Involvement of vasoactive intestinal peptide (VIP) on ovarian physiology

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Abstract

In addition to the central nervous system, vasoactive intestinal peptide (VIP) containing nerves have been described throughout the female genital tract. VIP is reported to be produced by nerves fibers innervating follicles at all stages of development in rodents. There is growing evidence that VIP and their receptors play important roles in the local regulation of ovarian physiology mostly through cAMP pathway. It has been reported that VIP regulates the ovarian follicle survival and growth, oocyte maturation, ovulation and steroidogenesis. Studies also demonstrated that VIP inhibits apoptosis of rat follicles and is an important factor for the growth of preantral follicles enclosed in caprine ovarian tissue. Even though the addition of VIP to the culture medium did not improve *in vitro* maturation and fertilization of oocytes, it has been shown to stimulate ovulation in perfused rat ovaries. VIP is also involved in the regulation of steroidogenic activity. Therefore, this review aims to summarize current data on the importance of VIP on ovarian physiology.

Keywords: ovarian follicles, ovulation, steroidogenesis, VIP.

Introduction

Ovarian activity is regulated not only by gonadotropins and steroids, but also by a number of neural inputs and paracrine regulatory mechanisms. The mammalian ovary is innervated by extrinsic nerves, which are both catecholaminergic and peptidergic in nature (Burden, 1985; Ojeda and Lara, 1989; Ojeda *et al.*, 1989). Peptidergic innervation of the ovary was verified, among other ways, by the presence of vasoactive intestinal peptide (VIP) (Ahmed *et al.*, 1986).

In addition to the central nervous system, VIP containing nerves have been described throughout the human female genital tract being most abundant in the vagina, cervix and clitoris and less numerous in the uterine body, oviduct and ovary (Ottesen and Fahrenkrug, 1995), which suggests that such peptides may not play an exclusively neuroendocrine role. The lack of radioimmunoassayable VIP levels following the transection of the ovarian nerves indicates that ovarian VIP derives mostly from extrinsic innervation of the gland (Dees *et al.*, 1986; Advis *et al.*, 1989). The VIP immunoreactive nerve fibers reach the ovary via the suspensory ligament (Dees *et al.*, 1986) and are located around blood vessels, in the interstitial gland and in the follicular wall of the ovary (Kannisto *et al.*, 1986; Ahmed *et al.*, 1986). However, the local ovarian synthesis of the peptide is suggested by the ability to detect VIP mRNA within rat ovarian tissue (Gozes and Tsafriri, 1986; Bruno *et al.*, 2011; State University of Ceará, Fortaleza, Brazil; personal communication). There is growing evidence that VIP plays an important role in the female reproductive system by acting as a potential local regulator of ovarian physiology, such as regulation of steroidogenesis, cAMP accumulation, plasminogen activator production and oocyte maturation (Ahmed *et al.*, 1986; Tornell *et al.*, 1988; Johnson and Tilly, 1988). This review will focus on the role of VIP and its receptors in ovarian physiology.

Expression of VIP and its receptors in the ovary

VIP is a member of the structurally related neuropeptide family including pituitary adenylate cyclase-activating polypeptide (PACAP)/secretin/ glucagons (Miyata *et al.*, 1989; Arimura, 1992; Gozes *et al.*, 1999). VIP and PACAP act by binding to three types of G protein-coupled VIP/PACAP receptors: PAC1-R, which exhibits a 300- to 1000-fold higher affinity for PACAP than for VIP (Harmar *et al.*, 1998); VPAC1-R and VPAC2-R, which exhibit similar affinities for the two neuropeptides (Lutz *et al.*, 1993).

Harikumar *et al.*, (2006) demonstrated, by using biophysical methods, that VPAC1-R and VPAC2-R formed constitutive homo- and heterodimers. Langer *et al.* (2006) studied the pharmacological properties of VPAC1-R and VPAC2-R co-expressed in Chinese-hamster ovary cells, and they confirmed by co-immunoprecipitation that the two receptors did interact and found that receptor co-expression did not modify VIP or selective agonist affinities. Similarly, the potency of agonists to stimulate adenylate cyclase activity was unaffected, suggesting that the properties of the selective ligands that were established in cell lines expressing a single population of VIP receptors were valid in cells expressing both receptors. Similarly, VIP receptor co-expression did not modify receptor

internalization and trafficking patterns following VIP or selective agonist exposure (Langer *et al.*, 2006).

In ovaries, VIP is reported to be produced by nerve fibers innervating follicles at all stages of development in rodent (Ahmed *et al.*, 1986) and avian (Johnson *et al.*, 1994) species. Hulshof *et al.* (1994) showed that bovine ovary is innervated by VIP-positive nerve fibers, beginning at the onset of follicular development and increasing with age. Furthermore, VIP and its receptors immunoreactivity has been found in close association with the ovarian vasculature (Hulshof *et al.*, 1994; Vaccari *et al.*, 2006).

Despite the presence of mRNA for VIP has been detected in ovaries of rats (Gozes and Tsafriri, 1986), its presence was not observed in mice (Barberi *et al.*, 2007). However, in goats, the presence of mRNA for VIP was detected in all follicular categories (primordial, primary, secondary and antral follicles) and cellular types (granulosa and theca cells) studied; Bruno *et al.*, 2011; State University of Ceará, Fortaleza, Brazil; personal communication, suggesting a local synthesis of this peptide. Furthermore, quantification of mRNA demonstrated that secondary follicles had significantly higher levels of VIP mRNA compared to the primordial follicle. Granulosa/theca cells from large antral follicles had higher levels of VIP mRNA than small antral follicles (Bruno *et al.*, 2011; State University of Ceará, Fortaleza, Brazil; personal communication).

Barberi *et al.* (2007) showed that the PACAP/VIP receptors are expressed in mouse granulosa cells *in vivo*. Furthermore, other authors have shown that VPAC1-R is the most abundant receptor in ovaries from juvenile mice, and that VPAC2-R levels are lower, while those of PAC1-R are very low (Cecconi *et al.*, 2004). In the rat preovulatory follicles, PAC1-R and VPAC2-R are expressed by granulosa cells, whereas theca/interstitial cells exclusively express VPAC1-R and VPAC2-R (Vaccari *et al.*, 2006). PAC1-R was the main receptor present in the ovary of rats, followed by VPAC2-R and VPAC1-R (Latini *et al.*, 2010).

Signaling pathways and effects of VIP on follicle survival

VIP has been shown to protect several cell types from apoptosis, including thymocytes, prostate cancer and neural cells (Delgado *et al.*, 1996; Said, 1996; Gutierrez-Canas *et al.*, 2003; Sastry *et al.*, 2006). In the ovary, previous *in vitro* studies also demonstrated that VIP inhibits apoptosis of rat follicles (Flaws *et al.*, 1995; Lee *et al.*, 1999). Moreover, it has been demonstrated that PACAP and VIP were able to prevent granulosa cell apoptosis of follicles cultured in serum free medium (Vaccari *et al.*, 2006; Barberi *et al.*, 2007). Morelli *et al.* (2008) have shown that VPAC1-R and VPAC2-R mRNAs were induced by gonadotropin stimulation and the apoptotic effect of serum

withdrawal from human granulosa-luteal cells culture was reverted by both PACAP and VIP. Both peptides showed the ability to reverse a decrease in procaspase-3 levels induced by culture in the absence of serum. Recently, VIP maintained the ultrastructural integrity of goat preantral follicles after seven days of ovarian tissue culture (Bruno *et al.*, 2010).

It is possible that VIP maintains cell viability through the cAMP pathway. The stimulation of cAMP formation by VIP is probably a transient response that occurs early after culture initiation. In support of this, the ability of VIP to suppress apoptosis was mimicked by treatment of follicles with the adenylyl cyclase activator (forskolin), and this substance increased cAMP production and accumulation within cultured follicles (Flaws *et al.*, 1995). These findings, along with previous reports on the role of the cAMP-protein kinase A (PKA) second messenger system in mediating the effects of VIP in target cells (Davoren and Hsueh, 1985; Johnson and Tilly, 1988; Ojeda *et al.*, 1989), provide evidence that VIP, potentially acting at least in part via the cAMP pathway, functions as a novel inhibitor of apoptosis.

Studies have shown that the neuroprotective effects of VIP are mediated partially via activation of PKA through PKC (Vaudry *et al.*, 2000) and cAMP-response element (CRE) binding protein (CREB) phosphorylation (Walton and Dragunow, 2000). The effect or mechanisms include inhibition of caspase-3 activity, upregulation of Bcl-2 and suppression of cytoplasmic cytochrome *c* translocation (reviewed by Filippatos *et al.*, 2001). Moreover, VIP activates mitogen-activated protein kinase (MAPK) cascade through the PKA pathway in the GH4C1 rat pituitary cell line (Le Péchon-Vallée *et al.*, 2000). Fernández *et al.* (2003) have shown previously that VIP-induced lactotroph proliferation is mediated by cAMP/PKA and MAPK signaling pathways. Studies have provided new insights into cAMP signal transduction and demonstrated that Rap1 can play a crucial role in mediating cAMP-induced MAPK activation in specific cell types (Vossler *et al.*, 1997, Alleaume *et al.*, 2003). These studies have shown that cAMP can stimulate Rap1 activation, leading to activation of B-Raf, MAPK/extracellular signal regulated kinase (ERK) kinase 1 (MEK1) and MAPK. More recently, Fernández *et al.* (2005) demonstrated that VIP-dependent activation of the ERK signaling pathway in GH4C1 cells requires the PKA-Rap1 pathway (Fernández *et al.*, 2005).

Conversely, it is plausible that mechanisms independent of PKA activity also partly mediate the actions of VIP on follicle survival. This possibility is supported by previous studies using a mouse pituitary cell line that demonstrated activation of voltage-sensitive calcium channels after VIP stimulation (Schecterson and McKnight, 1991). In this work, β-endorphin secretion by cells in response to VIP was

inhibited in the presence of Ca^{2+} chelator EGTA, in the absence of extracellular Ca^{2+}, or when Ca^{2+} channels were blocked. Based on these results, VIP appears to activate a voltage-sensitive Ca^{2+} channel which allows the influx of extracellular Ca^{2+} required to stimulate β-endorphin secretion. Several peptide hormones have more than one type of receptor, and these receptor subtypes can be coupled to completely different intracellular message systems. Malhotra et al. (1988) demonstrated that VIP mobilizes intracellular Ca^{2+} via inositol triphosphate generation to induce catecholamine secretion in the adrenal medulla, indicating that other signal transduction mechanisms are coupled to VIP receptors. Also, VIP has been shown to alter several voltage-sensitive conductances in GH_3/B_6 pituitary cells, and only part of these actions is mimicked by 8-bromo-aAMP (Hedlund et al., 1988).

In vitro effects of VIP on follicle growth

There are only a few papers dealing with VIP effects on the ovarian follicles. In vitro studies revealed that VIP stimulates the development of isolated bovine primary and early secondary follicles (Hulshof, 1995). We have recently demonstrated that VIP is an important factor for the growth of small preantral follicles enclosed in caprine ovarian tissue (Bruno et al., 2010). In addition to the increase of VIP immunoreactivity with the appearance of secondary and antral follicles in bovine ovaries (Hulshof et al., 1994), the contemporary presence of VIP and its receptors around preantral and antral follicles (Vaccari et al., 2006) may explain the role of VIP in follicle growth. Nevertheless, in mice, VIP did not affect follicular development and caused inhibition of follicular growth, antrum formation, granulosa cell proliferation, as well as estradiol production of follicles stimulated by FSH (Cecconi et al., 2004). These conflicting results may be due to study design, differences related to species, culture conditions and different follicular stages analyzed.

Influence of VIP on maturation and ovulation

Studies have shown that VIP stimulates maturation in follicle-enclosed oocytes, but could transiently inhibit or not affect spontaneous maturation of cumulus-enclosed oocytes (Tornell et al., 1988; Apa et al., 1997). Culture of bovine cumulus oocyte complexes in the presence of VIP did not affect nuclear maturation or cumulus expansion, but it retarded cytoplasmic maturation as reflected by delayed cortical granule migration (Beker et al., 2000). Moreover, the addition of VIP to the culture medium did not improve *in vitro* maturation and fertilization of sheep (Ledda et al., 1996) and buffalo (Nandi et al., 2003) oocytes.

Two possible explanations for the limited response to VIP in events of maturation can be suggested: first, depending on the experimental conditions, cAMP can either stimulate or inhibit meiosis (Hillensjo et al., 1978). It is possible that high doses of VIP sustain high levels of cAMP in the oocyte and prevent meiosis. Such a phenomenon is believed to occur in the presence of forskolin (Ekholm et al., 1984), dbcAMP, or phosphodiesterase inhibitors (Hillensjo et al., 1978). A second explanation for the limited response could be that VIP affects only one subpopulation of follicles or subpopulation of granulosa cells within each follicle. In fact, Kasson et al. (1985) have shown one predominantly VIP-sensitive and one FSH-sensitive subpopulation of granulosa cells.

Nevertheless, although VIP did not influence maturation, Hulshof et al. (1994) observed that VIP-containing cells were found exclusively in the granulosa layer of the preovulatory follicle around the time of the LH peak. This suggests a role of VIP in the final stage of follicle development. Indeed, VIP has been shown to stimulate ovulation in perfused rat ovaries (Schmidt et al., 1990). In the mammalian system, VIP or LH, acting via the adenylyl cyclase system, induce an increase in plasminogen activator activity (Beers, 1975; Wang and Leung, 1983; Liu et al., 1987), a serine protease that has been implicated in the process of follicular rupture at the time of ovulation (Beers, 1975; Reich et al., 1985) and in the process of follicular cumulus cell expansion and dispersion (Liu et al., 1986).

In addition, the localization of VIP and its receptors in association with blood vessels (Hulshof et al., 1994; Vaccari et al., 2006) suggests that this neuropeptide might be involved in the regulation of ovarian blood flow. In fact, VIP contributes to the increase in blood flow around preovulatory follicles observed after the LH surge (Acosta et al., 2003). This increased ovarian stromal blood flow may, in turn, lead to a greater delivery of gonadotropins to the granulosa cells of preovulatory follicles (Redmer and Reynolds, 1996), which will be important for the generation of a normal follicle and competent oocyte. The gonadotropins would consequently stimulate the production of PACAP in the preovulatory follicle. The fact that PACAP induces genes related to ovulation and luteinization, and mediates some of the effects of LH on granulosa cell differentiation at the time of ovulation (Gras et al., 1999; Lee et al., 1999, Park et al., 2000), suggests that PACAP may serve as an ovarian physiological mediator of gonadotropins in the ovulatory process. This is in agreement with a previous demonstration that provides direct evidence of the presence of PAC1-R and the absence of VPAC1-R and VPAC2-R on germinal vesicles oocytes (Vaccari et al., 2006). Further studies are warranted to evaluate the respective roles of PACAP and VIP in ovulatory process.

VIP stimulates ovarian steroidogenesis

Studies have shown that in the periphery, the denervation of ovaries during the early luteal phase of

the estrous cycle leads to changes in their morphology and impairs steroidogenic activity in pigs (Jana *et al*., 2005). Similarly, inhibition of ovarian secretory function and delayed pubertal onset were observed in rats after denervation (Ojeda *et al*., 1983; Lara *et al*., 1990; Forneris and Aguado, 2002). The alterations in gonadal endocrine function are attributed to the loss of the peptidergic supply (for example, VIP) of neuronal fibers (Ojeda *et al*., 1983; Lara *et al*., 1990; Forneris *et al*., 2002; Jana *et al*., 2005). Therefore, these studies show that VIP is implicated in ovarian steroidogenesis.

In addition, VIP is involved in the regulation of steroidogenic activity, stimulating estradiol and progesterone release from cultured granulosa cells and whole ovaries *in vitro* (Davoren and Hsueh, 1985; Ahmed *et al*., 1986; Parra *et al*., 2007; Kowalewski *et al*., 2010), progesterone release *in vivo* (Fredericks *et al*., 1983), and androgen release from ovarian fragments *in vitro* (Ahmed *et al*., 1986). These effects may be related to the ability of VIP to enhance the synthesis of the cholesterol side-chain cleavage enzyme complex (Trzeciak *et al*., 1986), the rate-limiting step in progesterone biosynthesis and the activity of the aromatase enzyme complex (George and Ojeda, 1987). Studies have provided evidence that VIP is capable of inducing aromatase activity before the ovary became responsive to gonadotropins (George and Ojeda, 1987; Mayerhofer *et al*., 1997). A possibility to consider is that VIP induction of estradiol release during the estrous cycle may also come from immature follicles. If true, this would suggest that VIP plays a complementary role to that of FSH, the primary mediator of estradiol biosynthesis, in determining the magnitude of estradiol increase under varying physiologic conditions (Parra *et al*., 2007). These findings suggest a role for this peptide in ovarian steroid biosynthesis.

Little is known about the molecular mechanisms of VIP-mediated steroidogenesis. It is known that VIP increases the levels of cAMP (Tornell *et al*., 1988, Apa *et al*., 1997; Vaccari *et al*., 2006) and subsequently leads to PKA activation, which in turn induces steroidogenesis in granulosa cells. The cAMP/PKA pathway is the major route in the trophic hormone-stimulated regulation of steroidogenic acute regulatory protein (StAR) expression and function. The activation of PKC has been shown to result in the effective transcription and translation of StAR. However, a further activation of the PKA pathway is required to effectively phosphorylate StAR and induce steroid production (Jo *et al*., 2005). On the basis of this phenomenon, using specific analogs of cAMP to preferentially activate either type I or type II PKA, Dyson *et al*. (2009) were able to show that type I PKA is more involved in StAR gene expression, while the activation of type II PKA is essential for efficient phosphorylation of StAR and consequently production of steroids. Indeed, Kowalewski *et al*. (2010) observed that VIP together with cAMP-analogs that activate Type

II PKA increased P-STAR and further increased steroidogenesis. StAR mediates the rate-limiting step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane, and it is the hormonal regulation of StAR expression and activity that allows tissues to accurately control their steroid production (Kowalewski *et al*., 2010). A reduction of StAR and 3β-hydroxysteroid dehydrogenase expression, enzyme converting pregnenolone to progesterone, accompanied by decreased serum levels of FSH was demonstrated in young VIP knockout mice (Lacombe *et al*., 2007). Additional signaling pathways may be involved because the receptors activate multiple intracellular pathways (Lutz *et al*., 1993; Spengler *et al*., 1993).

Final considerations

Collectively, these results suggest the role of VIP and its receptors in the control of ovarian folliculogenesis. VIP and its receptors, mostly through cAMP/PKA pathway regulate the follicle survival and growth, oocyte maturation and ovulation, as well as ovarian steroidogenesis. Further research in this field will greatly advance our understanding of ovarian physiology.

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