PRELIMINARY REPORT

Involvement of Nitric Oxide in Vasoactive Intestinal Peptide-Stimulated Prolactin Secretion in Normal Men

P. Chiodera, R. Volpi, and V. Coiro

To establish whether nitric-oxide (NO) participates in the regulation of prolactin (PRL) secretion in humans in basal conditions and/or under stimulation with vasoactive intestinal polypeptide (VIP), seven normal men were treated with a placebo (normal saline) or the NO synthase (NOS) inhibitor L-NAME (40 μg/kg injected plus 50 μg/kg infused intravenously over 60 minutes), which in previous studies has been found able to modify other pituitary hormone secretions. Experiments were performed either in basal conditions or during stimulation of PRL secretion with an intravenous infusion of VIP (4 pmol/kg min over 60 minutes). The administration of L-NAME was unable to change the basal secretion of PRL. In contrast, L-NAME significantly enhanced the PRL increase induced by VIP. These data argue against an involvement of NO in regulation of basal PRL secretion. In contrast, the stimulatory effect of L-NAME on VIP-induced PRL secretion suggests that NO exerts an inhibitory control of the PRL response to VIP.

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SEVERAL LINES OF EVIDENCE indicate that both vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) are involved in the control of anterior pituitary hormone secretions. Both VIP and NO have been located in the pituitary gland and in various hypothalamic structures, such as the suprachiasmatic nucleus, the paraventricular nucleus, and the median eminence of several mammalian species.[1-3] In addition, high concentrations of VIP have been detected in the pituitary portal blood.[1] Particularly, VIP is supposed to play a releasing role for PRL. In fact, both systemic administration of VIP in vivo and addition of VIP to hemipituitaries in vitro elicit significant increments in PRL secretion.[1,4] On the other hand, evidence has been provided of an inhibitory action of NO on PRL secretion at pituitary level.[5,6] NO synthase (NOS) has been found in the pituitary folliculostellate cells and in the gonadotrophs[5]; hence, it is supposed to diffuse to the lactotrophs.[7]

Recent studies of rat pituitaries in vitro suggest the possibility that NO plays a modulatory role on the PRL-releasing action of VIP. To substantiate this hypothesis, we tested the effect of the NOS inhibitor L-NAME on the PRL-releasing effect of VIP in humans in vivo. In addition, the effect of L-NAME given alone on basal PRL secretion was tested in the same subjects. In previous studies, the L-NAME infusion method provided useful informations on the influence of NO in the regulation of arginine vasopressin, oxytocin, corticotropin, thyroid-stimulating hormone, luteinizing hormone, and follicle-stimulating hormone secretion in humans.[8-11]

MATERIALS AND METHODS

Seven healthy men, aged 25 to 34 years, participated in this study. The subjects were informed of the purpose of the study, which was conducted in accordance with the Helsinki II Declaration. All men were in good health, without clinical and laboratory evidence of hepatic, renal, heart, or other organic disease. All subjects were within 10% of their ideal body weight. None was a smoker or was addicted to an excessive alcohol consumption (<300 mL ethanol week). None had taken any drug for at least 1 month before the study or was under drug therapy at the time of the tests. Each subject underwent four different tests that were performed in random order and were at least 7 days apart. All tests were conducted with the subjects lying in the recumbent position and fasting from the previous evening. At 8:30 AM on the experimental day, two intravenous cannulae were placed into two different antebrachial veins. One cannula was kept patent by a slow saline (NaCl 0.9%) infusion and was used for blood sampling; the other was used to administer L-NAME, VIP, or saline.

L-NAME Test and Saline Test

L-NAME (N^G-Nitro-L-Arginine-Methyl-Ester-Hydrochloride; Clinalfa, Laufelfingen, Switzerland) was injected as an intravenous bolus of 40 $\mu g \cdot kg^{-1}$ at time 0 and was followed by the constant infusion over 60 minutes of 50 $\mu g \cdot kg^{-1}$ of the drug diluted in 50 mL of normal saline. In the control test, normal saline was given instead of L-NAME. Blood samples were collected at time 0 and every 10 minutes for 60 minutes. The dose of L-NAME used here was the maximal dose that in a preliminary study (data not shown) was devoid of stimulatory effects on blood pressure, since an increase in blood pressure may change the anterior pituitary secretory pattern.

VIP Infusion Test

Basal samples were collected at time 0 before the infusion of 4 pmol/kg/min VIP (Clinalfa) diluted in 50 mL of normal saline (plus human serum albumin) for 60 minutes from time 0. Blood samples were withdrawn at time 0 and at 15-minute intervals for 90 minutes after time 0.

VIP Plus L-NAME Test

This test was performed as the previously described L-NAME test, except for the infusion of 4 pmol/kg/min VIP diluted in 50 mL of normal saline (plus human serum albumin) for 60 minutes from time 0.

Assays

Serum PRL levels were measured in all samples with an immunoradiometric assay, using commercial kits. The lower limit of sensitivity was 0.5 ng/mL for PRL.

Intraassay and inter-assay coefficients of variation were 4.0% and 7.7% for PRL. All samples from the same subject were measured in duplicate in the same assay. Pulse and sphygmomanometric blood pressure were monitored at each sampling time during tests.

Statistical analysis was performed with the Wilcoxon's matched-pair

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rank-sum test and ANOVA followed by a specific mean comparison test, as appropriate. Values are reported as means ± SE.

RESULTS

Saline and L-NAME Tests

The administration of saline or L-NAME did not change the plasma concentrations of PRL at any examined time point (Fig 1). Mean blood pressure did not change in any patient during tests. No side effects were observed.

VIP and L-NAME Tests

Serum PRL levels significantly increased in response to VIP ($P < .05$ at 15 minutes and 75 minutes; $P < .02$ at 30 minutes; $P < .01$ at 45 and 60 minutes *v* baseline values). The simultaneous treatment with L-NAME significantly increased the PRL response to VIP ($P < .02$ at time 45 and 60 minutes; $P < .05$ at time 30 and 75 minutes) (ANOVA followed by specific mean comparison test) (Fig 2).

VIP infusion slightly and progressively decreased blood pressure (mean arterial pressure at time 0, 92.8 ± 1.3 mm Hg; at time 60, 82.0 ± 1.4) and increased heart rate (time 0, 70.8 ± 3.5 beats/min; time 60, 94.7 ± 4.5). Similar values were observed during the VIP plus L-NAME test. Flushing of modest intensity was observed in all subjects after VIP administration.

DISCUSSION

The data presented here show that in normal men in basal conditions, the intravenous administration of the NOS inhibitor L-NAME does not change the circulating concentrations of PRL, arguing against the involvement of NO in regulation of basal PRL secretion. However, previous in vitro experiments showed slight stimulatory effects of L-NAME on PRL secretion; this effect of L-NAME, if present in vivo, may not be seen in the present experimental conditions. On the other hand, L-NAME significantly increased the PRL response to the concomitant administration of VIP, indicating an inhibitory involvement of NO in the control of VIP-stimulated PRL secretion. These findings agree with the results of previous studies showing interactions between VIP and NO at various levels in the CNS and peripheral nervous system.[12-14] Particularly, our data obtained in humans in vivo confirm the results of a previous report showing inhibitory effects of NO on the PRL secretion from rat hemipituitaries in vitro.[7]

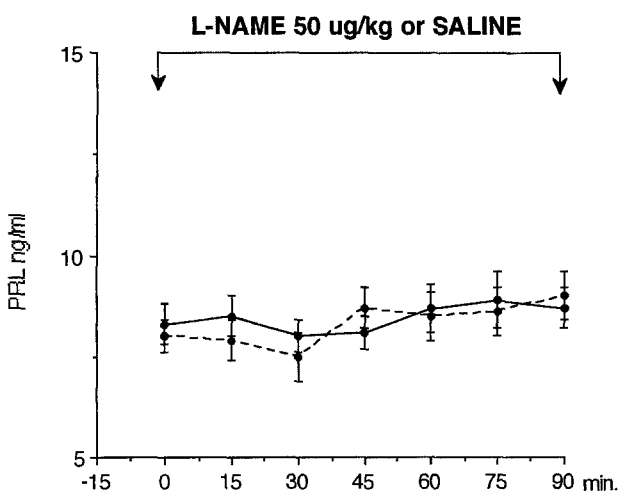

Fig 1. Effect of intravenous administration of L-NAME (●---●) or saline (●—●) on PRL secretion. Each point represents the mean ± SE of the observations.

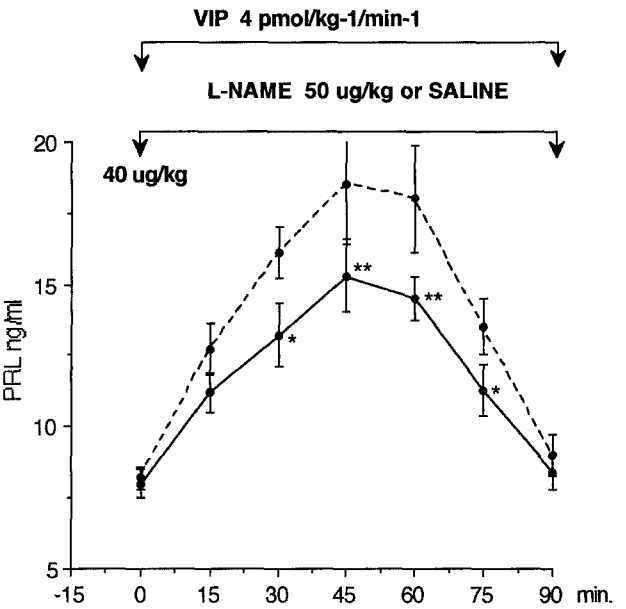

Fig 2. Serum PRL levels during VIP (●—●) and VIP + L-NAME administration (●---●). Each point represents the mean ± SE of the observations. *$P < .02$; **$P < .05$ between VIP and VIP plus L-NAME (ANOVA followed by specific mean comparison test).

The site of VIP-NO interaction and the mechanism by which NO inhibits VIP-induced PRL secretion remain uncertain, because our in vivo method is unable to clarify these issues. Both VIP and L-NAME are unable to cross the blood-brain barrier,[15] and thus a likely site of action of systemically administered drugs is represented by the pituitary gland, where immunoreactive VIP[1] and NOS[5] have been found. This possibility is supported by the aforementioned report of a VIP-NO interaction in the control of PRL secretion from rat hemipituitaries in vitro[7] and by the observation that in rhesus monkeys stimulation of PRL secretion is elicited by an intravenous administration of VIP even after pituitary stalk section.[1]

It cannot be excluded that L-NAME enhanced the PRL response to systemically administered VIP by acting at the level of hypothalamic structures located outside the blood-brain barrier. To explain the inhibitory effect of NO (reversed by L-NAME) on the PRL response to VIP, we might suppose that NO increases the release of a PRL-inhibiting factor, such as dopamine, from the hypothalamus.[6,7] However, in contrast with this hypothesis, studies in rats with intraventricular administration of NOS inhibitors have provided evidence that NO actions on releasing and/or inhibitory factors at the hypothalamic level stimulate rather than inhibit PRL secretion.[16]

In conclusion, the present study shows for the first time in humans in vivo that NO exerts an inhibitory role in the control of the PRL response to VIP. It is suggested that this interaction might take place at the pituitary level.

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