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Effects of Nitric Oxide on Neuroendocrine Function and Behavior

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Nitric oxide (NO) is an unusual chemical messenger. NO mediates blood vessel relaxation when produced by endothelial cells. When produced by macrophages, NO contributes to the cytotoxic function of these immune cells. NO also functions as a neurotransmitter and neuromodulator in the central and peripheral nervous systems. The effects on blood vessel tone and neuronal function form the basis for an important role of NO on neuroendocrine function and behavior. NO mediates hypothalamic portal blood flow and, thus, affects oxytocin and vasopression secretion; furthermore, NO mediates neuroendocrine function in the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal axes. NO influences several motivated behaviors including sexual, aggressive, and ingestive behaviors. Learning and memory are also influenced by NO. Taken together, NO is emerging as an important chemical mediator of neuroendocrine function and behavior. KEY WORDS: Hypothalamic releasing factors; endocrine secretion; hypothalamic–hypophyseal portal system; vascular tone; water balance; food intake; water intake; ingestive behavior; sexual behavior; penile erection; ovulation; aggression; copulation; learning; and memory. © 1997 Academic Press

INTRODUCTION

Research on the biological function of nitric oxide (NO)[1] has a relatively brief history, but a long past (15). The first biological function of NO was discovered

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[1] Abbreviations used: ACTH, adrenocorticotrophic hormone; BNST, bed nucleus of the stria terminalis; CRH, corticotropin-releasing hormone; cGMP, cyclic guanosine monophosphate; EDRF, endothelial-derived relaxing factor; FSH, follicle-stimulating hormone; GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; 5-HT, serotonin; HPA, hypothalamic–pituitary–adrenal axis; HPG, hypothalamic–pituitary–gonadal axis; IL-1, interleukin-1; L-NAME, *N*-nitro-L-arginine methyl ester; L-NNA, N^G-nitro-L-arginine; LTD, long-term depression; LTP, long-term potentiation; LH, luteinizing hormone; Me, medial nucleus of the amygdala; ME, median eminence; MPN, medial preoptic nucleus; MPOA, medial preoptic area; NADPH, nicotinamide adenine dinucleotide phosphate; NADPH-diaphorase; NANC, nonadrenergic and noncholinergic; NE, norepinephrine; 7-NI, 7-nitroindazole; NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; NMDA, *N*-methyl-D-aspartate; OLVT, organum vasculosum of the lamina terminalis; PGE_2, prostaglandin E_2; PVN, paraventricular nucleus; Ras-ERK, Ras-extracellular receptor kinase; SFO, subfornical organ; SNP, sodium nitroprusside; SON, supraoptic nuclei; Tyr, tyrosine; WT, wild-type.

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in the circulatory system. The ability of nitroglycerin and other organic nitrates to alleviate the pain of angina pectoralis was discovered in the nineteenth century (124), although the mechanisms by which nitrates worked were not discovered until 1980 (46). Relaxation of blood vessels in response to acetylcholine requires that the endothelium secretes a substance initially termed endothelial-derived relaxing factor (EDRF) (46). After many years of debate, it has become accepted that NO is EDRF (68, 123). NO is also the active metabolite of nitroglycerin, as well as other nitrates, and stimulates blood vessel dilation by activating guanylyl cyclase which induces cGMP formation (162). Thus, NO is an important endogenous mediator of blood vessel tone.

A second biological function of NO emerged in the late 1970s from an independent line of research documenting the carcinogenic risk of dietary nitrosamines. The discovery that both humans and non-human animals produce urinary nitrates in greater amounts than consumed and that this production increases during bacterial infections led to the realization that an endogenous source of nitrates existed (51). Macrophages convert L-arginine to L-citrulline and a reactive species that kills tumor cells *in vitro*, namely NO (59, 89). Thus, NO plays an important role in immune function.

It was soon discovered that NO was also released when cerebellar cultures were stimulated with glutamate (48). Pharmacological inhibition of the synthetic enzyme NO synthase (NOS) blocked the elevation of cGMP levels in brain slices coincident with activation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (18, 49). Since then, many studies of NO affecting neural function have been reported (reviewed in 15, 34, 35). By acting on the neurons and endothelial cells in the circulatory system, NO exerts profound effects on neuroendocrine function and behavior. Our goal is to review these effects of NO on neuroendocrine function and behavior.

NO is an endogenous gas that has biochemical properties of a free-radical. NO is very labile, with a half-life of <5 s; consequently, many studies have manipulated NO indirectly by affecting its synthetic enzyme, NOS, that transforms L-arginine into NO and L-citrulline (Fig. 1). As noted above, studies that have used this approach indicate that NO plays several critical physiological roles. Three distinct isoforms of NOS have been identified: (i) in the endothelial tissue of blood vessels (eNOS; type III), (ii) an inducible form acting from macrophages (iNOS; type II), and (iii) in neural tissue (nNOS; type I). Suppression of NO formation either by elimination of arginine or by use of *N*-methyl-arginine or *N*-nitro-L-arginine, potent NOS inhibitors, affects all three isoforms of NOS. Recent development of drugs that inhibit specific isoforms of NOS and the development of mice with targeted disruption of the genes encoding specific isoforms of NOS are beginning to clarify the precise role of each isoform of NOS in neuroendocrine function and behavior.

The use of pharmacological agents that block synthesis of NO and production of knockout mice that lack one of the various isoforms of NOS have revealed several intriguing behavioral and neuroendocrinological effects of NO (e.g., 2, 22–24, 112). NO appears to affect neuroendocrine and behavioral processes generally in one of two ways: (i) indirectly through eNOS mediation of blood

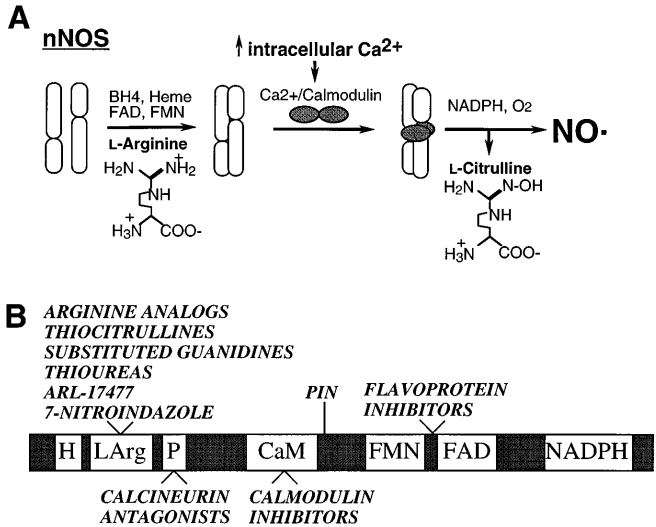

FIG. 1. Nitric oxide synthase. (A) NOS monomers loosely dimerize in the presence of tetrahydrobiopterin (BH_4) heme, the flavoproteins, FMN and FAD, and L-arginine. Rapid increase in intracellular calcium activates calmodulin. The calcium/calmodulin complex associates with the NOS dimers promoting tight dimerization and permitting the flow of electrons from the reductase moiety to the catalytic site where L-arginine is converted to L-citrulline and NO in the presence of NADPH and oxygen. (B) Nitric oxide synthase converts L-arginine to L-citrulline in the presence of tetrahydrobiopterin (BH_4), oxygen, NADPH, heme, and calmodulin. There are multiple regulatory sites on NOS that can be exploited for regulating enzyme activity. Many agents are targeted toward the catalytic site such as arginine analogs, thiocitrulline compounds, gaunidines, thioureas, and ARL-17477. Of these agents, the arginine analogs and thiocitrulline agents are nonspecific for all three NOS isoforms. The substituted guanidines and thiourea agents have some selectivity for iNOS over nNOS and eNOS. ARL-17477 is a selective inhibitor of nNOS. 7-Nitroindazole is selective for nNOS and targets the catalytic site to promote monomerization. The endogenous inhibitor of nNOS, protein inhibitor of NOS (PIN), also promotes monomerization to inhibit enzyme activity. The active enzyme is a dimer which forms on binding of calmodulin. Calmodulin antagonists interfere with this activity and inhibit NO formation. Once the protein monomers dimerize on the binding of calmodulin, electrons are shuttled from NADPH through the flavins. Inhibition of this electron shuttling with flavoprotein inhibitors such as diphenyleneiodonium will inhibit NO formation. NOS has several consensus sites for phosphorylation and is a substrate for the phosphatase, calcineurin. Inhibition of calcineurin activity with FK506 or cyclosporin A prevents activation of NOS by dephosphorylation.

flow, which affects neuroendocrine secretion rates, or (ii) directly as a result of nNOS effects on neuronal functioning. Both types of regulation of NO in neuroendocrinological and behavioral processes are reviewed below. First, a description of the neuroanatomical distribution, as well as the physiology of NOS, will be presented.

Neuroanatomy of NO

nNOS neurons are distributed in a unique manner, distinct from that of any other previously described neurochemically defined cell population (Fig. 2). The

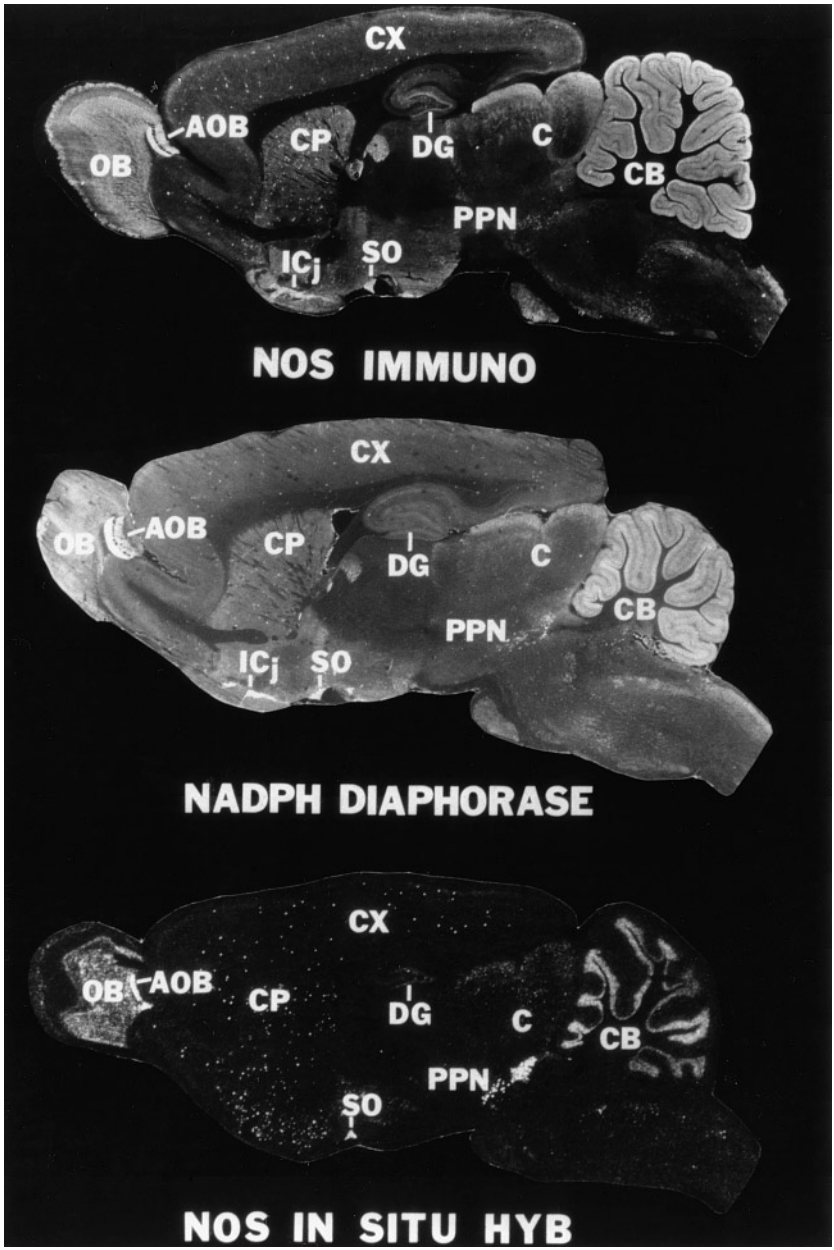

FIG. 2. Localization of nNOS, NADPH-diaphorase and nNOS mRNA in rat brain. Adjacent saggital sections from rat brain were processed for (top) nNOS immunohistochemistry, (middle) NADPH-diaphorase staining, and (bottom) *in situ* hybridization with specific probes to nNOS. The highest density of nNOS expression is in the accessory olfactory bulb, peduncular pontine tegmental nucleus, and cerebellum. Moderate expression of nNOS is observed in the dentate gyrus of the hippocampus, main olfactory bulb, superior and inferior colliculi, and supraoptic nucleus. Intensely labeled isolated cells are visualized in the cortex, caudate-putamen, and basal forebrain. Some regions, including the molecular layer of the cerebellum, islands of Callejae, and the neurophil of

highest densities of nNOS are within the cerebellum and nNOS occurs within glutaminergic granule cells as well as GABAergic basket cells (16, 169). nNOS is highly concentrated in the olfactory bulb and is especially concentrated within the accessory olfactory bulb. nNOS is also highly concentrated within the pedunculopontine tegmental nucleus, the superior and inferior colliculi, the islands of Callejae, the caudate-putamen, and the dentate gyrus of the hippocampus. Within the cerebral cortex and striatum, nNOS is localized and scattered in isolated cells that are medium to large aspiny neurons (15). A dense cluster of nNOS is found in the nucleus of the diagonal band of Broca and within the medial septal nucleus. Positive nNOS fibers are present within the fornix and the diagonal band and the lateral septal nucleus. The entorhinal cortex contains a moderate dense fiber plexus and in addition contains a scattered population of large multipolar intensely stained cells that are scattered throughout the entorhinal cortex (15). Large, multipolar, intensely stained neurons are scattered throughout the amygdaloid complex, in particular, in the basolateral nucleus and the preamygdaloid area. Many neurons of the medial amygdaloid nucleus contain nNOS, whereas a few single fibers are present within the central nucleus. The bed nucleus of the stria terminalis contains many moderately stained nNOS neurons, particularly in the posterior lateral division.

In the hypothalamo–neurohypophyseal system of the rat, nNOS positive neurons are distributed within the supraoptic nucleus, the retrochiasmatic supraoptic nucleus, as well as the medial and lateral portions of the paraventricular nucleus (3). All accessory nuclei, including the nucleus circularis, periventricular nuclei, anterior commissural nucleus, nuclei of the medial forebrain bundle, posterior fornical nucleus, and other scattered neurons, are positive for nNOS in the magnocellular neurosecretory system (45). Axons of the paraventricular nucleus project to and through the interior layer of the median eminence and terminate within the posterior pituitary. These axons have large varicosities near the median eminence and within the posterior pituitary. The posterior pituitary stains intensely for nNOS, and nNOS staining is localized mainly to variable-sized varicosities within the posterior pituitary. Some nNOS positive cells are also observed in the anterior and medial lobes of the pituitary.

nNOS does not seem to colocalize with a single neurotransmitter. For instance, in the cerebral cortex and corpus striatum, NOS neurons are colocalized with somatostatin and neuropeptide Y (33). In the pedunculopontine tegmental nucleus of the brain stem, NOS neurons lack somatostatin and neuropeptide Y, but stain for choline acetyltransferase. Within the hypothala-

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the caudate-putamen and cortex, are enriched for nNOS protein and NADPH-diaphorase but do not contain nNOS mRNA, suggesting that the nNOS has been transported to nerve fibers distant from the site of protein synthesis. Reproduced with permission of the publisher (Bredt *et al.*, 1991). Abbreviations: AOB, accessory olfactory bulb; C, superior and inferior colliculi; CB, cerebellum; CP, caudate-putamen; CX, cortex; DG, dentate gyrus of the hippocampus; ICj, islands of Cellejae; OB, main olfactory bulb; PPN, peduncular pontine tegmental nucleus; SO, supraoptic nucleus.

mus, some nNOS neurons stain positively for oxytocin and vasopressin (45). Despite the various neurotransmitters and neuropeptides that are distributed within nNOS neurons, all nNOS neurons identified colocalize with a histochemical stain, NADPH-diaphorase (NDP) (33, 62). NDP is a histochemical stain originally described by Thomas and Pearse (160) in which enzymes that possess diaphorase activity reduce tetrazolium dyes in the presence of NADPH, but not NADH to a dark blue formazan precipitant. NOS catalytic activity accounts for diaphorase staining because cultured human kidney 293 cells transfected with nNOS cDNA produces cells that stain for both nNOS and NADPH-diaphorase (33). Furthermore, mice lacking the gene for nNOS are completely devoid of NADPH-diaphorase staining (64). The coincidence of nNOS immunoreactivity and NDP staining in neurons is observed only under appropriate paraformaldehyde fixation. Presumably, paraformaldehyde fixation inactivates virtually all NADPH-dependent oxidative enzymes, except for NOS (92).

In addition to localization to endothelial cells of blood vessels where NO release mediates relaxation of blood vessels, eNOS is also localized to neurons. eNOS is highly concentrated within pyramidal cells of the CA1 through CA3 region of the hippocampus, as well as granule cells of the dentate gyrus. This markedly contrasts with the staining for nNOS, which is very low in the CA1 pyramidal neurons, but is concentrated within GABAergic interneurons of the hippocampus (39). In some brain regions such as the cerebellum and olfactory bulb both eNOS and nNOS occur in the same cell populations, although in different proportions.

Physiology of NO

The biological outcome of NO is critically dependent upon the local microenvironment in which it is generated (Fig. 3). The biologic function of NO is probably dependent upon the various oxidation states of NO (85). NO·, the free radical form, the oxidized NO^+ (nitrosonium ion), and the reduced NO^- (nitroxide ion) of NO may exist in the brain and, in part, may be responsible for some of the conflicting activities attributed to NO. The NO free radical (NO·) is the most likely form of NO that is produced on stimulation of NOS.

The heme moiety of guanylyl cyclase is sensitive to NO which induces a conformational change that activates the enzyme and results in cGMP formation. Guanylyl cyclase is the best known target of NO and most studies have focused on the NO/guanylyl/cGMP system as the physiologic mediator of NO actions. However, NO has a number of potential physiologic targets and these may turn out to be more important transducers of NO physiologic actions. NO increases prostaglandin production by activating another heme containing enzyme, cyclooxygenase (141). Non-heme iron in iron sulfur clusters in numerous enzymes is sensitive to NO. Peroxynitrite, a reaction product of NO and $O_2^{\cdot -}$, rather than NO, may react with iron sulfur clusters (26). In contrast to the reversible reaction of NO with heme, dissolution of the iron sulfur cluster

Nitric Oxide Species	Putative Cellular Targets
NO· (Nitric Oxide)	**Proteins containing Heme:** Guanylyl cyclase Nitric Oxide Synthase Cyclooxygenase 1 & 2 Cytochrome P450 Hemoglobin and Myoglobin **Mono-ADP ribosylation:** GAPDH
NO· + O_2^{-}· → ONOO- (Peroxynitrite)	**Proteins with iron-sulfur clusters:** cis-Aconitase (IRE-BP) Mitochondrial aconitase Ribonucleotide reductase Mitochondrial complex I Mitochondrial complex I I Ferritin and transferrin **Nitration of proteins:** SOD Neurofilaments *trk*-receptors **Reaction with DNA:** DNA deamination DNA strand breaks PARS activation
NO· + CM → CM-NO+ or CM-NO- (CM, Carrier Molecule)	**Nitrosylation of proteins:** G proteins/$p21^{ras}$ NMDA-Receptor Glutathione Protein Kinase C GAPDH Adenylyl cylcase Tissue Plasminogen activator NADPH Oxidase SNAP-25 GAP-43 Albumin

FIG. 3. Putative cellular targets affected by NO formation. Substantial biochemical evidence indicates that NOS produces the free radical NO·. NO· can react with O_2·− to form peroxynitrite (ONOO−) which is a potent oxidant that can also chemically behave similar to ·OH and NO_2·. Additionally, NO· can react with a carrier molecule (CM) and subsequently be donated to a cellular target molecule as NO+ or NO−. While there are reports of interactions due to NO+ and NO−, it is important to note that these agents do not exist freely in solution but are bound to a carrier molecule and donated to another molecule in this valence state. There are multiple putative cellular targets that can react with NO·. A partial list is presented. Several factors regulate the biologic outcome of NO· formation. NO· chemically reacts with cellular targets. These interactions are determined by the concentration of NO present, the concentration of potential cellular targets, and the rate of reaction between NO and the cellular target. For example, the rate of reaction of NO with the heme iron of guanylyl cyclase is relatively fast, resulting in the formation of cGMP. This is the basis of one of the most widely used indirect markers of NO· formation. NO· can also react with iron-sulfur clusters in proteins such as aconitase. However, the reaction rate is slower and therefore more NO· and more aconitase is required for a significant effect. Understanding the biologic outcome of NO formation is further complicated by the formation of ONOO− and carrier protein complexes. The chemistry of these species differs from that of NO·. For example, while NO weakly reacts with aconitase, ONOO− is a potent reactant with the iron-sulfur centers in aconitase. Therefore, the final biologic outcome of NO formation is also dependent on the local chemical environment which determines whether NO reaches cellular targets as a free radical, as peroxynitrite or complexed to a carrier molecule.

occurs after the reaction with NO (57). Through these interactions NO may inhibit oxidative respiration and cause cytotoxicity.

Another major target for NO may be protein thiols. *S*-nitrosoglutathione and the nitrosothiol of serum albumin have been isolated and characterized (156). Another potentially important regulatory system accounting for some of the physiologic actions of NO is the nitrosylation of proteins. Protein-associated targets of NO include heme, cysteine (Cys), and tyrosine (Tyr) residues. Cys residues are efficiently nitrosylated at physiological pH, while other reactions such as further oxidation of nitrosothiol to sulfonic acid and nitration of Tyr occur at a much slower rate (98). NO may physiologically modulate glutamatergic neurotransmission through nitrosylation of the NMDA receptor (85). The cysteine residue of hemoglobin is sensitive to NO and forms *S*-nitrosyl hemoglobin (70). During arterial–venous transit NO is released from *S*-nitrosyl hemoglobin and appears to control blood pressure, which may facilitate efficient oxygen delivery to tissues.

The best evidence for a transmitter role of NO comes from studies of the peripheral nervous system. nNOS is localized to the myenteric plexus (17) and nonadrenergic and noncholinergic (NANC)-mediated relaxation of the gastrointestinal tract is blocked by NOS inhibitors (101). Further support for a role of nNOS in gastrointestinal function comes from studies in mice with targeted disruption of the nNOS gene (64). These mice have marked enlargement of the stomach and hypertrophy of the inner circular muscle layer resembling infantile hypertrophic pyloric stenosis in male infants (166). The pyloric sphincter of mice with targeted disruption of the nNOS gene (nNOS−/−) fails to relax properly in response to a food bolus, leading to the grossly distended stomach and hypertrophy of the inner circular muscle layer. NO acts as an inhibitory neurotransmitter that mediates the slow component of inhibitory junction potentials in circular smooth muscle of gastric fundus (90). Interestingly, the nNOS gene is a susceptibility locus for infantile pyloric stenosis (30).

A similar mechanism may be at work in the bladder. Male nNOS−/− mice exhibit higher urination frequency than WT males (22); nNOS−/− mice possess hypertrophic dilated bladders and dysfunctional urinary outlets that do not relax in response to electrical field stimulation or L-arginine (22). The abnormal micturition patterns of nNOS−/− mice mimic idiopathic voiding disorders in humans.

NO and Neurotransmitter Release

In the central nervous system, the physiologic roles of NO are less well described. NO may be involved in light phase shifts of circadian rhythm in that NOS inhibitors block light-induced resetting of behavioral rhythms (40). NO may also be involved in long-term potentiation (LTP) in the hippocampus and long-term depression (LTD) in the cerebellum (see below). Neurotransmitter release is influenced by NO presumably through cGMP activation of cGMP-dependent protein kinases to augment the phosphorylation of synaptic vesicle

proteins associated with neurotransmitter release. NMDA receptor-mediated neurotransmitter release occurs, in part, through the actions of NO as inhibitors of NMDA receptor-mediated neurotransmitter release from the cerebral cortex and striatal synaptosomes (60, 102). Hypothalamic gonadotropin-releasing hormone (GnRH) release mediated by glutamate or norepinephrine may also involve NO (146; and see below). The mechanism of how NO influences neurotransmitter release is unclear. However, NO may act on both calcium-sensitive and calcium-insensitive pools of synaptic vesicles (96). The actions of NO on neurotransmitter release could involve cGMP activation of cGMP-dependent protein kinase to augment phosphorylation of synaptic vesicle proteins or through activation of cyclic GMP dependent-cation channels (1). NO may also influence transmitter release through nitrosylation of sulfhydryl groups of protein(s) involved in the release process. For instance, NO facilitates vesicle docking/fusion events by promoting VAMP/SNAP-25/syntaxin 1a complex formation and by inhibiting n-sec1 binding to syntaxin 1a (95). Thus, there may be multiple molecular targets of NO acting in concert to modulate neuronal and neuroendocrine secretion.

NO and Water Balance

Magnocellular neurons in the paraventricular and supraoptic nuclei in the hypothalamus of rats contain nNOS and these neurons contain a variety of neuropeptides, most notably vasopressin and oxytocin. NO appears to play a prominent role in the regulation of hypothalamic portal blood flow (27, 140). Various manipulations such as hypophysectomy salt loading, and water deprivation can induce marked changes in the expression of vasopressin and oxytocin (97), as well as nNOS (167, 168). The exact role of the upregulation of nNOS following hypophysectomy has not been specified, but it may contribute to the regeneration of the "new neural lobe" at the median eminence level or facilitate portal blood flow (61).

The upregulation of nNOS following salt loading may play an indirect role in diuresis. For instance central inhibition of NOS enhances the release oxytocin during dehydration that would favor the release of vasopressin, thus enhancing the antidiurectic effect (61, 159). NO appears to influence water intake in other ways as it may be a major inhibitory modulator in the rat subfornical organ (SFO) mediating antidipsogenic effects (132). Furthermore, NO influences renin secretion at the level of kidney macula densa (133).

NEUROENDOCRINE EFFECTS OF NO

Hypothalamic–Pituitary–Adrenal Axis

As noted above, NOS is localized throughout discrete brain areas, including the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus

(17, 19, 54, 171). These nuclei represent the major source of neurons containing corticotropin-releasing hormone (CRH) (74, 84, 121). In response to stimulation by CRH release (primarily from neurons in the PVN), the anterior pituitary secretes adrenocorticotropic hormone (ACTH) into the hypophyseal portal system. During a stress response, ACTH acts on the adrenal cortex to stimulate the production and secretion of glucocorticoids (121).

Numerous factors can influence the secretion of ACTH. These factors may act directly at the level of the pituitary or further upstream by acting on hypothalamic CRH neurons. For example, the cytokines interleukin-1 (IL-1), IL-1β, IL-2, and IL-2β increase CRH secretion both *in vivo* and *in vitro* (73, 137, 138). Likewise, neurotransmitters such as acetylcholine, norepinephrine, and prostaglandins, in addition to neurohormones, such as oxytocin and vasopressin, are capable of influencing the secretion of CRH (47, 73, 138, 147). Recent evidence suggests that the ability of cytokines, catecholamines, prostaglandins, and hormones to modulate the secretion of CRH or ACTH may involve, or depend upon, the actions of NO.

Cytokines can act in the central nervous system to modulate the secretion of CRH (53, 73, 137). For example, IL-1β and IL-2 increase CRH secretion in incubated hypothalami (31, 73). Basal and IL-2-mediated release of CRH from hypothalami maintained *in vitro* is augmented by the addition of L-arginine (the precursor for NO production) to the preparation (73). Incubation of hypothalami with inhibitors of NO synthase such as N^G-monomethyl-L-arginine (NMMA) suppresses IL-2-induced release of CRH (73). Contrary to these findings, one study reported that IL-1β-induced secretion of CRH is inhibited by the addition of L-arginine to the incubation medium (31). *In vivo,* intravenous (iv) injections of IL-1β increase plasma ACTH and corticosterone concentrations; this increase in CRH is attenuated by pretreatment with *N*-nitro-L-arginine-methyl ester (L-NAME), a potent NOS inhibitor, lending further support for a modulatory role of NO in CRH release (138).

In common with the interleukins, several neurotransmitters and neurohormones are capable of regulating CRH secretion (47, 73, 138, 147). For example, carbachol, a muscarinic receptor agonist, increases IL-2β-induced CRH release; NMMA administration blocks IL-2β potentiation of CRH release (73). Rats injected iv with oxytocin or arginine vasopressin increase CRH release; this augmentation is inhibited by pretreatment with L-NAME (138). Norepinephrine is also capable of increasing CRH secretion, but NO does not appear to be involved in this response (73, 131). These data suggest that both *in vivo* and *in vitro* CRH release is regulated by a complex interaction among several endogenous factors, many of which may be regulated by NO.

Cytokines are capable of activating NOS (78, 81), and NO can stimulate the release of several factors, including catecholamines, prostaglandins, and neurohormones (125, 147, 173). Thus, it is possible that the interleukins act on the hypothalamic–pituitary–adrenal (HPA) axis by affecting NOS activity; NO then acts indirectly on the HPA axis by increasing neuromodulators that act at the level of either the hypothalamus or the pituitary. This possibility has received some support; L-NAME does not augment cytokine-induced increases

in plasma ACTH if prostaglandins are inhibited by ibuprofen administration (137). Similar results are obtained with *in vitro* hypothalamic preparations (142).

Taken together, these studies suggest that NO is intimately involved in the regulation of the HPA axis. Whether NO acts indirectly through other neuromodulators or directly on hypothalamic nuclei containing CRH remains to be determined. Regardless of mechanisms, NO is well-positioned to modulate neuroendocrine–immune effects on the HPA axis. Conceivably, under conditions of moderate immune stimulation, NO release from macrophages may act to regulate activation of the HPA axis to ameliorate adverse physiological effects due to the "stress" of mounting an immune response (138). The observation that L-NAME administration augments cytokine-induced increases in ACTH supports this contention (137, 138).

Hypothalamic–Pituitary–Gonadal Axis

The neuropeptide gonadotropin-releasing hormone (GnRH) represents the primary link between the brain and the reproductive system (50). In mammalian species, GnRH neurons are widely distributed and form a loose continuum from the telencephalic diagonal band of Broca and more dorsal septal areas (i.e., medial and triangular septal nuclei) to the bed nucleus of the stria terminalis and dinencephalic areas (i.e., periventricular area, medial and lateral preoptic areas, anterior hypothalamus, and retrochiasmatic zone medial to the optic tract) (150). GnRH neurons send axonal projections primarily to the median eminence to regulate anterior pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), collectively known as the gonadotropins. In turn, the gonadotropins act on the gonads to regulate steroidogenesis and gametogenesis (LH and FSH, respectively).

The excitatory amino acids (EEAs) are ubiquitous in the central nervous system and are capable of regulating gonadotropin secretion. Specifically, both *in vivo* and *in vitro*, glutamate administration leads to an increase in GnRH and LH concentrations (14, 41, 119). Glutamate often acts on the NMDA receptor and may act on this subtype of glutamate receptor to regulate GnRH and gonadotropin secretion. For example, icv infusions of NMDA lead to increased LH secretion in gonadectomized male and female rats (120, 129). Moreover, NMDA receptor antagonists lead to reductions in GnRH and LH levels (5). Because glutamate binds to NMDA receptors resulting in the influx of Ca^{2+}, thereby increasing NOS activity, nitric oxide may play a role in GnRH regulation.

NOS-containing neurons are localized in brain regions dense with GnRH neurons and fibers (e.g., medial preoptic area (MPOA), organum vasculosum of the lamina terminalis (OVLT), hypothalamus, median eminence (ME)); however, neither nNOS nor nNOS mRNA is typically expressed in the same neurons as GnRH, although nNOS cells often surround GnRH neurons (54, 58).

This observation lends further support to the hypothesis that NO may regulate GnRH release. Indeed, sodium nitroprusside (SNP), an NO donor, stimulates hypothalamic GnRH release in a dose-dependent manner *in vitro* (103, 136). Conversely, icv injections of NOS inhibitors reduce pulsatile LH release within 20 min (134). Incubation of hypothalamic explants with the NOS inhibitor NMMA blocks the increased GnRH release typically observed after the addition of glutamic acid to the preparation (136). Likewise, pretreatment with L-NAME antagonizes the NMDA-induced increase in plasma LH seen in male and female rats (129).

These data suggest that glutamate may act on the NMDA receptor to modulate NOS activity and, in turn, NOS acts on GnRH neurons to regulate the hypothalamic–pituitary gonadal (HPG) axis. Because norepinephrine (NE) is capable of increasing GnRH secretion (135), it is possible that NO acts to increase levels of NE to regulate GnRH or, alternatively, glutamate acts on NE neurons, and NE stimulates the production and release of NO to modulate GnRH. In one study, glutamic acid-induced release of GnRH was prevented by the α receptor blocker, phentolamine; however, increased GnRH release induced by sodium nitroprusside was not blocked by phentolamine (71). This study suggests that glutamate activates the release of NE, and NE acts on α receptors on NOergic neurons to increase NOS activity; NO then increases the secretion of GnRH. Further support for this pathway is provided by the observation that glutamate increases NE release *in vitro* (111), and incubation of hypothalamic explants with NE increases NOS activity; this increased NOS activity is blocked by the addition of prazosine, an α_1 adrenergic receptor antagonist (25). Soluble guanylyl cyclase has not been colocalized with GnRH in neurons. NO probably acts to increase GnRH release by activating other molecules or by affecting soluble guanylyl cyclase in adjacent cells; cGMP levels are elevated in hypothalamic explants treated with SNP (103).

Previous reports suggest that prostaglandin E_2 (PGE_2) is required for the release of GnRH (117, 118). Norepinephrine increases PGE_2 from hypothalamic explants; this increase in PGE_2 is blocked by the addition of NMMA to the medium (135). These data suggest that activation of NOS and the resulting production of NO by norepinephrine may lead to PGE_2 production required to induce the release of GnRH. There is some support for this hypothesis (reviewed in 94). In order for PGE_2 to be released, phospholipase A_2 (PLA_2) must be activated for membrane phospholipids to be converted into arachidonate, the substrate for cyclooxygenase (94). Because intracellular calcium can activate PLA_2, it is conceivable that NE-induced activation of NOS may lead to increased intracellular calcium via increased cGMP levels (reviewed in 94). In turn, this increased intracellular calcium could activate PLA_2, leading to activation of PGE_2 and GnRH secretion.

Recent evidence suggests that GnRH secretion may also be regulated through NO-modulation of γ-aminobutyric acid (GABA) (146). NO can stimulate the release of GABA; SNP increases GABA release in cortical neurons and MBH explants *in vitro* (116, 146). In turn, GABA can have dual effects on GnRH

secretion in males and females (91, 93); GABA leads to inhibition of GnRH in the MPOA of females, and stimulation of GnRH release in the arcuate nucleus-median eminence region (93). Additionally, one recent study has shown that GABA attenuates the release of GnRH from MBH explants (146). Taken together, these data provide indirect evidence that NO may stimulate the release of GABA, leading to alterations in GnRH release. Presumably, in the MBH, the increased release of GnRH by NE stimulation of NO may be regulated by an opposing decrease in GnRH secretion by NO-induced elevation in GABA (146).

Another mechanism through which GnRH release may be regulated is through interactions between endogenous opioids and NO. It is well-established that opiates, particularly β-endorphin, exert an inhibitory effect on GnRH release (43, 76). Endogenous opioids may inhibit GnRH secretion by modulating NO synthesis (128). Naloxone, an opiate antagonist, was capable of increasing cGMP/NO efflux in the MPOA of rats concomitant with an increase in pituitary LH (128). This increase in cGMP/NO efflux and LH release was attenuated by pretreatment with morphine, a μ opiate receptor agonist (128). In sum, these data provide preliminary evidence that GnRH secretion may be dually regulated through excitatory influences (mediated through the NMDA subtype of glutamate receptor) and inhibitory effects (mediated through endogenous opioids effects on the μ receptor) on NO and cGMP.

In addition to the effects of NO on GnRH secretion, NO is also involved directly in ovulatory processes. Treatment with L-NAME inhibits ovulation in rats (94, 149). Fertility is reduced among nNOS−/− female mice. To test for physiological defects, which could account for reduced fertility, the rate of ovulation, as determined by the number of rupture sites on the ovaries, as well as the number of oocytes recovered from the oviducts, was evaluated. Female wild-type (WT) and nNOS−/− mice did not differ in these parameters after mating with a WT male (75). Female nNOS−/− mice had fewer ovulation rupture sites than WT females after GnRH treatment. Despite equivalent numbers of ovulation rupture sites after a superovulating dose of pregnant mare serum gonadotropin (PMSG), fewer oocytes were recovered from the oviducts of nNOS−/− mice compared to WT mice (75). Thus, nNOS−/− female mice display normal ovulation during natural estrus or after estrus induction with GnRH injections, suggesting that the central mechanisms of ovulation are intact. Furthermore, ova capture by the fallopian tubes was impaired during superovulation. Taken together, these results indicate that nNOS contributes to female fertility. Additional studies must be conducted to tease apart the contribution of eNOS and nNOS to normal ovulation.

In all vertebrate species examined thus far, GnRH is required for the regulation of gonadal steroids, gamete production, and ovulation. Likewise, a tightly regulated hormonal milieu is required for the appropriate expression of mating behavior. Thus, nitric oxide may play a critical role in regulating mating behavior and other gonadal steroid-dependent behavioral and physiological processes. Evidence will be presented below in support of these possibilities.

BEHAVIORAL EFFECTS OF NO

Reproductive Behaviors

NO affects reproductive behavior. There have been reports that both sexual motivation and performance are influenced by NO, although much of the effects of NO on male sexual performance reflect the direct consequences of the well-established effects of NO on penile erectile function (23). Generally, sexual motivation in male rats is not affected; treatment of male rats with NOS inhibitors (i.e., L-NAME) did not reduce the latency to first mount or the number of mounting attempts of estrous females by male rats (11, 66). Male nNOS−/− mice displayed normal sexual motivation to mount with estrous females; however, male nNOS−/− mice exhibited inappropriate persistent mounting attempts of anestrous females (112).

Administration (ip) of L-arginine, the natural substrate for NOS, increased the percentage of naive male rats copulating and improved sexual performance in sexually experienced males (9). These behavioral effects were reversed in rats receiving systemic injections of L-NAME. Intracerebroventricular (icv) administration of L-arginine did not affect reproductive behavior. L-NAME infusion (icv) prevented ejaculation of naive rats (100 µg/rat), but had no effects on ejaculation of experienced male rats at doses up to 300 µg/rat (9). Systemic treatment of sexually naive male rats with 30 or 60 mg/kg of L-NAME reduced the number of ejaculations by 43 and 86%, respectively (11). In both experimental groups, the number of intromissions was reduced, although mounting behaviors were elevated (11); increased number of mounts is common with penile erectile dysfunction in rats (e.g., 130). The number of ultrasonic vocalizations increased; these vocalizations are an important component of sexual behavior in rats (8). Systemic L-NAME administration impaired copulation, reduced the number of *ex copula* penile erections, increased the number of *ex copula* seminal emissions, and decreased the latency of first seminal emission of male rats (66). Treatment with L-arginine increased the number of penile reflexes, but had no other effects on male rat reproductive behavior (66). There is no obvious explanation for the conflicting reports on the behavioral effects of L-arginine treatment in rats (e.g., 9, 66). Male mice with targeted deletion of the gene encoding nNOS exhibited penile intromissions and ejaculations equivalent to those in WT males when paired with estrous females (24). This outcome was surprising because it had been firmly established using L-NAME and other NOS inhibitors that NO mediated penile erectile function (reviewed in 20, 21, 86). However, it appears that eNOS is upregulated in nNOS−/− animals and that eNOS, not nNOS, mediates penile erections (24).

NO functions in the brain to affect sexual behavior. Expression of NOS mRNA in the paraventricular nucleus of the hypothalamus was nearly doubled in sexually potent male rats compared to impotent males (9). Reproductive performance in male golden hamsters (*Mesocricetus auratus*) is dependent upon chemosensory and endocrine stimuli processed in limbic neural circuits (127). Neurons expressing NOS in hamster brains were discovered in the

medial amygdala nucleus (Me), the bed nucleus of the stria terminalis (BNST), and the MPOA (55). NOS-positive fibers were observed in the stria terminalis and the ventral amygdalo–fugal pathway; this pathway connects the Me with the BNST and MPOA. Several of the NOS-positive neurons in the ME and the medial preotic nucleus (MPN) also contained androgen receptors. Castration decreased the number of NOS-positive neurons in the MPN, suggesting that NOS may be regulated by gonadal steroids (55).

The involvement of NO in male reproductive behavior appears to be conserved throughout vertebrate evolution. NO plays an important role during courtship of urodele crested newts (*Triturus carnifex*) (174). Courtship is complex in this species with four distinct stages of courtship. Females that are receptive remain relatively immobile and close to the male during his approach, fanning, lashing, and spermatophore deposition, whereas nonreceptive females move away from courting males (174). Some males do not court in the presence of receptive females. Brain NOS was measured in receptive and nonreceptive females, in males at various points during courtship, and in noncourting males. Mating success of male newts was characterized by high brain NOS activity that progressively increased during each of the courtship phases and returned to baseline levels after spermatophore deposition (174). Inactive males exhibited the lowest brain NOS activity. There was no difference among female newts regardless of their receptive state (174). Thus, NO appears important in male, but not female, newt reproductive behavior.

NO appears to mediate female sexual behavior in rats (88). Receptive behavior can be elicited in estrogen-primed, ovariectomized rats with progresterone treatment. Receptive behavior is usually indicated by the display of the mating posture, lordosis, in response to male mounting behavior (113). When N^G-monomethyl-L-arginine was microinjected icv into the third cerebral ventricle of awake and freely moving female rats, lordosis was not observed after progesterone administration (88). Microinjections (icv) of N^G-monomethyl-D-arginine, a compound that does not inhibit NO production, did not inhibit lordosis after progesterone administration. Microinjections (icv) of SNP, a substance that spontaneously releases NO, promoted the display of lordosis of estrogen-primed rats in the absence of progesterone. Lordosis that was evoked in estrogen-primed female rats by either progesterone or SNP could be blocked by microinjections (icv) of GnRH antiserum. Thus, it appears that progesterone normally causes the release of NO that stimulates GnRH secretion from the hypothalamus which facilitates lordosis in rats.

In contrast, mating behavior appeared normal in nNOS−/− female mice, although fertility rates are about 50% of those of WT mice (75, 112). nNOS−/− females did not differ from WT females in the latency to first lordosis posture or in the number of lordosis postures during a 30-min mating test. If impregnated, then nNOS−/− and WT females had equivalent litter sizes at birth and at weaning, and their litters displayed normal sex ratios (75). As noted above, NO appears to be involved in the mechanisms of ovulation and the reduction of fertility among nNOS−/− females might reflect this physiological defect.

Aggressive Behaviors

There are no published reports that L-NAME or other nonspecific NOS inhibitors affect aggression. However, nNOS−/− males are dramatically more aggressive and sexually persistent than WT mice (112). When examined in an intruder-resident test of aggression, these nNOS−/− mice engaged in three to four times more aggressive encounters than WT mice. Nearly 90% of the aggressive encounters were initiated by the nNOS−/− animals. Similar results were obtained in dyadic or group encounters in neutral arenas. In all test situations, male nNOS−/− mice rarely displayed submissive behaviors.

Behavioral studies of mice with targeted deletion of specific genes suffer from the criticism that the gene product is not only missing during the testing period, but is also missing throughout development when critical ontogenetic processes, including activation of compensatory mechanisms, may be affected. To address this criticism, mice were treated with 7-nitroindazole (7-NI) (50 mg/kg ip), which specifically inhibits nNOS formation *in vivo* (37). Mice treated with 7-NI displayed substantially increased aggression in two different tests of aggressiveness compared to control animals (37). Drug treatment did not affect nonspecific locomotor activities. Importantly, NOS activity in brain homogenates was reduced >90% in 7-NI-treated mice. Similarly, immunohistochemical staining for citrulline revealed a dramatic reduction in 7-NI-treated animals (37). Taken together, 7-NI inhibited NOS staining in the limbic system and reduced citrulline immunohistochemical staining, suggesting that NO formation was virtually eliminated in these experimental animals. Because 7-NI treatment in WT mice caused aggression to be elevated to the levels displayed by nNOS−/− mice, it appears that nNOS is an important mediator of aggression.

Plasma androgen concentrations can affect the display of aggressive behavior. There were no differences between nNOS−/− and WT mice in blood testosterone concentrations either before or after agonistic encounters. However, recent data on castrated nNOS−/− males suggest that testosterone is necessary, but not sufficient, to promote increased aggression (77). Castrated nNOS−/− mice displayed low levels of aggression that were equivalent to the aggression observed among castrated WT males. Androgen-replacement therapy restored the elevated levels of aggression in nNOS−/− mice. Importantly, inappropriate aggressiveness was never observed among female nNOS−/− mice; however, aggressive behavior was not examined in female nNOS−/− mice in the context of maternal aggression during which WT females are highly aggressive toward an intruder. There were no sensorimotor deficits among the mutant mice to account for the increased aggression.

Regulation of Feeding

There has been increasing evidence that nitric oxide is involved in the regulation of food intake. NOS has been localized to areas known to be involved

in the control of feeding, particularly in the paraventricular nucleus of the hypothalamus and the nucleus tractus solitarius (see above; 151, 169). In rodents, ip injections of the NOS inhibitor, N^G-nitro-L-arginine (L-NNA), lead to a dose-dependent suppression of feeding in food-deprived mice (104–106) and rats (36, 153). When L-arginine is coadministered with L-NNA, the effects of the NOS inhibitors on food intake are abated (105, 106). Similar results are obtained when NOS inhibitors are administered icv, providing further evidence for a central role of NO in regulating feeding (36). These effects are not limited to mammals; ip or icv administration of NOS inhibitors decreases feeding behavior in chickens (28, 29). The effects of pharmacological treatment do not appear to be the result of nausea, leading to decreased food intake. In both chickens and mice, long-term food consumption is unaffected by drug treatment, suggesting that treatment with NOS inhibitors did not result in a conditioned taste aversion (indicative of illness) (29, 104).

In genetically obese Zucker rats, L-NAME or L-NNA maintains the ability to decrease food intake (153, 155, 158). Likewise, NOS inhibitors reduce food intake in the obese (ob/ob) mouse, another genetic animal model of obesity (107). These data provide further support for a role of NO in food intake and may offer new insights into treatments for obesity.

Because NOS inhibitors limit food intake in normal and genetically obese mice, one would expect NOS levels to be increased in the brains of food-deprived or obese animals compared to animals fed *ad lib* or lean mice. This possibility has received some support. For example, ob/ob mice have increased levels of NOS and NOS mRNA in the hypothalamus compared to lean controls (108). Likewise, food deprivation increased diencephalic NOS activity in the brains of rats (153). Contrary to these findings, other studies report the opposite pattern of NOS activity; NOS levels are decreased in the hypothalamus of Zucker rats (109). Likewise, NOS mRNA levels were reduced in the SON and PVN of rats that were food-deprived for 4 days (122). Regardless of these conflicting findings, these data implicate nitric oxide in feeding behavior and potentially the regulation of body mass.

It is possible that NO regulates feeding through other neurotransmitter systems. Central NE administration increases food intake in rats (52). This increase is reduced by coadministration of α_2 but not by α_1 antagonists, suggesting that NE acts on α_2 receptors to modulate feeding (143). In chickens, a specific α_2 agonist, clonidine, increases food intake; coadministration of L-NNA attenuates this increased food intake in a dose-dependent manner (28). Taken together, these data suggest that NO may interact with the α_2 adrenergic system to regulate food intake.

Recent evidence suggests that NO may influence feeding through its actions on the central serotonergic system (5-HT). Food deprivation leads to a reduction in brain 5-HT levels in rats (154). As previously mentioned, L-NNA (i.e., a NOS inhibitor) reduces food intake in food-deprived animals (36, 104–106, 155). Additionally, administration of L-NNA increases 5-HT levels in the diencephalon of rats (153). This inverse relationship between 5-HT and NOS levels suggests that NO may regulate feeding through a 5-HT mechanism. In rats,

pretreatment with metergoline (a 5-HT receptor antagonist) attenuated the hypophasia induced by administration of L-NNA (154). The 5-HT receptor antagonists, methylsergide and ritanserin, had no effect on the reduced food intake caused by NOS inhibitors in mice (65). In Zucker rats, NOS inhibitors increase brain serotonin metabolism after either acute or chronic administration of L-NNA (153). However, lean littermate controls of Zucker rats increase brain 5-HT metabolism after acute, but not chronic, L-NNA treatment (153). Taken together, these studies suggest that NO may act through central serotonergic mechanisms, and 5-HT metabolism differences may explain obesity in Zucker rats.

In sum, NO appears to be involved in the regulation of food intake. Future studies are necessary to determine the mechanism(s) through which NO operates to modulate food intake. Conceivably, NO may act through central neurotransmitter systems, peripheral means, or a combination of both mechanisms. Recent studies have shown that NO may be involved in relaxation of the esophageal sphincter and may be involved in the relaxation of the stomach to accommodate food (38, 172). These data provide evidence for a peripheral role for nitric oxide in regulating food intake. Regardless of mechanism, future studies are likely to uncover additional important roles for nitric oxide in feeding.

Learning and Memory

Inhibition of NOS impairs spatial learning in mice (165) and rats (13, 42, 99, 100). It appears that NO is important during initial acquisition, but according to many workers the role of NO upon the mechanisms of spatial learning remains unspecified (6, 87; see below). NOS inhibitors might affect learning by affecting blood pressure or other nitrogen-dependent processes (15). NOS inhibition has also been reported to impair social and olfactory memory (13), as well as performance in some inhibitory avoidance learning paradigms in rats (10, 63). Other labs have reported no effect of NOS inhibition on passive avoidance (e.g., 165). Inhibition of NOS does not impair visual or spatial discrimination (161) or sensorimotor or motivational processes (42). Sensorimotor skills and sexual motivation are not impaired in nNOS−/− mice (112).

The role of NO in learning and memory appears to have been conserved throughout the animal kingdom. NOS inhibition has been reported to impair learning and memory processes in honeybees (*Apis mellifera*) (110), crickets (*Pteronemobius* spp) (69), octopuses (*Octopus vulgaris*) (139), and goldfish (*Carassius auratus*) (139).

Many reports suggest that NO is part of the mechanisms underlying learning and memory. LTP and LTD are stable and enduring increases or decreases, respectively, in the magnitude of neuronal responses after afferent cells to the region have been stimulated with bursts of electrical stimulation of relatively

high or low frequency, respectively. Because afferent neural activity affects firing patterns, the existence of retrograde messengers has been proposed (144, 170). NO is a candidate molecule to serve as the retrograde messenger in LTP and LTD (19), although there have been conflicting reports about the role of NO in the mediation of LTP and LTD (87, 157, 163, 164).

Again, LTP and LTD are forms of synaptic plasticity that are thought to be involved with learning and memory (12, 83). Calcium influx through postsynaptic NMDA receptor channels and enhancement of glutamate release by Schaeffer collaterals are thought to be required for induction of LTP in the CA1 region of hippocampal slices; further, it has been hypothesized that LTP requires release of a retrograde messenger from postsynaptic CA1 pyramidal cells. NO may act as a retrograde messenger because NO produces an increase in the frequency of spontaneous miniature excitatory postsynaptic potentials and observations that direct application of NO elicits LTP. The establishment of LTP is blocked by NOS inhibitors and hemoglobin that binds NO blocks LTP. Furthermore, NOS inhibitors directly injected into CA1 pyramidal neurons block LTP (13, 56, 115, 145). Support for the contention that postsynaptically generated NO acts directly on the postsynaptic neuron to produce LTP (4) is the observation that intracellular application of a NO scavenger or use of a UV-sensitive NO donor elicits LTP. Similar evidence exists for a role of NO in LTD (32, 148). Genetic knockout of nNOS or eNOS failed to support the role of NO in LTP. LTP in nNOS null or eNOS null mice appeared normal and can still be blocked by NOS inhibition (114, 152). Kandel and colleagues recently generated double knockout mice lacking both nNOS and eNOS expression and reported that hippocampal LTP in the striatum radiatum of CA1 was markedly reduced (152). These mutant mice showed normal LTP in the striatum oriens of hippocampal CA1. Thus, there is both NO-dependent and NO-independent LTP. LTP in wild-type mice may be mediated through eNOS, as adenovirus-mediated disruption of eNOS activity in wild-type hippocampal slices eliminates NO-dependent LTP (72). Recent studies suggest that NO might help regulate the threshold for LTP induction (87).

Our recent studies also support a role for NO in synaptic plasticity. Calcium-dependent activation of nNOS and NO generation via stimulation of the NMDA receptor in cultured cortical neurons activates the Ras/ERK (extracellular signal receptor kinase) pathway (H.Y. Yun, M. Gonzalez-Zulueta, V.L. Dawson, and T.M. Dawson, submitted for publication). NMDA-stimulated phosphorylation of cyclic AMP-response element binding protein (CREB), a downstream effector of ERK, is also NO-dependent (Yun *et al.*, submitted for publication). Activation of the Ras/ERK pathway by NO may be mediated by direct activation of Ras GTPase activity presumably by nitrosylation of cysteine through a redox-sensitive interaction (79, 80). Because calcium-dependent activation of Ras-MAPK pathway is considered to be a major pathway of neural activity-dependent long-term changes in nervous system (44), NO may be a key mediator linking activity to gene expression and long-term plasticity.

CONCLUSIONS

During the past decade, the known biological functions of NO have continued to increase dramatically. NO is emerging as an important regulator of neuroendocrine and neuronal function; this molecule has also become a well-established mediator of behavior and probably serves in the interplay between behavior and neuroendocrine function. NO appears to regulate many hypothalamic–hypophyseal neuroendocrine processes from hormone secretion to portal blood flow. Thus, NO may serve as an important chemical signal for intercell communication and may play a fundamental role in neuroendocrine function and behavior. It must be noted that most experiments conducted to date do not specifically reveal the involvement of NO in a particular neuroendocrine regulatory system. The extent to which studies of NO and neuroendocrine function reflects only a general role of NO in neural activation remains unspecified. With the availability of specific NOS inhibitors, and mice with conditional targeted gene inactivation of specific isoforms of NOS, additional physiological and behavioral roles of NO are likely to be discovered.

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