The Mechanism of Action of Cytokines to Control the Release of Hypothalamic and Pituitary Hormones in Infection

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ABSTRACT: **During infection, bacterial and viral products, such as bacterial lipopolysaccharide (LPS), cause the release of cytokines from immune cells. These cytokines can reach the brain by several routes. Furthermore, cytokines, such as interleukin-1 (IL-1), are induced in neurons within the brain by systemic injection of LPS. These cytokines determine the pattern of hypothalamic–pituitary secretion that characterizes infection. IL-2, by stimulation of cholinergic neurons, activates neural nitric oxide synthase (nNOS). The nitric oxide (NO) released diffuses into corticotropin-releasing hormone (CRH)–secreting neurons and releases CRH. IL-2 also acts in the pituitary to stimulate adrenocorticotropic hormone (ACTH) secretion. On the other hand, IL-1α blocks the NO-induced release of luteinizing hormone-releasing hormone (LHRH) from LHRH neurons, thereby blocking pulsatile LH but not follicle-stimulating hormone (FSH) release and also inhibiting sex behavior that is induced by LHRH. IL-1α and granulocyte macrophage colony-stimulating factor (GMCSF) block the response of the LHRH terminals to NO. The mechanism of action of GMCSF to inhibit LHRH release is as follows. It acts on its receptors on γ-aminobutyric acid (GABA)ergic neurons to stimulate GABA release. GABA acts on GABAa receptors on the LHRH neuronal terminal to block NOergic stimulation of LHRH release. IL-1α inhibits growth hormone (GH) release by inhibiting GH-releasing hormone (GHRH) release, which is mediated by NO, and stimulating somatostatin release, also mediated by NO. IL-1α-induced stimulation of PRL release is also mediated by intrahypothlamic action of NO, which inhibits release of the PRL-inhibiting hormone dopamine. The actions of NO are brought about by its combined activation of guanylate cyclase-liberating cyclic guanosine monophosphate (cGMP) and activation of cyclooxygenase (COX) and lipoxygenase (LOX) with liberation of prostaglandin E_2 and leukotrienes, respectively. Thus, NO plays a key role in inducing the changes in release of hypothalamic peptides induced in infection by cytokines. Cytokines, such as IL-1β, also act in the anterior pituitary**

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gland, at least in part via induction of inducible NOS. The NO produced inhibits its release of ACTH. The adipocyte hormone leptin, a member of the cytokine family, has largely opposite actions to those of the proinflammatory cytokines, stimulating the release of FSHRF and LHRH from the hypothalamus and FSH and LH from the pituitary directly by NO.

INTRODUCTION

Our knowledge of neuroimmunomodulation has undergone explosive growth since the discovery of the structure of many pro- and antiinflammatory cytokines and the revelation that certain other hormones are members of the cytokine family, which includes PRL, growth hormone (GH), and the newly discovered adipocyte hormone leptin. Nearly all of these cytokines have roles as autocrine, paracrine, and hormonal agents that play an important part not only in normal homeostasis of the body, but also in the response to infections.

The induction of fever following injection of bacterial lipopolysaccharide (LPS) was the first example of neuroimmunomodulation, because it was early shown that LPS not only apparently induced fever on its own, but also released an endogenous pyrogen that circulated to the brain and induced fever. In the early 1960s, it was discovered that injection of purified LPS into dogs induced fever after a delay and a concomitant increase in plasma cortisol. These findings suggested that endogenous pyrogen reached the brain and induced not only fever, but also release of corticotropin-releasing hormone (CRH) that activated adrenocorticotropic hormone (ACTH) followed by cortisol release.[1] Because of the high potency of cytokines, the amounts circulating in the blood after LPS were too small to isolate and determine structure of these compounds. In the 1980s and 1990s, however, the structure of many of them was revealed, and it is now apparent that endogenous pyrogen was at least in part interleukin-1 (IL-1).

Understanding of the mechanism of action of cytokines to alter hypothalamic–pituitary function was also made possible by discovery of the many classical transmitters and hypothalamic peptides that control the release of the various pituitary hormones from the anterior lobe of the pituitary gland. The release of the various pituitary hormones is controlled by neurohormones that are released into the hypophyseal portal vessels that transport them to the anterior pituitary gland where they stimulate or inhibit particular pituitary cell types.[2,3] There is a family of peptides that stimulates the release of the pituitary hormones, namely, corticotropin-releasing hormone (CRH), luteinizing hormone-releasing hormone (LHRH), follicle-stimulating hormone (FSH)-releasing factor (FSHRF), GH-releasing hormone (GHRH), GH release-inhibiting hormone (somatostatin), prolactin (PRL)-inhibiting and -stimulating factors, and thyrotropin (T)RH. Several other peptides, in particular vasopressin, oxytocin, and atrial natriuretic peptide, have modifying actions on the release of pituitary hormones by actions directly on the gland, whereas catecholamines can also influence the secretion of pituitary hormones by direct action on the gland, the principal effect being the inhibitory action of dopamine (DA), the most potent PRL-inhibiting hormone. The pattern of release of pituitary hormones in infection is brought about by cytokine-induced release of hypothalamic peptides, which alter the release of pituitary hormones. Furthermore, direct actions of these cytokines on the pituitary gland itself

can alter pituitary hormone release and responsiveness of the gland to hypothalamic peptides.[4]

Introduction of bacteria into the body causes the liberation of toxic, soluble products of the bacterial cell wall, for example, LPS, which circulates in the blood and acts on immune cells, particularly monocytes and macrophages. LPS combines with its receptors on these cells and induces the synthesis and release of various cytokines, such as IL-1, tumor necrosis factor (TNF), IL-6, IL-2, γ-interferon, and others. The pattern of release probably depends on the infective agent and the severity of the infection.[4]

Because there is no arterial blood supply to the anterior pituitary gland, cytokines released into the circulation only reach the hypophyseal portal capillaries in the median eminence (ME) of the tuber cinereum via the anterior hypophyseal arteries.[5] Cytokines (molecular mass 15 kDa) diffuse into the ME, because there is little or no blood–brain barrier there. Therefore, the concentration of cytokines delivered to the anterior lobe sinusoids by the long hypophyseal portal veins will be lower than in arterial blood. The concentration of cytokines in blood reaching the anterior lobe via the short portal vessels draining the neural lobe of the pituitary is similarly reduced by diffusion into neural lobe tissue. One-third of the blood supply of the anterior lobe is provided by these vessels.[5]

Transport of cytokines to the hypothalamus presents a more difficult problem except in regions where the blood–brain barrier is defective in the ME and other circumventricular organs: the organum vasculosum lamina terminalis, the subfornical organ, the subcommissural organ, the area postrema, and the pineal gland.[6] Permeability is probably also enhanced in the choroid plexus. Banks and Kastin[7] have reported a transport system that carries IL-1 and other cytokines into the brain. Clearly, peripherally injected cytokines effectively reach the brain, because IL-1 injected intravenously (i.v.) can induce fever and increase ACTH secretion by hypothalamic action.[4]

Evidence is also mounting for the production of various cytokines by glial elements within the brain. This appears to be the case for IL-1, IL-2, and IL-6 and perhaps for others.[6] Bacterial LPS appears to be capable of increasing the production of cytokines such as IL-6 in the anterior pituitary.[8]

In addition, a neuronal system that produces IL-1β has been described in humans.[9] The cell bodies of these neurons are located in the paraventricular nucleus (PVN) with axons projecting to the ME, so that IL-1β released from these neurons could reach the anterior lobe and even the peripheral circulation after uptake by portal vessels.

The research to be reviewed here indicates that NO has a powerful influence on the secretion of not only the hypothalamic peptides, but also classical synaptic transmitters, such as catecholamines and γ-amino butyric acid (GABA). NO also has a powerful effect in suppressing or stimulating the release of pituitary hormones directly. NO is formed in the body by NOS, an enzyme that converts arginine in the presence of oxygen and several cofactors into equimolar quantities of citrulline and NO. In aqueous solutions, NO, a free radical, decomposes to nitrate, which, in the presence of superoxide, further decomposes into two free radicals, nitrite and hydroxyl ions. All of these free radicals are powerful oxidizing agents. The half-life of NO in aqueous solutions is 5 to 10 seconds. However, the half-life of the soluble

gas in living systems is prolonged by combination with other substances to form nitroso compounds, which slowly release the gas.[10]

There are three isoforms of the enzyme. One, termed inducible NOS (iNOS), is formed principally by immune cells, such as macrophages, but also by other cells, such as vascular endothelial cells. Bacterial infection leads to release of products of their cell walls, such as bacterial LPS, which combines with receptors on the surface of macrophages and these other cells. The LPS–receptor combination acts in the nucleus to induce synthesis of iNOS mRNA, which then synthesizes iNOS. The induction of iNOS mRNA occurs within an hour or two, and NO synthesis begins within 2 hours. It reaches a peak at 18 hours, declining to nearly control levels by 24 hours following a single injection of LPS. LPS also induces mRNA for various cytokines, such as IL-1, -2, -6, and TNF-α, which are then synthesized. These also act on the cell surface receptors of the above-mentioned cells to produce iNOS mRNA and iNOS formation. The large quantities of NO produced then interact with the bacteria or viruses and cause cell death by inactivating metabolic enzymes. These amounts of NO are also toxic to neighboring cells, but not to the cells containing iNOS, and cause apoptosis and cell death in the region of production of NO.[10,11]

The endothelial NOS (eNOS) is formed in vascular endothelial cells following cholinergic stimulation. It is a constitutive enzyme that requires an increase in intracellular free calcium (CA^{2+}) stimulated by the activated muscarinic-type cholinergic receptors on the endothelial cells. The increased [CA^{2+}] interacts with calmodulin and activates the enzyme. The activated enzyme produces NO, which diffuses to overlying smooth muscle, and activates soluble guanylate cyclase (GC), which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). This relaxes the vascular smooth muscle. It probably plays an important role in control of vascular tone, and the large amounts of NO produced by iNOS during infection can cause vascular collapse in the toxic shock syndrome.[10]

Garthwaite *et al.*[12] found evidence for NO production in incubates of hippocampal slices that were stimulated to produce long-term potentiation. Palmer *et al.*[13] showed that this substance was indeed NO, leading Bredt and Snyder[14] to isolate neural NOS (nNOS). They studied the distribution of the enzyme by immunocytochemistry. Thus, NO is the first gaseous transmitter that, instead of acting on cell surface receptors, diffuses into the cell to activate or inhibit intracellular enzymes.

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 numbers of nNOS-containing neurons were also found in the hypothalamus, particularly in the paraventricular and supraoptic nuclei with axons projecting to the ME and neural lobe, which also contained large amounts of nNOS. These findings indicated that the enzyme was synthesized at all levels of the neuron from perikaryon to axon terminals.[15]

Because of this distribution in the hypothalamus in regions that contain peptidergic neurons that control pituitary hormone secretion, we decided to determine the role of this soluble gas in hypothalamic–pituitary function. The approach was to incubate medial basal hypothalamic (MBH) explants with sodium nitroprusside (NP), which spontaneously liberates NO, and determine whether this altered the release of various hypothalamic transmitters. Hemoglobin, which scavenges NO by a reaction with the heme group on the molecule, and inhibitors of NOS (such as N^G-monomethyl-L-

arginine (NMMA), a competitive inhibitor of NOS), was also used to determine the effects of decreased NO. Two types of studies were performed. In the first set of experiments, MBH explants were preincubated *in vitro* and then exposed to neurotransmitters that modify the release of the various hypothalamic peptides in the presence or absence of inhibitors of the release of NO. The response to NO itself, provided by sodium NP, was also evaluated. Anterior pituitaries were incubated similarly *in vitro*, and the effects of these compounds that increase or decrease the release of NO into the tissue were examined.

In order to determine whether the results *in vitro* also held *in vivo*, substances were injected into the third ventricle of the brain of conscious, freely moving animals to determine the effect on pituitary hormone release.

ROLE OF THE HYPOTHALAMIC–PITUITARY–ADRENAL SYSTEM IN INFECTION

The hypothalamic–pituitary response to infection can be mimicked by the injection of bacterial LPS i.v. or i.p. This induces an identical pattern of pituitary hormone secretion as that seen in infection. There is a very rapid increase in plasma ACTH and PRL within a few minutes following i.v. injection of LPS. The response is dose-related and is accompanied by a rapid inhibition of LH and TSH but not FSH secretion. GH secretion is stimulated in humans but suppressed in the rat.[16]

Recent work indicates that central nervous system infection is a powerful inducer of cytokine production in glia and neurons of the brain, which causes induction of iNOS and production of potentially toxic quantities of NO. Following i.v. injection of an intermediate dose of LPS, there was an induction of IL-1α immunoreactive neurons in the preoptic–hypothalamic region.[16] These cells were shown to be neurons by the fact that double staining revealed the presence of neuron-specific enolase. The neurons were found in saline-injected control animals, suggesting that they are normally present, but they increased in number by a factor of two within two hours after injection of LPS. They are located in a region that also contains the thermosensitive neurons. They may be the neurons that are stimulated to induce fever following injection of LPS. They have short axons that did not clearly project to the areas containing the various hypothalamic-releasing and -inhibiting hormones, but they could also be involved in the stimulation or inhibition of their release, which occurs following infection.

This study led to further research, which demonstrated that i.p. injection of a moderate dose of LPS induced IL-1β and iNOS mRNA in the brain, anterior pituitary, and pineal glands. The results were very exciting because an induction of IL-1β and iNOS mRNA occurred with the same timecourse as found in the periphery following injection of LPS, namely, clear induction of IL-1β followed by iNOS mRNA within two hours, reaching a peak in 4–6 hours, followed by a decline to near basal levels at the next measurement by 24 hours after the injection. The induction of both mRNAs occurred in the meninges; the choroid plexus; the circumventricular organs, such as the subfornical organ and ME; in the ependymal cells lining the ventricular system; and very suprisingly in parvocellular neurons of the PVN and arcuate nucleus (AN), areas of particular interest because they contain the hypothalamic-releasing

and -inhibiting hormone-producing neurons and also other neurotransmitters controlled by NO.[17]

The greatest induction occurred in the anterior lobe of the pituitary, where the iNOS mRNA was increased at two hours by a factor of 45 and in the pineal where the activity was increased by a factor of 7 at six hours, whereas the increase in the PVN was fivefold. At six hours, the MBH was found to have an increased content of NOS measured *in vitro*, and the collected cerebrospinal fluid (CSF) had increased concentrations of the NO metabolite nitrate. These results indicate that the increase in iNOS mRNA was followed by de novo synthesis of iNOS that liberated NO into the tissue and also into the CSF. Presumably, LPS was bound to its receptors in the circumventricular organs and in the choroid plexus. These receptors, as in macrophages, activated DNA-directed IL-1β mRNA synthesis which, in turn, caused the synthesis of IL-1β. IL-1β then activated iNOS mRNA and synthesis.[17]

How can neurons in the AN and PVN be activated if they are inside the blood–brain barrier? In the case of the AN, the neurons may have axons that project to the ME. These neurons may have LPS receptors on their cell surfaces which then induce IL-β mRNA and IL-1β synthesis. IL-1β then induces iNOS mRNA followed by NO synthesis. Alternatively, LPS acting on its receptors may simultaneously induce IL-β mRNA and iNOS mRNA.

Active transport mechanisms for IL-1 and other cytokines,[7] and perhaps LPS, are present in the choroid plexus. On the basis of our results, the cells of the choroid plexus must have LPS receptors on them. LPS then stimulates IL-1β and iNOS mRNA, followed by synthesis of IL-1β and iNOS in the choroid plexus. LPS and IL-1β are then transported into the CSF. LPS is carried by CSF flow to the third ventricle, where it either crosses the ependyma or acts on terminals of PVN neurons in the ependyma to induce IL-1β and iNOS mRNA.

This massive delayed increased NO production should further increase the effects of NO to maintain the pattern of hypothalamic hormone secretion already induced by LPS. Unfortunately, the effect of inhibitors of NOS on these later stages in the response to LPS or infection have not yet been studied. Interestingly enough, in studies on LHRH release induced by NO, it has been shown that increasing concentrations of NO provided by release from sodium NP produce a bell-shaped dose–response curve in terms of LHRH release with values reaching a peak and then declining as the concentration of NO increases.[18] Therefore, the massive increase in NO produced by iNOS, several hours after injection of LPS, might actually reduce the effects of NO on releasing hormone discharge below the peaks achieved earlier.

In addition to inducing production of proinflammatory cytokines such as IL-1, IL-2, IL-6, and TNFα, LPS also induces production of antiinflammatory cytokines, such as IL-10 and IL-13 and IL-1 receptor antagonist in the brain, pituitary, and pineal gland.[42] In the periphery these inhibit the inflammatory response induced by the proinflammatory cytokines. Limited studies indicate that these antiinflammatory cytokines antagonize the actions of the proinflammatory cytokines in the brain as well as the hypothalamic–pituitary response to infection.[19]

The initial response to LPS is mediated by the constitutive nNOS present in the brain. There is no participation of the NO synthesized by iNOS in this initial response. Indeed, the initial response must be due to action on receptors for LPS on the endings of vagal afferents and also in areas where the blood–brain barrier is not

present, such as the choroid plexus, ME, OVLT, area postrema, and other circumventricular organs. Input to the hypothalamus from LPS by vagal afferents occurs at least in part by activation of the locus ceruleus that sends noradrenergic axons to the hypothalamus to activate CRH release.[20] The noradrenergic axons apparently synapse on cholinergic interneurons in the region of the PVN.[21]

CRH release from hypothalami incubated *in vitro* is controlled by muscarinic cholinergic receptors because it can be blocked by atropine.[21,22] The acetylcholine-producing interneurons in the hypothalamus release acetylcholine, which stimulates a muscarinic-type receptor, which in turn stimulates CRH release from the CRH neurons. Nicotinic receptors also appear to play a stimulatory role.[22] nNOS has been located in neurons in the PVN of the hypothalamus. Stimulated CRH release can be blocked by NMMA, a competitive inhibitor of all forms of NOS. Consequently, CRH release from the neurons in the PVN is stimulated by cholinergic neurons that synapse on these NOergic neurons to activate NOS. NOS synthesizes NO, which diffuses into the CRH neurons and activates CRH release by activating cyclooxygenase I (COX I), leading to the generation of prostaglandin E_2 (PGE_2) from arachidonate (AA). PGE_2 activates CRH via activation of adenylyl cyclase (AC) and generation of cyclic adenosine monophosphate (cAMP). cAMP activates protein kinase A (PKA), which induces exocytosis of CRH secretory granules into the hypophyseal portal vessels which then activates ACTH release from the corticotrophs of the anterior pituitary gland. NO activates not only COX, but also lipoxygenase (LOX), which also plays a role in the activation of CRH release.[23] NO also activates GC, which converts guanosine triphosphate into cGMP. cGMP is postulated to increase the intracellular [Ca^{2+}] required to activate phospholipase A_2 (PLA_2), which converts membrane phospholipids into AA, the substrate for COX and LOX, permitting generation of PGs and leukotrienes, respectively.[21,24]

Activation of CRH release can be blocked by the synthetic glucocorticoid dexamethasone[23,24] and also by blockers of the three pathways of AA metabolism, such as clotrimazol, which blocks epoxygenase which converts AA into epoxides; indomethacin, which inhibits COX; and by 5′8′11-eicisotrionoic acid, which blocks LOX. Thus, CRH release is activated by the AA cascade.[23] α-Melanotcyte-stimulating hormone (αMSH) also inhibits CRH release.[24] Cyclosporin inhibits CRH release as well,[25] probably by inhibiting calcineurin. Calcineurin dephosphorylates NOS, rendering it inactive.

Of the many proinflammatory cytokines, it has been shown that IL-1α or β, TNFα, IL-6, and IL-2 can stimulate ACTH release from the anterior pituitary *in vitro* and *in vivo*.[4] The principal action is probably, at least acutely, on the release of CRH and vasopressin from the hypothalamus, but there are also clear effects at the pituitary level. There have been few studies on the mechanism of this direct pituitary action of cytokines; however, several cytokines such as IL-6 have been found to be produced in pituitaries, and nNOS is also present in the gland as indicated earlier. There are indications that NO participates in inhibiting the response of ACTH to vasopressin.[26] Whether it plays a role in the stimulatory action of the various proinflammatory cytokines on ACTH secretion has not yet been studied.

In our studies LPS itself had no acute effect on ACTH release from hemianterior pituitaries *in vitro*.[16] However, LPS induces cytokine production in the pituitary. Cytokine production would be increased in a few hours and undoubtedly would

modify the responses of the pituitary to the continued altered secretion of releasing and inhibiting hormones.

In addition to the proinflammatory cytokines that we have discussed extensively, it is now clear that there are a number of antiinflammatory cytokines, the first one to be discovered being IL-I receptor antagonist, but IL-10 and IL-13 also serve this role, as indicated above. These are also induced in the brain by LPS and may play roles at the hypothalamic and pituitary levels to diminish the response to the proinflammatory cytokines.

EFFECT OF CYTOKINES AND NO ON THE RELEASE OF LHRH

Our most extensive studies were carried out with regard to the release of LHRH, which controls the release of LH from the pituitary gland. LHRH release is not continuous, but instead is pulsatile, with the frequency of pulses determined by the species and gonadal hormone status, with considerable variation in individual animals of a given species.[2,3] LH then circulates to the gonads and causes the production of gonadal steroids. In the female, after secretion of FSH to develop the ovarian follicles, LH produces ovulation and formation of the corpus luteum. Not only does LHRH act after its secretion into the hypophyseal–portal vessels to stimulate LH and to a lesser extent FSH release, but it also induces mating behavior in rats.[27]

Our experiments showed that release of NO from NP promoted LHRH release in vitro and that the action was blocked by hemoglobin, a scavenger of NO.[28] NP also caused an increased release of PGE_2 from the tissue,[29] which previous experiments showed played an important role in the release of LHRH. Furthermore, it caused the biosynthesis and release of prostanoids from [^{14}C]AA. The effect was most pronounced for PGE_2, but there also was release of LOX products, which have been shown to play a role in LHRH release. Inhibitors of COX, the enzyme responsible for prostanoid synthesis, such as indomethacin and salicylic acid, blocked the release of LHRH induced by norepinephrine (NE), providing further evidence for the role of NO in the control of LHRH release via the activation of COX I.[30] Needleman's group[31] later showed that NO activates COX I and COX II in cultured fibroblasts. The action is probably mediated by a combination of NO with the heme group of COX altering its conformation. The action on LOX is similar; although it contains ferrous iron, the actual presence of heme in LOX has yet to be demonstrated.

The previously accepted pathway for the physiologic action of NO is by activation of soluble GC by interaction of NO with the heme group of this enzyme, thereby causing conversion of GTP into cGMP, which mediates the effects on smooth muscle by decreasing the intracellular [Ca^{2+}] as described above. On the other hand, Muallem's group[32] has shown in incubated pancreatic acinar cells that cGMP has a biphasic effect on intracellular [Ca^{2+}], elevating it at low concentrations and lowering it at higher concentrations. We postulate that the NO released from the NOergic neurons near the LHRH neuronal terminals diffuses into the terminals and activates GC. The cGMP synthesized increases the intracellular [Ca^{2+}] required to activate PLA_2. PLA_2 causes the conversion of membrane phospholipids in the LHRH terminal to AA, which then can be converted to PGE_2 via the activated COX. The released PGE_2 activates AC, causing an increase in cAMP release, which activates PKA,

leading to exocytosis of LHRH secretory granules into the hypophyseal–portal capillaries for transmission to the anterior pituitary gland.[30]

NE has previously been shown to be a powerful releasor of LHRH. In the present experiments, we show it acted by activation of the NOergic neurons, since the activation of these neurons and the release of LHRH could be blocked by phentolamine, an α-receptor blocker, and prazosine, an α_1-receptor blocker. Therefore, the action is by α_1-adrenergic receptors.[28,30]

We measured the effect of NE on the content of nNOS in the MBH explants at the end of the experiments by homogenizing the tissue and adding [^{14}C]arginine and measuring its conversion to citrulline on incubation of the homogenate, a modification of the method of Bredt and Snyder.[15,30] Because arginine is converted to equimolar quantities of NO and citrulline, measurement of citrulline production provides a convenient estimate of the activity of the enzyme. The NO disappears rapidly, making its measurement very difficult. NE caused an increase in citrulline formation, suggesting that NE had increased the content of nNOS during the 30-minute incubation of MBH explants. We confirmed that we had actually increased the content of enzyme by isolating the enzyme according to the method of Bredt and Snyder[15] and then measuring the conversion of labeled arginine to citrulline. The conversion was highly significantly increased by NE.[30]

Glutamic acid, at least in part by means of *n*-methyl-D-aspartate (NMDA) receptors, also plays a physiologically significant role in controlling the release of LHRH. Therefore, we evaluated where glutamic acid fit into the picture. It also acted via NO to stimulate LHRH release, but we showed that the effect of glutamic acid could be completely obliterated by the α-receptor blocker phentolamine. Consequently, we concluded that glutamic acid acts by stimulation of the noradrenergic terminals in the MBH to release NE, which then initiated NO release and stimulation of LHRH release.[33]

Oxytocin has actions within the brain to promote mating behavior in the female and penile erection in the male rat. Because LHRH mediates mating behavior, we hypothesized that oxytocin would stimulate LHRH release, which mediates LH release from the pituitary. Consequently, we incubated MBH explants and demonstrated that oxytocin induced concentration-dependent LHRH release (10^{-7}–10^{-10} M) via NE stimulation of nNOS. Therefore, oxytocin may be very important as a stimulator of LHRH release. Furthermore, the released NO acts as a negative feedback to inhibit oxytocin release.[34]

One of the few receptors to be identified on LHRH neurons is the GABAa receptor. Consequently, we evaluated the role of GABA on LHRH release and the participation of NO in this. The experiments showed that GABA blocked the response of the LHRH neurons to NP, which acts directly on the LHRH terminals. We concluded that GABA suppressed LHRH release by blocking their response to NE. Additional experiments showed that NO stimulated the release of GABA, providing thereby an inhibitory feed-forward pathway to inhibit the pulsatile release of LHRH initiated by NE. As NE stimulated the release of NO, this would stimulate the release of GABA, which would then block the response of the LHRH neuron to the NO released by NE.[35]

Other studies indicated that NO would suppress the release of DA and NE. We have already described the ability of NE to stimulate LHRH release. DA also acts as

a stimulatory transmitter in the pathway. Therefore, there is an ultrashort-loop negative feedback mechanism to terminate the pulsatile release of LHRH, because the NO released by NE would diffuse to the noradrenergic terminals and inhibit the release of NE, thereby terminating the pulse of NE, LHRH, and finally LH.[36]

β-Endorphin blocks release of LHRH into the hypophyseal portal vessels by stimulating μ opiate receptors, thereby inhibiting secretion of LH. Our results indicate that β-endorphin stimulates μ opioid receptors on NOergic neurons to inhibit the activation and consequent synthesis of NOS in the MBH. β-Endorphin also blocks the action of NO on PGE_2 release, and consequently on LHRH release, by stimulating GABAergic inhibitory input to LHRH terminals that blocks NO-induced activation of COX and consequent PGE_2 secretion.[37]

We further examined the possibility that other products from this system might have inhibitory actions. Indeed, we found that as we added increasing amounts of NP, we obtained a bell-shaped dose–response curve of the release of LHRH, such that the release increased with increasing concentrations of NP up to a maximum at around 600 μM and then declined with higher concentrations. When the effect of NP on NOS content at the end of the experiment was measured, we found that high concentrations of NP lowered the NOS content. Furthermore, NP could directly decrease NOS content when incubated with MBH homogenates, results that indicate a direct inhibitory effect on NOS probably by interaction of NO with the heme group on the enzyme. Thus, when large quantities of NO are released, as could occur following induction of iNOS in the brain during infections, the release of NO would be decreased by an inhibitory action on the enzyme at these high concentrations. Furthermore, cGMP released by NO also acted in the explants or even the homogenates at high concentrations to suppress the activation of NOS. This pathway could also be active in the presence of high concentrations of NO, as in infection.[18] Thus, high concentrations of NO produced in the MBH by iNOS produced by arcuate neurons could inactive NOS, leading to decreased NO stimulation of LHRH and decreased LH released and loss of libido.

EFFECT OF CYTOKINES (IL-1 AND GMCSF) ON THE NOergic CONTROL OF LHRH RELEASE

The cytokines so far tested, for example, interleukin-1 and granulocyte macrophage colony-stimulating factor (GMCSF), act within the hypothalamus to suppress the release of LHRH as revealed in both *in vivo* and *in vitro* studies. We have examined the mechanism of this effect and found that for IL-1, it occurs by inhibition of COX, as shown by the fact that there is a blockade of the conversion of labeled AA to prostanoids, particularly PGE_2, and the release of PGE_2 induced by NE is also blocked.[38] LOX was also inhibited by IL-1α, as indicated by decreased leukotriene formation. Leukotrienes also increase LHRH release.

A principal mechanism of action is by suppression of the LHRH release induced by NO donors such as NP.[38] We first believed that there were IL-1 and GMCSF receptors on the LHRH neuron that blocked the response of the neuron to NO. However, since we had also shown that GABA blocks the response to NP and earlier work had shown that GABA receptors are present on the LHRH neurons, we evaluated the

possibility that the action of cytokines could be mediated by stimulation of GABAergic neurons in the MBH. Indeed, in the case of GMCSF, its inhibitory action on LHRH release can be reversed by GABAa receptor blocker, bicuculline, which also blocks the inhibitory action of GABA itself on the response of the LHRH terminals to NO. Therefore, we believe that the inhibitory action of cytokines on LHRH release is mediated, at least in part, by stimulation of GABA neurons.[39]

ROLE OF NO IN MATING BEHAVIOR

LHRH controls lordosis behavior in the female rat and is also involved in mediating male sex behavior. Studies in vivo have shown that NO stimulates the release of the LHRH involved in inducing sex behavior. This behavior can be blocked by inhibitors of NOS. Apparently, there are two LHRH neuronal systems: one with axons terminating on the hypophyseal portal vessels; and the other with axons terminating on neurons that mediate sex behavior.[27] NO is also involved in inducing penile erection by the release of NO from NOergic neurons innervating the corpora cavernosa penis. The role of NO in sex behavior in both sexes has led us to refer to NO as the "sexual gas".[40] The suppression of LHRH release by cytokines may be responsible for the decreased libido characteristic of infections.

EFFECT OF CYTOKINES AND NO ON THE RELEASE OF OTHER HYPOTHALAMIC PEPTIDES

Pulsatile GH release is controlled by GHRH release, and this can be blocked by intraventricular injection of NMMA, indicating that NO is also responsible for GHRH release.[41] Somatostatin release and mRNA synthesis are also stimulated by NO.[42] Injection of IL-1α into the third ventricle inhibits GH release by blocking the release of GHRH and stimulating the release of somatostatin.[37] NO induces the PRL release from injection of IL-1α by inhibiting release of the PRL-inhibiting hormone, DA.[43]

ACTION OF CYTOKINES AND NO TO CONTROL RELEASE OF ANTERIOR PITUITARY HORMONES

NOS is localized in LH gonadotropes and folliculostellate cells, which are modified glial cells that bear a resemblance to macrophages, as revealed by immunocytochemistry.[44] When pituitaries are incubated *in vitro*, most pituitary hormones are secreted only in small quantities. The exception to this rule is PRL, which is secreted in large amounts because of removal of inhibitory hypothalamic control by DA.[45] The other anterior pituitary hormones are secreted at low levels because of lost stimulatory hypothalamic input, and NO donors have little effect on this basal release in the case of GH. On the other hand, in the case of PRL, which is released in large amounts, NO donors suppress the release of the hormone; and inhibitors of NOS

usually enhance the release, indicating that there is still some capability for the gland to increase release of PRL *in vitro*.

DA is the most important PRL-inhibiting hormone by action on D^2 receptors in the gland. The dramatic inhibitory action of DA can be prevented by D^2 receptor blockers and also is prevented by incubation in the presence of inhibitors of NOS. Therefore, we conclude that the primary inhibitory action of DA is mediated by its action to stimulate D^2 receptors on the NOS-containing cells in the pituitary gland with resultant release of NO, which diffuses to the lactotropes and activates GC, causing the release of cGMP which mediates the inhibition of PRL secretion. Consistent with this hypothesis is the fact that NO donors suppress PRL release and the addition of cGMP can also lower the release of the hormone from incubated pituitaries. NO probably also inhibits ACTH release.[45] By contrast NO mediates the stimulation of FSH and LH induced by FSHRF, LHRH, and leptin, presumably by activation of specific receptors on the gonadotropes. NO converts GTP to cGMP that induces release of FSH and LH secretory granules.

During infection, cytokines, secreted by folliculostellate cells, are also released within the pituitary gland, resulting in activation of iNOS leading to generation of NO.[17] Therefore, NO should mediate, at least in part, the actions of cytokines directly on the pituitary gland.

THE ADIPOCYTE HORMONE LEPTIN

Leptin is a member of the cytokine family of hormones and has actions that are quite different from those of the inflammatory cytokines, which in some ways resemble those of the antiinflammatory cytokines. Instead of inhibiting LHRH release, as is the case with the proinflammatory cytokines, leptin combines with its receptors in the arcuate region to activate LHRH release by stimulating the release of NO from NOergic neurons. Rather surprisingly, it also activates not only FSH, but also LH release from hemipituitaries incubated *in vitro* at concentrations similar to those of LHRH itself. Again, the action is mediated by NO[47] and is modified by estrogen.[47,49] Evidence is mounting that these actions of leptin to stimulate gonadotropin secretion are of physiological importance and may play an important role in induction of puberty and in the amenorhea that follows malnutrition, as in the case of anorexia nervosa.[50]

Like other cytokines, leptin release is stimulated by LPS. The mechanism of this has yet to be determined, but the increase in leptin can be blocked completely by the glucocorticoid dexamethasone. The action of leptin on the hypothalamic–pituitary axis in this situation has not yet been studied, but leptin also elevates body temperature and inhibits feeding. These are responses to LPS and it may be that leptin plays a role in the anorexia, decreased libido and fever induced by infection.[51]

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