VIP/PACAP signaling as an alternative target during hyperoxic exposure in preterm newborns

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Summary

The use of oxygen therapy (high doses of oxygen - hyperoxia) in the treatment of premature infants results in their survival. However, it also results in a high incidence of chronic lung disease known as bronchopulmonary dysplasia, a disease in which airway hyper-responsiveness and pulmonary hypertension are well known as consequences. In our previous studies, we have shown that hyperoxia causes airway hyper-reactivity, characterized by an increased constrictive and impaired airway smooth muscle relaxation due to a reduced release of relaxant molecules such as nitric oxide, measured under *in vivo* and *in vitro* conditions (extra- and intrapulmonary) airways. In addition, the relaxation pathway of the vasoactive intestinal peptide (VIP) and/or pituitary adenylate cyclase activating peptide (PACAP) is another part of this system that plays an important role in the airway caliber. Peptide, which activates VIP cyclase and pituitary adenylate cyclase, has prolonged airway smooth muscle activity. It has long been known that VIP inhibits airway smooth muscle cell proliferation in a mouse model of asthma, but there is no data about its role in the regulation of airway and tracheal smooth muscle contractility during hyperoxic exposure of preterm newborns.

Key words: lung; bronchopulmonary dysplasia; hyperoxia; vasoactive intestinal peptide; pituitary adenylate cyclase-activating polypeptide; preterm newborns.

Introduction

Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) was first described in 1967 by Northway *et al*. as a chronic lung disease in premature infants, (usually those treated with high oxygen partial pressure), because their alveoli are not enough developed to perform respiration (Jobe & Bancalari 2001). Airway hyper-responsiveness and pulmonary hypertension (PH) are well known consequences of BPD (Hershenson *et al*. 1994). Previous studies have shown that hyperoxia (treatment with high doses of oxygen) causes airway smooth muscle (ASM) hyperreactivity due to the reduced release of relaxant molecules such as nitric oxide (NO), changes in prostaglandin E_2 (PGE_2) levels, etc (Sopi *et al*. 2012; Stamenkovska *et al.* 2020). The data published through last few decades indicates that hyperreactivity involves many different molecular signaling mechanisms, among which the non-adrenergic-noncholinergic inhibitory system (iNANC) (Anaid *et al*. 2007), is one of the mainly affected systems. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating polypeptide (VIP/PACAP) relaxation pathway, is considered to be another part of this system and plays an important role in the airway caliber (Ao *et al*. 2011).

The hyperoxic exposure leads to generation of reactive oxygen species (ROS) in the lungs, such as superoxide radical anion ($O_2^{\cdot -}$), peroxyl radicals ($ROO^{\cdot}$), and hydroxyl radical ($HO^{\cdot}$). The non-radical derivatives of molecular oxygen (O_2), like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^{\cdot}$), are all strongly associated with the pathophysiology of BPD (Berkelhamer *et al*. 2013). Another major risk factor for developing BPD is pneumonia, which occurs when pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), receptor of chemokine 2 (CXCR2), and interleukin 11 (IL-11), are released in response to prenatal and neonatal trigger factors such as

mechanical ventilation (Federico *et al*. 2007). Many of these pro-inflammatory cytokines have been detected in aspirated fluids of neonates with BPD (Bose *et al*. 2008).

VIP/PACAP (Vasoactive Intestinal Peptide/Pituitary Adenylate Cyclase-Activating Polypeptide)

The vasoactive intestinal peptide (VIP), also known as the vasoactive intestinal polypeptide, is a 28-amino acid peptide first isolated from the upper intestine in 1975 by Said and later found in many mammalian organs and tissues including the intestines (Costa & Furness 1983), lungs (Dey *et al*. 1981), kidneys (Barajas *et al*. 1983), heart (Weihe & Remecke 1981), skin (Bloom & Polak, 1983), pancreas, suprachiasmatic nuclei of the hypothalamus, and widely distributed in the central and peripheral nervous systems (Said 1986), with approximately two minutes of blood half-life (Henning & Sawmiller 2001). The human VIP gene located in the chromosome *6q24* contains 7 introns and 6 exons, of which 5 are encoded (Hahm & Eidem 1998), whereas this gene in the rat is located in the chromosome *1p11* (Lamperti *et al*. 1991). VIP belongs to the super-family of structurally related peptide hormones which includes glucagon, glucagon-like peptide (GLP), helodermin, secretin, gastric inhibitory polypeptide (GIP), growth hormone releasing factor (GRF), and ligand II protein-receptors (Umetsu *et al*. 2011). VIPs may also contain sequences, encoding several additional biological neuroendocrine peptides, including the peptide histidine isoleucine [PHI; in low mammals] (Tatemoto & Mutt 1981), peptide histidine methionine [PHM]; the human equivalent of PHI (Itoh 1983), histidine valine peptide [PHV] and C-terminal extended form of the PHI and PHM (Yiangou 1987). PHI, PHM, and PHV presumably perform their biological function through the same receptors as VIP (Fahrenkrug 1993).

The pituitary adenylate cyclase (AC), activating polypeptide (PACAP) was firstly isolated from ovine hypothalamic tissue in the 1980s as a new member of the glucagon vazoactive/secretin superfamily, and shows high homology to VIP, sharing 68% similarities in the amino acid sequence (Sherwood 2000). PACAP is also found in a variety of peripheral tissues, including the gastrointestinal tract, adrenal glands, and testes, which are involved in a variety of biological functions, such as anterior pituitary secretion control, vasodilation, adrenaline secretion, insulin secretion, and immunosuppression (Arimura & Shioda 1995, Ghatei *et al*. 1993). Its half-life in human blood ranges between 5 and 10 min (Mentlein 1999). PACAP in humans is encoded by the *ADCYAP1* gene and is located in the chromosome *18p11* (Hosoya *et al*. 1992). Two types of this peptide have been identified to date: 38 amino acid peptides (PACAP-38) isolated from the sheep hypothalamus that stimulates AC in rat anterior pituitary cells in culture (Miyata *et al*. 1989) and 27 amino acid peptide (PACAP-27), isolated from the same source (Miyata *et al*. 1990) (Table 1).

VIP/PACAP receptors in the airways

The biological effects of VIP and PACAP are mediated by three types of G-protein-coupled receptors (GPCR), VPAC1, VPAC2 and PAC1. VPAC1 and VPAC2 receptors are binding sites for both VIP and PACAP, while PAC1 is a binding site for PACAP only (Laburthe *et al*. 2002; Ito *et al*. 2001) (Fig. 1). The G protein receptor family is classified into 3 groups (A, B and C), generally as 7-pass trans-membrane protein receptors. The VIP/PACAP receptor belongs to group B from the GPCR family, which consists of 437-459 amino acid residues (Ulrich *et al.* 1998). VPAC1 was the first VIP and PACAP receptor isolated from rat lungs by (Ishihara *et al*. 1992). VPAC1 is also found in the central nervous system (CNS), predominantly in the cerebral cortex and hippocampus (Ishihara *et al*. 1992; Usdin *et al*. 1994), in peripheral tissues including the liver, lungs, intestines [Usdin *et al*. 1994, Sreedharan *et al*. 1995), as well as in T lymphocytes (Delgado

et al. 1996). VPAC2 is the second receptor to respond to VIP and PACAP, cloned by Harmar and coworkers (1995), from a rat's odor bulb and later confirmed by (Usdin *et al*. 1994). Messenger RNA encoding the VPAC2 receptor is also found in the central nervous system (CNS), and most commonly in the thalamus and supra chiasmic nucleus, as well as in the lower parts like hippocampus, brainstem, spinal cord, and dorsal root ganglia (Ito *et al*. 2001). The receptor is also present in many peripheral tissues, including the smooth muscles of the cardiovascular, gastrointestinal, and reproductive system (Adamou *et al*. 1995, Wei & Mojsov 1996). The PAC1 receptor for the first time was cloned by Pisegna and Wank in 1993, from the acinar pancreatic carcinoma cell line (AR4-2J) in rats, with a much greater ability to bind to PACAP-27 and PACAP-38 in comparison to VIP. The DNA sequences of the related mouse (Hashimoto *et al*. 1996a), bovine (Miyamoto *et al*. 1994), human (Ogi *et al*. 1993) and a series of rat receptors were published independently by several groups of authors (Hashimoto *et al*. 1993, Svoboda *et al*. 1993). PAC1 is highly expressed in the CNS, in the olfactory bulb, thalamus, hypothalamus, hippocampus, granular cells of the cerebellum [Hashimoto *et al*. 1996b, Shioda *et al*. 1997)] and in a number of peripheral tissues, most commonly in the adrenal medulla (Moller *et al*. 1996) (Fig. 1).

VIP/PACAP signaling pathway in the airways

High-density VIP and PACAP expressing nerve fibers are found in the tracheobronchial tree, especially in the smooth muscle layer around submucosal, mucousal and serousal glands, in the lamina propria, and the walls of pulmonary and bronchial arteries (Dey *et al*. 1981). As mentioned before the physiological effects of VIP and PACAP are mediated by three types of G-protein-coupled receptors VPAC1, VPAC2, and PAC1. These physiological actions include relaxation of the airways smooth muscle, bronchodilation (Diamond *et al*. 1983, Kanazawa *et al*.

1996), and pulmonary vasodilation (Linden *et al*. 1999). In different *in vivo* and *in vitro* studies, with various subjects including guinea pigs, rabbits, dogs and humans, VIP was shown to cause a reduction of the constrictive effects of histamine, prostaglandin $F_{2\alpha}$, kallikrein, leukotriene D_4, neurokinins A and B and endothelin in isolated tracheal or bronchial segments (Hamasaki *et al*. 1983, Boomsma *et al*. 1990). On the other hand, calcium (Ca^{2+}) ions as an important player in the mechanisms of the muscle contraction/relaxation processes, may be released by the sarcoplasmic reticulum (SR), or transported from extracellular space (Groneberg *et al*. 2001, Kuo *et al*. 2003). After Ca^{2+} binding to the calmodulin, the myosin light chain kinase (MLCK) activates-(phosphorylate) myosin light chains (MLC), and allows the myosin cross-bridge to bind to the actin filaments, leading to contraction (Roux *et al*. 1997). In relation to VIP/PACAP, it was found that after their binding to corresponding receptors, they causes activation of the membrane-bound AC, which further generates cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) (Robinson & Colbran 2013, Ganz *et al*. 1986). The intracellular accumulation of cAMP on the level of the airways causes activation of a group of cAMP-dependent protein kinases A (PKA) (Francis *et al*. 1988, Hedlund *et al*. 1995). PKA phosphorylates phospholamban (PLN), a protein that normally interferes with the Ca^{2+} pump within the membrane of the SR. Reducing the level of free cytoplasmic Ca^{2+} or increasing Ca^{2+} uptake by internal stores like SR or mitochondria, results with smooth muscle relaxation (Mueller *et al*. 1979, Somlyo & Somlyo 1994). However, it is important to note that Ca^{2+} uptake by mitochondria is not cAMP regulated (Borie 1981). Other previous studies in rats, guinea pigs and humans, suggests that cAMP induces relaxation of ASM by interacting with various signaling pathways, including *K^+ channels*, more likely by membrane hyperpolarisation followed by a reduction in the Ca^{2+} influx via *voltage-dependent Ca^{2+} channels* (Nuttle & Farley 1996, Prakash *et al*. 1997). In addition, there is evidence that the reduction in the intracellular Na^+ by the *Na^+/K^+ ATPase*, caused increased Ca^{2+} efflux via

Na^+/Ca^{2+} exchanger; (the exchanger could be activated by PKA or directly by cAMP). The interaction of these channels would therefore be expected to induce ASM relaxation (Hall 2000, McGrogan *et al*. 1995, Gunst & Strop 1988). Additional mechanisms may contribute to the decreasing in the intracellular Ca^{2+} concentration, like inositol 1,4,5-triphosphate (*IP_3*)-*gated Ca^{2+} release channels* in the membrane of SR. IP_3 plays a substantial role in the opening of these channels, and different studies suggest for PKA prevented formation of the intracellular IP_3, consequently followed by a reduced concentration of the intracellular Ca^{2+} (Yang *et al*. 1996, Ding *et al*. 1997). Moreover, activated PKA usually causes MLCK inactivation and reduces its ability to activate the MLCs, which is essential for ASM contraction, and bronchodilatation (Giembicz & Newton 2006).

In addition, VIP is degraded by proteases that are present at/or near the airway mucosa, including mast-cell tryptase and chymase and by neutral endopeptidase ("enkephalinase") (Caughey *et al*. 1988, Goetzl *et al*. 1989), whereas, PACAP is metabolized by dipeptidyl peptidase IV (Li *et al*. 2007).

Involvement of the VIP/PACAP signalling in the inflammation

As indicated before, another major risk factor for the development of BPD is inflammation. Particular types of pro-inflammatory cytokines and chemokines such as TNFα, IL-1β, IL-6, chemokine receptor 2 (CXCR2) and CXCL8, IL-11 and IL-12 are related to inflammation. Numerous studies, in animal and human models, showed that VIP/PACAP signaling plays a key role in the balance between pro- and anti-inflammatory factors and possesses essential role in the successful control of inflammation (Gomariz *et al*. 2006, Ambalavanan *et al*. 2009). Transcription of the nuclear factor κB (NF-κB), leads to increased production of TNF-α, IL-1β and IL-6. VIP/PACAP on the other hand is able to inhibit NF-κB translocation through a cAMP independent

mechanism, further stimulating production of anti-inflammatory cytokines, such as IL-10, IL-11 and transforming growth factor-β (TGF-β), and at the same time prevent inflammation (Delgado *et al.* 1998; Trepicchio *et al*. 1996; Tsunawaki *et al*. 1988; Delgado *et al*. 1999). The VIP/PACAP cause inhibition of the production of pro-inflammatory cytokines mainly by involvement of the VPAC1-receptor, and lesser involvement of the VPAC2-receptor too (Delgado & Genea 1999, Di Benedetto *et al*. 2019). The main producers of cytokines are macrophages (Laskin & Pendino 1995, Juarranz *et al.* 2004). Moreover, VIP/PACAP was found to modulate inflammatory responses by regulation of the different functions in other cells, including the mast cells, microglia, dendritic cells and synovial fibroblasts (Tuncel *et al*. 2000, Abad *et al*. 2003). VIP also reduces the pro-inflammatory T helper1 (Th1) and T helper 17 (Th17) responses (Delgado *et al*. 2001, Abad *et al.* 2011, Benitez *et al*. 2018, Austin & Loyd 2014).

Involvement of the VIP/PACAP signaling in the pulmonary hypertension

Another well-known consequence of BPD is pulmonary hypertension (PH), which pathobiology is not yet completely clear. PH represents high blood pressure in the arteries of the lungs, which occurs when blood vessels in the lungs are narrowed, blocked or destroyed, and as a consequence blood flow through the lungs slows (Lau *et al*. 2017, Maarman *et al.* 2017). Other major determinants in the prognosis of the PH, are pulmonary artery pressure greater than 25 mmHg and right ventricular hypertrophy (Maarman *et al*. 2017). Several abnormal signaling pathways related to the PH have been identified, including reduced synthesis of prostacyclin and nitric oxide, and increased production of thromboxane and endothelin-1 (Giaida & Saleh 1995, Petkov *et al*. 2003). The recent studies have focused on the possible implication of the VIP/PACAP system in patients with PH. A low level of VIP in the lungs is found in patients suffering from PH with an over-expression of both types VPAC receptors. Conversely, Said *et al*. (2007), have shown

that VIP inhalation improves hemodynamics and lung capacity in the patients suffering from PH, proposing the peptide as a potential new treatment for PH. Previous observations in mice suggested that genetic knockout of the VIP gene, led to hemodynamic and histomorphological features of arterial PH, whereas intraperitoneal injections of VIP, has been shown to improve vascular pulmonary and right ventricular remodeling (Busto *et al.* 2000). Same as in other organs and tissues, the effect of VIP/PACAP in human pulmonary artery smooth muscle cells is mediated by VIP receptors VPAC1, VPAC2 and PAC1, which are primarily Gαs-coupled receptors (Said *et al.* 2007). The VPAC2 receptor is highly expressed in human pulmonary artery smooth muscle cells (Said *et al.* 2007). Gαs-coupled receptor activation causes an increase in cAMP, by activating AC, which can increase the activity of downstream mediators such as PKA, or induce expression of the protein directly activated by cAMP. PKA also phosphorylates targets such as MLCK to decrease its activity, resulting with vasodilatation and decreased proliferation of pulmonary artery smooth muscle cells (Fig. 2).

Conclusion

This review describes the physiological importance of VIP and PACAP in pulmonary diseases including BPD and PH. VIP/PACAP expresses a variety of actions, including potent dilatory actions in the pulmonary blood vessels and ASM and a potent anti-inflammatory and anti-proliferative actions. Based on all mentioned above, our opinion is that VIP/PACAP signaling might have an important role in the regulation of airway and tracheal smooth muscle contractility during hyperoxic exposure of preterm newborns.

Directions for future research

The need for additional investigation may be suggested, that will lead VIP/PACAP or some other player from their airway/tracheal signaling to be classified as a medication in the potential treatment of BPD and PH.

Author agreement

We certify that all authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Conflict of Interest

None.

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Table:

Table 1. The amino acid sequences of VIP/PACAP and its related peptides

Figure legends

Figure 1: Schematic representation of the signal transduction pathways of vasoactive intestinal peptide (VIP) / pituitary adenylate cyclase activating polipeptide (PACAP) receptors. Three receptors to PACAP have been described: VPAC1, VPAC2 and PAC1. VIP and PACAP show similar affinity for VPAC1 and VPAC2, whereas PACAP is more selective for PAC1 receptor.

Figure 2: Molecular actions of VIP/PACAP in induction of relaxation in airway smooth muscle cells. AC- adenylyl cyclase; cAMP - cyclic adenosine monophosphate; ATP - adenosine triphosphate; PKA - protein kinases A; IP_3 -inositol 1,4,5-triphosphate; PLN - phospholamban; MLCK - myosin light chain kinase.

Table 1.

Peptide	Amino acid sequence
	5 10 15 20 25 30 35 40 45
VIP	HSDAV | FTDNY | TRLRK | QMAVK | KYLNS | ILN
PACAP38	HSDGI | FTDSY | SRYRK | QMAVK | KYLAA | VLGKR | YKQRV | KNK
PACAP27	HSDGI | FTDSY | SRYRK | QMAVK | KYLAA | VL
PHI	HADGV | FTSDF | SRLLG | QLSAK | KYLES | LI
Helodermin	HSDAI | FTOOY | SKLLA | KLALO | KYLAS | ILGSR | TSPPP
Secretin	HSDGT | FTSEL | SRLRD | SARLQ | RLLQG | LV
Glucagon	HSQGT | FTSDY | SKYLD | SRRAQ | DFVQV | LMNT
GRF	YADAI | FTNSY | RKVLG | QLSAR | KLLQD | YMSRQ | QGESN | QERGA | RARL
GIP	YADGT | TFISD | YSIAM | DKIHO | ODFVN | WLLAO | KGKKN | DVKHN | ITO

Fig. 1

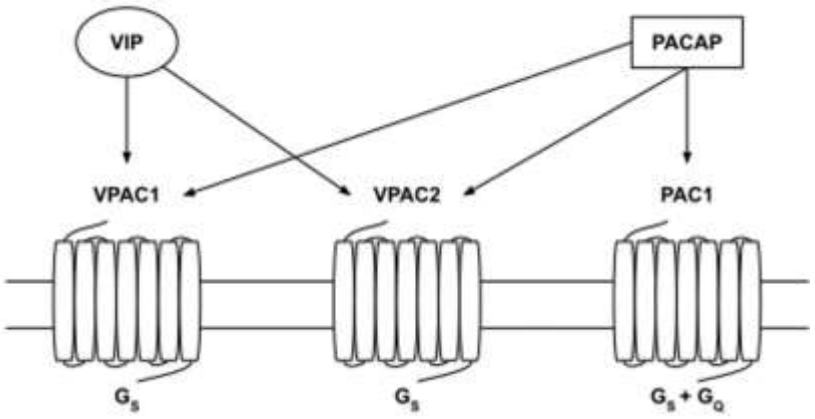

Fig. 2