes and increase in steroid synthesis, including the transcription of steroidogenic genes. Many clinical and experimental observations indicate that there could be dissociation between plasma ACTH concentrations and cortisol/corticosterone secretion. Since the sympathetic nervous system responds earlier than the hypothalamus-pituitary-adrenal axis to stressors, corticosterone release prior to the ACTH action may be due to earlier stimulation of the adrenal cortex via the splanchic nerves, possibly via NO release. The effect of ACTH on adrenal function may involve NO since NOS was found expressed in the adrenal gland. Endothelial and neuronal cells have a close anatomical proximity to steroidogenic cells in the adrenal gland, and these cells release NO. In fact, one study suggested that the production of NO by eNOS or nNOS could be effective in regulating the blood flow and steroid release. Moreover, NO can also be generated within steroid-producing cells and may be involved in steroidogenesis.
On the other hand there are controversial reports on the effects of NO and PGs on the steroidogenic pathway.\textsuperscript{68} All of these studies used dispersed cells, thereby eliminating the normal architecture of the gland. NO has been postulated as an autocrine/paracrine regulator of steroidogenesis in several tissues, with effects on adrenal steroid secretion.\textsuperscript{64,68} We investigated the role of NO and the participation of PGE\textsubscript{2} in corticosterone release stimulated from rat adrenal glands by ACTH in vitro.\textsuperscript{69}

Our results indicate that NO stimulates corticosterone release from adrenals. We believe that NO leads to a rapid release of stored corticosterone from the adrenals, as the released at 15 min was nearly as great as at 30 min, and corticosterone adrenal content was lower than control, indicating that any \textit{de novo} synthesis of corticosterone occurred during this period. The effect of NO and ACTH on acute release of corticosterone could be mediated by PGE\textsubscript{2}, since PGE\textsubscript{2} can release corticosterone and this effect is blocked by indomethacin, a COX inhibitor.\textsuperscript{69}

We hypothesized that ACTH activates NOS that increases NO that activates COX that generates PGE\textsubscript{2}, which in turn, releases corticosterone. The mechanism by which PGE\textsubscript{2} causes exocytosis remains to be determined.

\textit{Effect of radiation on NO production in submandibular gland}

The exposure to ionising radiation during therapy for head and neck tumours results in alterations of salivary glands such as sialoadenitis and xerostomia followed by dysphagia, mucositis, rampant dental caries, increased tooth decay, oral infections, oesophagitis and gustatory dysfunction.\textsuperscript{70,71} There has been a continuous effort to understand this salivary gland dysfunction; however, the specific mechanisms that underline such damage remain poorly understood. Salivary glands are composed of highly differentiated almost non cycling cells and differences in radio sensitivity of the various cell types have been observed.\textsuperscript{72} In the submandibular gland (SMG), it has been shown that serous cells are far more radiosensitive and this is in concordance with the observed decrease in salivary flow after irradiation.\textsuperscript{71}

NOS is widely distributed in different regions of SMG.\textsuperscript{73} In previous publications of our group we have shown that nNOS is clearly present in nerve terminals and together with iNOS in the apical membrane of the excretory and striated ducts of SMG. Interestingly, NOS was absent in the acinar cells.\textsuperscript{74} Previously reported results from our group indicate that NO has a stimulatory effect on salivary secretion.\textsuperscript{75}
therefore we hypothesize that radiation could affect salivary secretion by modulating the NOS activity in the SMG. The inducible NOS expressed in immune cells produces the greatest quantities of NO and many macrophages were seen within the gland.\textsuperscript{75} We observed that iNOS activity was significantly reduced after radiation indicating that the decrease in the levels of NO in SMG could mediate the decreased salivary secretion.\textsuperscript{76} Also we found an increase in the PGE content in the SMG that could contribute to the decrease in salivary secretion as we have shown previously.\textsuperscript{77} Furthermore, we found an increase in lipid peroxidation, oxidative stress and an increase in mitochondrial NOS expression in SMG that could lead to a tissue damage observed 180 days after radiation. At 365 days post radiation there were structural changes that could account for irreversible damage such as fibrosis with loss of salivary secretion.

**Effect of prenatal stress on NO production in the CNS of the offspring**

The developing CNS is especially vulnerable to stress-induced damages. The effect of stress during the first stages of development induces both, immediate and later alterations, which reflect changes in the neuroendocrine, cognitive and behavioral systems. Stress during pregnancy triggers physiological and behavioral abnormalities in the offspring.\textsuperscript{78}

The hippocampus is one of the classic structures associated to cognitive processes. However, lately, the cerebellum has been redefined as a structure with a much wider functionality.\textsuperscript{87} Certain investigations in humans and animal models involve the cerebellum in cognitive functions. We evaluated the effect of stress during pregnancy in the offspring at 30, 60 and 90 postnatal days on the nNOS and iNOS activities and protein contents in the hippocampus and cerebellum. Also we tested the learning and memory capabilities at the same postnatal days.\textsuperscript{80}

When protein levels from cerebellum and hippocampus were studied in the offspring of stressed animals, an increase in nNOS was found in both tissues at different ages. At the same time, inducible isoform, iNOS expression did not vary in a significant manner in the analyzed tissues.\textsuperscript{89} Also, we found an increase in the activity in calcium-dependent NOS in cerebellum homogenates in prenatal stressed animals respect to controls.\textsuperscript{80}

When spatial memory test was performed it showed that offspring from stressed mothers needed more time to complete the task than control offspring animals. The
differences were highly significant at postnatal day 30 and maintained at day 60 and 90. Also, prenatal stress induced a deficit in the inhibitory avoidance task. As predicted, the latency for the control group increased in a significant manner, which reflects that both, information acquisition and consolidation provided by the context were successful. On the contrary, in the prenatal stressed group, no significant differences were observed when the training latency was compared with the test latency. Also, significant differences were found between control and stressed animals for the test latency values from postnatal day 45. So far, it has been demonstrated that, from a behavioral point of view, prenatal stress induced a decrease in the ability to resolve spatial navigation tasks, along with a learning deficit in the inhibitory avoidance test. 79

The results indicate that prenatal stress model used is able to induce a decrease in the offspring learning and memory capabilities. These alterations correlate with an increase in the expression and activity of the enzyme NOS in different parts of the brain of stressed rats.

Therefore, we are able to conclude that the changes in NOS profiles and activities might constitute primary events during the development of the nervous system that may manifest in adulthood as significant changes in the animal behavior.

CONCLUSIONS

Nitric Oxide regulates numerous and diverse physiological processes, including reproduction and neurotransmission. The data presented above indicate that there are many areas in the brain where there is regular periodic physiological release of NO throughout the life span. This occurs in the hippocampus, cerebellum, and, in particular, in the hypothalamus, in which NO controls most of the hypothalamic peptidergic neurons (such as CRH, LHRH, GHRH, somatostatin, OT, and VP) and also activates the release of GABA and inhibits that of NE and DA.

Because of the ubiquitous nature of NO, the inappropriate release of this mediator has been linked to pathogenesis of a number of disease states including stress, neurodegenerative disorders, inflammation and septic shock. It is already well known, that stress and infections with release of viral or bacterial products, such as LPS, cause the induction of cytokines, which are released and travel through the bloodstream. LPS and the released cytokines combine with their receptors, induce iNOS expression in neurons, glia and many other immune cells, producing high amount of NO, than would be released in basal conditions. NO itself or combined to other free radicals and other
substances produces toxic effects in cells that bring about cell injury and death. On the other hand, there appears to be a temporal and functional relation between de hypothalamo-pituitary-adrenal (HPA) axis response and NO formation at every level of the axis, suggesting a fundamental role of NO as a key modulator of the HPA to maintain homeostasis in these pathological states.

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receptor subtypes are involved in the regulation of opiate-dependent and independent


FIGURE LEGEND

Diagram of the postulated participation of NO in LHRH release. Glutamic acid (Glut) released from its neurons binds to NMDA receptors located on catecholaminergic neurons inducing NE release, which acts on α-adrenergic receptors activating nNOS and therefore the production of NO. NO exerts its stimulatory actions on LHRH release by the interaction with the heme group of, at least, two enzymes. One is the activation of COX, present in LHRH neurons, producing the conversion of arachidonic acid (AA) into PGE$_2$ that stimulates AC activity with the consequent increase in cAMP production that induces LHRH secretion via cAMP-dependent protein kinase (PKA). The other is activation of GC, increasing cGMP production that stimulates LHRH secretion via cGMP-dependent PKG. Also, by releasing AA from membrane phospholipids, enhances PGE$_2$ production. On the other hand, NO can produce inhibitory actions on LHRH release. NO depolarizes GABAergic neurons and therefore increases GABA release that acts on GABA receptors located on LHRH neurons producing the inhibition of LHRH release. Also, NO blocks the depolarization of the catecholaminergic terminals, inhibiting NE release and therefore the NE-stimulatory pathway. Both inhibitory actions of NO probably are necessary to terminate LHRH pulses. Solid arrows indicate stimulation. Dashed arrows indicate inhibition.