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Interaction between NO and oxytocin: Influence on LHRH release

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Abstract

Nitric oxide synthase (NOS)-containing neurons have been localized in various parts of the CNS. These neurons occur in the hypothalamus, mostly in the paraventricular and supraoptic nuclei and their axons project to the neural lobe of the pituitary gland. We have found that nitric oxide (NO) controls luteinizing hormone-releasing hormone (LHRH) release from the hypothalamus acting as a signal transducer in norepinephrine (NE)-induced LHRH release. LHRH not only releases LH from the pituitary but also induces sexual behavior. On the other hand, it is known that oxytocin also stimulates mating behavior and there is some evidence that oxytocin can increase NE release. Therefore, it occurred to us that oxytocin may also stimulate LHRH release via NE and NO. To test this hypothesis, we incubated medial basal hypothalamic (MBH) explants from adult male rats *in vitro*. Following a preincubation period of 30 min, MBH fragments were incubated in Krebs-Ringer bicarbonate buffer in the presence of various concentrations of oxytocin. Oxytocin released LHRH at concentrations ranging from 0.1 nM to 1 µM with a maximal stimulatory effect ($P<0.001$) at 0.1 µM, but with no stimulatory effect at 10 µM. That these effects were mediated by NO was shown by the fact that incubation of the tissues with N^G-monomethyl-L-arginine (NMMA), a competitive inhibitor of NOS, blocked the stimulatory effects. Furthermore, the release of LHRH by oxytocin was also blocked by prazocin, an α_1-adrenergic receptor antagonist, indicating that NE mediated this effect. Oxytocin at the same concentrations also increased the activity of NOS ($P<0.01$) as measured by the conversion of [^{14}C]arginine to citrulline, which is produced in equimolar amounts with NO by the action of NOS. The release of LHRH induced by oxytocin was also accompanied by a significant ($P<0.02$) increase in the release of prostaglandin E_2 (PGE_2), a mediator of LHRH release that is released by NO. On the other hand, incubation of neural lobes with various concentrations of sodium nitroprusside (NP) (300 or 600 µM), a releaser of NO, revealed that NO acts to suppress ($P<0.01$) the release of oxytocin. Therefore, our results indicate that oxytocin releases LHRH by stimulating NOS via NE, resulting in an increased release of NO, which increases PGE_2 release that in turn induces LHRH release. Furthermore, the released NO can act back on oxytocinergic terminals to suppress the release of oxytocin in an ultrashort-loop negative feedback.

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- Citrulline
- NMMA
- Sodium nitroprusside

Introduction

Little is known about the function of oxytocin, even though it is one of the two most abundant peptides in the body, the other being vasopressin (1). In the female, oxytocin has been shown to be essential for milk ejection and to play an important role in parturition by inducing uterine contractions (1). More recently, oxytocin has been shown to have a role in maternal and mating behavior (2) since its intraventricular injection can induce these behaviors in female rats. Even though the quantity of oxytocin present in the neurohypophysis of males is equal to that in females, its role in the male is almost completely unknown. As indicated at this meeting, oxytocin induces natriuresis in both sexes and there is now evidence that this effect may be caused by its stimulation of the release of atrial natriuretic peptide from the atria (3).

Intraventricular injection of oxytocin induces penile erection in male rats (4) and lordosis behavior in female rats (5). The oxytocin-mediated penile erection can be inhibited by injecting inhibitors of nitric oxide synthase (NOS) into the ventricular system (4). Previous studies indicated that mating behavior induced in estrogen-primed ovariectomized rats is mediated by nitric oxide (NO) which stimulates the release of luteinizing hormone-releasing hormone (LHRH) from LHRH neurons that activate brain stem neurons involved in the induction of lordosis (6). In addition, we have also shown that NO releases LHRH into the hypophyseal portal vessels to bring about release of LH from the pituitary gland (7). Therefore, it occurred to us that oxytocin might control the release of LHRH into the portal vessels to stimulate release of LH from the anterior pituitary gland, which would constitute another important role for this peptide in both sexes. Since the release of LHRH is controlled by NO, we thought it might also mediate the LHRH-releasing action of oxytocin, a hypothesis that was tested in the present study.

A relationship between the NOS-containing neurons (NOergic neurons) and the oxytocinergic and LHRH neuronal systems is further suggested by the anatomical relationship between these three neuronal systems. Many of the oxytocin neurons in the paraventricular and supraoptic nuclei also contain NOS and the enzyme is also present in abundance in axons projecting to the median eminence and neural lobe (8). There is a plexus of NOergic fibers and cell bodies in the region of the arcuate nucleus in close juxtaposition to the axons of the LHRH neurons (9). Therefore, the anatomical substrate for the postulated interactions between oxytocin, NO, and LHRH is present.

In the present study, we investigated the role of oxytocin in the release of NO and LHRH from incubated medial basal hypothalamic explants.

Material and Methods

The methods used were previously reported (7,9). Briefly, after decapitation and removal of the brain, the medial basal hypothalamus (MBH) was dissected and the hypothalami were preincubated in Krebs-Ringer bicarbonate-buffered medium, pH 7.4, containing 0.1% glucose (KRB) for 30 min prior to replacement with fresh medium or medium containing the substances to be tested. The incubation was continued for 30 min followed by removal of the medium and storage of samples at -20°C prior to the LHRH assay. Incubations were carried out in a Dubnoff metabolic shaker (50 cycles per min; 95% O_2/5% CO_2) (7,9). LHRH was measured by radioimmunoassay (RIA) using a highly specific LHRH antiserum kindly provided by A. Barnea (The University of Texas Southwestern Medical Center, Dallas). The sensitivity of the assay was 0.2 pg per tube and the curve was linear up to 100 pg of LHRH. Prostaglandin E_2 (PGE_2) was

also measured in media in some experiments by RIA (Peninsula Laboratories, Belmont, CA).

For determination of NO release from the incubated MBH explants, we used a modification of the method of Bredt and Snyder (10), which measures the conversion of [^{14}C]arginine into [^{14}C]citrulline in a homogenate of the explants at the end of the experiment (9). Since citrulline remains in the sample, whereas the equimolar amounts of NO produced are rapidly destroyed, this method provides a measure of the activity of the enzyme. We have shown that this modification provides an estimate of the activity of the enzyme in the sample.

For incubation of the neurohypophysis, the neural lobe was removed from the pituitaries and one lobe was placed in each tube together with 500 µl of KRB. The neural lobes were preincubated for 30 min and then incubated for an additional 30 min in fresh KRB containing the compounds to be tested. Oxytocin released into the medium was measured by RIA using a kit supplied by Peninsula Laboratories, Belmont, CA. All experiments were repeated at least once.

The following compounds were purchased from Sigma: sodium nitroprusside (NP), N^G-monomethyl-L-arginine (NMMA), and hemoglobin. Oxytocin was obtained from Peninsula Laboratories, Belmont, CA.

Results

Effect of oxytocin on LHRH release from MBH explants

Relatively high concentrations of oxytocin (1-10 µM) did not induce LHRH release, whereas lower concentrations produced highly significant increases in LHRH release with the maximum effect obtained at 0.1 µM. The dose-response curve was flat, so that a significantly increased release was seen at the lowest concentration tested (0.1 nM). Thus, the MBH is extremely sensitive to the LHRH-releasing action of oxytocin, while relatively high concentrations result in the loss of stimulation.

Effect of oxytocin on PGE_2 release from MBH explants

Since we have previously shown that the stimulatory effect of NO on LHRH release is mediated by the activation of cyclooxygenase and subsequent release of PGE_2 (7,9), we examined the effect of oxytocin on PGE_2 release. Indeed, a significant release occurred with the most effective concentration of oxytocin (0.1 µM).

Effect of inhibition of NOS on LHRH release from MBH explants

Our previous experiments indicated that NMMA, a competitive inhibitor of NOS that blocks the conversion of arginine into citrulline and NO, suppressed the stimulation of LHRH release induced by norepinephrine (NE) (7). NMMA completely blocked the release of LHRH induced by oxytocin, suggesting a role for NO in the LHRH-releasing action of oxytocin.

Effect of oxytocin on NOS activity in MBH explants

Other stimuli, which increased LHRH release via NO, such as NE (7,9) and N-methyl-D-aspartic acid (NMDA) (data not shown), also increased the activity of NOS as measured by the modified citrulline method at the end of the experiment. Therefore, we examined the action of oxytocin on NOS activity and found that it increased the content of NOS at doses of oxytocin (1-100 nM) which increased the release of LHRH.

Effect of prazocin on oxytocin-induced LHRH release

The α_1-noradrenergic receptor blocker,

prazocin, completely blocked the release of LHRH induced by NE (9). Therefore, we hypothesized that oxytocin might act to release LHRH via activation of NE release. Indeed, prazocin completely blocked the release of LHRH induced by 100 nM oxytocin.

Effect of NO on oxytocin release from the neural lobe of the pituitary gland

We have discovered a number of feedback mechanisms in the NOergic system controlling LHRH release and therefore we evaluated the effect of NO released by NP on the release of oxytocin from oxytocinergic terminals in the neural lobe of the hypophysis. At the concentrations of NP tested (300 and 600 µM), which are highly effective to release LHRH, NO induced a highly significant reduction in the release of oxytocin from the neural lobe. Hemoglobin, a scavenger of NO, completely blocked the action of NP.

Discussion

The present results show that oxytocin at low concentrations (0.1 nM to 1 µM) releases LHRH from MBH explants of male rats. These concentrations may exist *in vivo* in view of the close association between oxytocin neuronal terminals and LHRH neuronal terminals in the median eminence. The reason why high concentrations were not effective is unknown, but we have often found dose-response relationships with peptides in which high concentrations failed to have the effect (9). As is the case for other stimulators of LHRH release, the action of oxytocin is accompanied and probably caused by PGE_2 release. The action appears to be mediated via release of NO since blockade of NOS with NMMA prevented the stimulatory action of oxytocin. Furthermore, oxytocin induced an increase in the content of the enzyme measured by the citrulline method at the end of the experiment as is the case for other stimulators of LHRH release, such as NE and glutamic acid (9). Oxytocin appears to act via noradrenergic terminals since its action was blocked by the α_1 receptor blocker prazocin, which also inhibits the action of norepinephrine (7,9).

Presumably, the increase in enzyme content measured at the end of the experiment was caused by oxytocin stimulation of the noradrenergic terminals which, in turn, activated α_1 receptors on the NOergic neurons by increasing intracellular free calcium that combined with calmodulin to activate neural NOS (9). NO diffused to the LHRH terminal and activated release of LHRH by the activation of guanylate cyclase and generation of cyclic GMP, as well as by the activation of cyclooxygenase and generation of PGE_2 (9) as described earlier.

The increase in cyclic GMP presumably increased intracellular free calcium concentrations to activate phospholipase-A_2 which converted membrane phospholipids into arachidonate. Arachidonate served as a substrate for the NO-activated cyclooxygenase leading to the production of PGE_2. PGE_2 then activated adenylate cyclase leading to the production of cyclic AMP that in turn activated protein kinase A leading to the extrusion of LHRH secretory granules (9). The mechanism of activation of NOergic neurons via stimulation of the release of NE is supported by other experiments which demonstrated that oxytocin releases norepinephrine from hypothalami *in vivo* (11).

Unexpectedly, NO released by nitroprusside suppressed the release of oxytocin from the neural lobe suggesting that there is a negative feedback mechanism by which oxytocin stimulates the release of NO, which in turn acts to inhibit oxytocin release.

Therefore, it appears that oxytocin stimulates LHRH release. In the MBH, it stimulates the release of LHRH which enters the hypophyseal portal capillaries and reaches the pituitary gland where it releases LH, but oxytocin may also stimulate LHRH release

from LHRH neurons that project to brain stem neurons that activate mating behavior in the male rat since intraventricular injection of oxytocin produced penile erection. Oxytocin appears to have a major role in the reproduction of male rats which may be added to its probable role in the control of body fluid homeostasis. In female rats, oxytocin may act similarly via NO-induced LHRH release to stimulate LH release and lordosis.

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