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Vasoactive Intestinal Peptide (VIP) Mediates the Effect of Estrogens on the Dopaminergic Tone in the Hypothalamic–Pituitary Axis of Ovariectomized (OVX) Rats

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The role of vasoactive intestinal peptide (VIP) in the regulation of dopamine (DA) concentration in mediobasal hypothalamus (MBH), posterior and anterior pituitary of ovariectomized (OVX) estrogenized rats was studied using passive immunization against VIP with a specific antiserum (a-VIP). Chronic estradiol administration decreased DA concentration in MBH, and in posterior and anterior pituitary, compared to OVX control rats. DA tissue concentration increased following a-VIP administration to control and estrogenized OVX rats. In vitro study of VIP and a-VIP on DA release from MBH in chronically estrogenized OVX rats showed that estrogens decreased DA evoked-release from MBH; a-VIP increased DA evoked-release from MBH of control OVX and estrogenized rats. VIP decreased DA evoked-release from MBH of OVX rats, but had no effect on estrogenized rats. VIP decreased DA tissue concentration in MBH of OVX control but not of estrogenized rats. It is suggested that VIP decreases DA synthesis and release from hypothalamic neurons in female rats, and that VIP partially mediates the inhibitory effect of long-term estrogen administration on DA release from MBH.

Key Words: Vasoactive intestinal peptide (VIP); dopamine (DA); estradiol; mediobasal hypothalamus (MBH); pituitary gland.

Introduction

Vasoactive intestinal peptide (VIP) participates in the regulation of the anterior and posterior pituitary functions and has a pivotal role as a physiological prolactin (PRL)-releasing factor *(1)*. VIP acts directly at the pituitary level *(2)* antagonizing inhibitory effects of dopamine (DA) and gamma-aminobutyric acid (GABA) on PRL secretion in vitro *(3)*. The PRL-releasing effect of VIP is independent of interruptions of dopaminergic tone, as observed for TRH *(4)*.

Some evidence suggests that VIP may exert paracrine or autocrine control on PRL secretion *(5,6)*. Immunohistochemical techniques show VIP in lactotrophs, in a subset of galanin-containing lactotrophs after estradiol treatment and in large stellate cells *(7)*. Estrogens increase VIP content *(8)* and mRNA VIP levels *(9)* in the anterior pituitary.

VIP-positive fibers and mRNA for VIP have been identified in the hypothalamus *(10)*. Some evidence suggests that hypothalamic VIP is involved in regulating pituitary PRL secretion *(11)*. The authors observed that VIP antiserum (a-VIP) administration reduces serum PRL levels in chronically estrogenized rats *(12)*. The dramatic blockage of hyperprolactinemia by passive a-VIP immunization suggests that VIP affects some neurotransmitter systems involved in control of PRL secretion. In fact, VIP has been shown to affect dopaminergic *(13)* and GABAergic neuronal activity *(14)*.

The tuberoinfundibular (TIDA) and tuberohypophysial dopaminergic (THDA) systems have perikarya in the rostral arcuate nucleus. TIDA neurons terminate in the stalk median eminence whereas THDA neurons project to the intermediate and neural lobes of the posterior pituitary. Both systems contribute to the regulation of PRL secretion *(15)*.

Since estrogens reduce dopaminergic inhibitory tone *(15)* and increase VIP release *(8)*, the question was whether VIP could mediate that effect. Therefore, passive a-VIP immunization on DA concentration in mediobasal hypothalamus (MBH) and anterior and posterior pituitary gland of control and estrogenized ovariectomized (OVX) rats was investigated. The in vitro effect of VIP and a-VIP on the content and release of DA from MBH in OVX rats chronically treated with estrogens was also examined.

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Materials and Methods

Female adult Wistar rats (175–200 g) were housed under controlled temperature and lighting. Food and water were available ad libitum. The drugs were from Sigma (St. Louis, MO), except VIP, purchased from Peninsula Lab.

Anti-VIP Serum

The a-VIP serum was obtained as previously reported *(12)*. It showed no cross-reactivity with glucagon, GHRH, secretin, gastrin inhibitory polypeptide, peptide histidine isoleucine, substance P, neurokinin A and B, or LHRH when these peptides were tested at doses at least 100-times higher than VIP.

Chronic Estrogen Treatment

Female rats were OVX and immediately implanted with Silastic capsules containing 5 mg of 17-β-estradiol. Control rats were implanted with empty Silastic capsules. Two weeks later the animals were anesthetized with ether and injected iv with a-VIP (0.25 mL/rat) or with normal rabbit serum (NRS) and killed 24 h later.

All rats were killed by decapitation and the brain was quickly removed. The posterior pituitary was gently separated from the anterior pituitary, and MBH was dissected out.

Dopamine Release In Vitro

Tubes containing two MBH were incubated in a Dubnoff shaker at 37°C in an atmosphere of 95% O_2–5% CO_2 in 500 µL of Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4 containing 25 m*M* HEPES, 0.1% bovine albumin, 100 µ*M* ascorbic acid, 100 µ*M* bacitracin, and 10 µ*M* tyrosine. Media containing 40 m*M* K^+ were prepared by substituting NaCl for KCl on a molar basis. Tissues were allowed to equilibrate for 15 min and the medium was discarded. After a 30 min period the medium was changed and tissues were exposed to 40 m*M* K^+ medium for 30 min. VIP (10^{-6}*M*), a-VIP (1/200) and NRS were present during both periods of incubation. Medium samples for K^+-evoked release were collected in ice-cold 0.1*N* perchloric acid (final concentration) and centrifuged at 5000*g*. Tissues and medium were frozen at –70°C until analyzed for DA.

Analytical Methods

In in vitro studies the tissues were homogenized after incubation. In in vivo studies the tissues were homogenized immediately after killing.

Tissues were homogenized in 300 µL of ice-cold 0.1*N* perchloric acid containing 100 pg of 3,4-dihydroxybenzylamine (DHBA) as an internal standard. DA was extracted by absorption onto alumina with 60–80% recovery of DHBA. DA was determined by reverse-phase HPLC with electrochemical detection. Elution times for DHBA and DA were 5.5 and 8.5 min, respectively. The detection limit for DA was 18 pg. The intra-assay coefficient of variance was 4%.

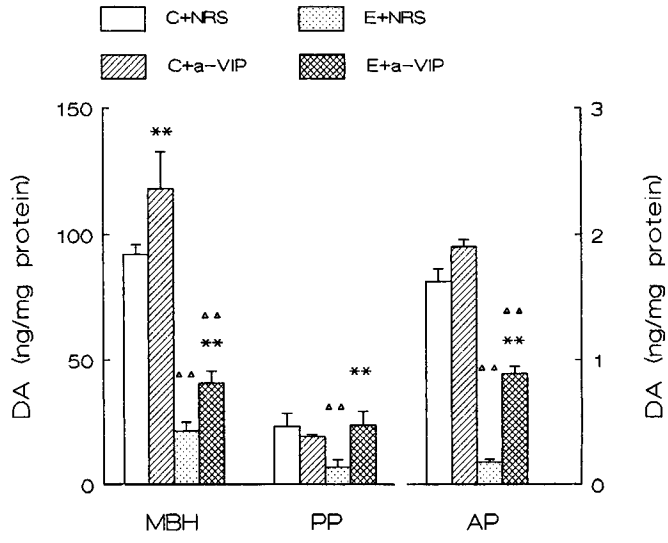

Fig. 1. Effect of a-VIP administration on DA content in MBH and in posterior and anterior pituitary of OVX and OVX estrogenized rats. **, $p < 0.01$ vs respective control (NRS) without a-VIP; ^^, $p < 0.01$ vs respective control without estradiol. The results were expressed as the mean ± SE and were analyzed by ANOVA ($n = 8$).

Statistical Analysis

Data were evaluated using two-way ANOVA with interaction terms or a Student *t*-test. Differences between mean values were considered significant when $p < 0.05$.

Results

Effect of a-VIP Administration on DA Content

In order to determine whether VIP could mediate the effect of estrogens on DA concentration, the authors studied the effect of passive immunization against VIP on DA concentration in MBH, posterior and anterior pituitary after in vivo a-VIP administration to OVX rats chronically treated with estrogens. Chronic estradiol administration markedly decreased DA levels in the three tissues. In OVX control rats, the injection of a-VIP increased DA concentration only in MBH. However, in OVX-estrogenized rats, a-VIP administration significantly increased DA concentration in MBH and in both posterior and anterior pituitary glands, although it only completely reversed the inhibitory effect of estrogens in the posterior pituitary (Fig. 1).

In Vitro Effect of VIP and a-VIP on Hypothalamic DA Release and Content

The authors investigated the effect of endogenous and exogenous VIP on DA release from MBH of OVX estrogenized rats by incubating the tissue in the presence of a-VIP or VIP, respectively.

Chronic estradiol administration significantly lowered evoked-DA release. a-VIP significantly increased evoked-DA release from MBH of both control OVX and estro-

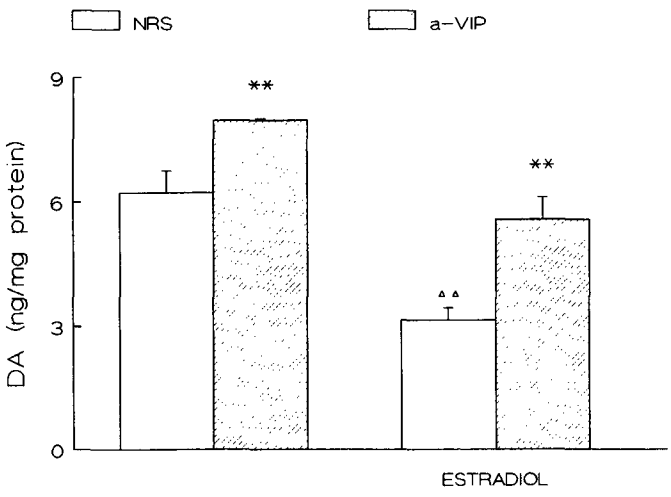

Fig. 2. In vitro effect of a-VIP (1/200) on DA release from MBH of OVX and OVX estrogenized rats. **, $p < 0.01$ vs respective control (NRS) without a-VIP; ^^, $p < 0.01$ vs respective control without estradiol. The results were expressed as the mean ± SE and were analyzed by ANOVA ($n = 6$).

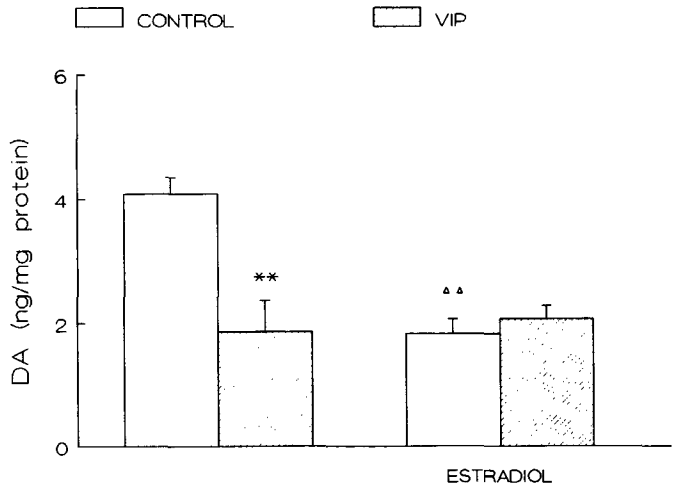

Fig. 3. In vitro effect of VIP ($10^{-6}M$) on DA evoked-release from MBH of OVX and OVX estrogenized rats. **, $p < 0.01$ vs respective control without VIP; ^^, $p < 0.01$ vs respective control without estradiol. The results were expressed as the mean ± SE and were analyzed by ANOVA ($n = 6$).

genized rats (Fig. 2). a-VIP did not modify DA tissue concentrations (data not shown).

VIP decreased hypothalamic evoked-DA release from control OVX rats but had no effect on DA release from MBH of estrogenized OVX rats (Fig. 3). A similar inhibitory effect of VIP was observed in DA tissue concentration. (Control: 20.31 ± 5.46 ng/mg protein; VIP: 5.01 ± 0.90, $p < 0.01$; E2: 9.78 ± 3.81, $p < 0.01$; E2 + VIP: 5.37 ± 0.90, N.S. vs E2, $n = 6$.)

Discussion

This study shows that passive immunization against VIP increases hypothalamic DA concentration and reduces the inhibitory effect of chronic estrogen treatment on DA concentration in MBH, and in anterior and posterior pituitary glands. Since TIDA neurons lack a high affinity transport system for DA *(15)* and DA is released to portal blood and is unavailable for reuptake, the increase in hypothalamic DA concentration may indicate increased hypothalamic DA release.

Long-term estrogen treatment inhibits the synthesis of DA in the median eminence *(15)*, and reduces hypothalamic potassium-stimulated DA release *(16)*. Estrogens also increase hypothalamic VIP release *(8)*. Likewise, the authors observed that chronic estrogen treatment decreased DA content and release in MBH. The neutralization of endogenous VIP increased DA evoked-release and content in MBH of OVX control and estrogenized rats; this indicates that VIP may mediate estradiol action on hypothalamic dopaminergic neurons. Supporting this idea, it was observed that VIP inhibited DA evoked-release in OVX control rats, although this effect was not observed in OVX estrogenized rats, probably because DA release was already at very low levels. The results disagree with a recent report *(17)* that shows a transient stimulatory effect on DA metabolism by central administration of VIP in estrogenized rats. However, PRL responses to stress *(18,19)* and suckling *(18)*, and proestrous PRL surge *(19)*, are, at least, partially mediated by VIP. Since dopaminergic tone is depressed in all these physiological states *(15)* and under estrogenization, it is possible to speculate that VIP stimulates PRL secretion through an inhibitory action on TIDA neurons.

Intravenous administration of anti-VIP sera can also block the hyperprolactinemia induced by central administration of serotonin *(20)*, prostaglandins *(21)*, cholecystokinin *(22)*, and galanin *(23)*, indicating that these effects are also mediated by hypothalamic VIP. Furthermore, it has been shown that oxytocin mediates the hypothalamic action of VIP to stimulate PRL secretion *(24)*. Thus, it seems that VIP may act at more than one locus in the hypothalamic–pituitary axis on the regulation of PRL secretion.

The localization of VIP immunoreactivity and mRNA both in lactotrophs and in folliculo stellate cells *(7)* suggests that VIP may be a paracrine factor with diverse functional roles *(5)*. The changes in DA concentration in the anterior pituitary gland could result from modifications in the release of DA from the median eminence or posterior pituitary, and/or from a direct effect on the metabolism of DA in the anterior pituitary. Whatever the mechanism may be, the VIP-induced decrease in DA pituitary content could facilitate its stimulatory action on PRL secretion. Recent data *(25)* indicates that pituitary VIP mediates PRL response to DA withdrawal through an autocrine or paracrine mechanism, and that inhibition of VIP synthesis and release is necessary for the inhibitory dopaminergic control of PRL secretion. Thus, a relationship between VIP and DA at the anterior pituitary level is relevant to the control of PRL release.

In conclusion, VIP may be involved in the inhibitory action of estrogens on DA concentration and release from MBH. Estrogens increase hypothalamic VIP release, which, in turn, could decrease DA release. Since, VIP also has an inhibitory effect on DA concentration in anterior and posterior pituitary glands in estrogenized rats, these effects could be involved in the stimulatory effect of estrogens on PRL release.

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