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Abstract

Pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide with a widespread distribution both in the nervous system and peripheral organs. The peptide is also present in the female gonadal system, indicating its role in reproductive functions. While a lot of data are known on PACAP-induced effects in oogenesis and in the regulation of gonadotropin secretion at pituitary level, its placental effects are somewhat neglected in spite of the documented implantation deficit in mice lacking endogenous PACAP. The aim of the present review is to give a brief summary on the occurrence and actions of PACAP and its receptors in the placenta. Radioimmunoassay (RIA) measurements revealed increased serum PACAP levels during the third trimester and several changes in placental PACAP content in obstetrical pathological conditions, further supporting the function of PACAP during pregnancy.

Both the peptide and its receptors have been shown in different parts of the placenta and the umbilical cord. PACAP influences blood vessel and smooth muscle contractility of the uteroplacental unit and is involved in regulation of local hormone secretion. The effects of PACAP on trophoblast cells have been mainly studied in vitro. Effects of PACAP on cell survival, angiogenesis and invasion/proliferation have been described in different trophoblast cell lines. PACAP increases proliferation and decreases invasion in proliferative extravillous trophoblast cells, but not in primary trophoblast cells, where PACAP decreased the secretion of various angiogenic markers. PACAP pretreatment enhances survival of non-tumorous primary trophoblast cells exposed to oxidative stress, but it does not influence the cell death-inducing effects of methotrexate in proliferative extravillous cytotrophoblast cells. Interestingly, PACAP has pro-apoptotic effect in choriocarcinoma cells suggesting that the effect of PACAP depends on the type of trophoblast cells. These data strongly support that PACAP plays a role in normal and pathological pregnancies and our review provides an overview of currently available experimental data worth to be further investigated to elucidate the exact role of this peptide in the placenta.

Keywords (separated by " - ")

Pregnancy - Trophoblast - Proliferation - Migration - Placenta - Human

Chapter 23
Occurrence and Functions of PACAP in the Placenta

Gabriella Horvath, Jozsef Nemeth, Reka Brubel, Balazs Opper, Miklos Koppan, Andrea Tamas, Laszlo Szereday, and Dora Reglodi

Abstract Pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide with a widespread distribution both in the nervous system and peripheral organs. The peptide is also present in the female gonadal system, indicating its role in reproductive functions. While a lot of data are known on PACAP-induced effects in oogenesis and in the regulation of gonadotropin secretion at pituitary level, its placental effects are somewhat neglected in spite of the documented implantation deficit in mice lacking endogenous PACAP. The aim of the present review is to give a brief summary on the occurrence and actions of PACAP and its receptors in the placenta. Radioimmunoassay (RIA) measurements revealed increased serum PACAP levels during the third trimester and several changes in placental PACAP content in obstetrical pathological conditions, further supporting the function of PACAP during pregnancy.

Both the peptide and its receptors have been shown in different parts of the placenta and the umbilical cord. PACAP influences blood vessel and smooth muscle contractility of the uteroplacental unit and is involved in regulation of local hormone secretion. The effects of PACAP on trophoblast cells have been mainly studied in vitro. Effects of PACAP on cell survival, angiogenesis and invasion/proliferation have been described in different trophoblast cell lines. PACAP increases proliferation and decreases invasion in proliferative extravillous trophoblast cells, but not in primary trophoblast cells, where PACAP decreased the

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secretion of various angiogenic markers. PACAP pretreatment enhances survival of non-tumorous primary trophoblast cells exposed to oxidative stress, but it does not influence the cell death-inducing effects of methotrexate in proliferative extravillous cytotrophoblast cells. Interestingly, PACAP has pro-apoptotic effect in choriocarcinoma cells suggesting that the effect of PACAP depends on the type of trophoblast cells. These data strongly support that PACAP plays a role in normal and pathological pregnancies and our review provides an overview of currently available experimental data worth to be further investigated to elucidate the exact role of this peptide in the placenta.

Keywords Pregnancy • Trophoblast • Proliferation • Migration • Placenta • Human

Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP) was first described as a hypothalamic neuropeptide acting on the pituitary [1]. Numerous subsequent studies have described its regulatory effects in the hypothalamo-hypophyseal-endocrine gland axis at all levels. Shortly after its discovery it became evident that PACAP occurs at high levels in several peripheral organs, especially in the gonads. Arimura and coworkers showed that after the hypothalamus, highest PACAP levels are found in the testis [2]. This drew the attention to the peptide as a regulator of male fertility and reproduction. Indeed, PACAP was found to influence spermatogenesis at various levels [3–6].

PACAP is also involved in female reproductive functions. Although our knowledge on PACAP in reproductive functions is still limited, currently available data clearly indicate that the neuropeptide plays an important regulatory role in female reproductive physiology and pathology (rev. [7]). Briefly, PACAP, at the hypothalamic level, influences receptive behavior in female rodents, in association with gonadotropin releasing hormone and steroids [8], and plays an important modulatory role in pituitary hormone production. The role of PACAP in the hypothalamo-pituitary-gonadal axis has been reviewed several times previously [9–13] and is reviewed in the present book in two chapters (11. and 12.). PACAP is present in the ovary, in the ovarian follicular fluid and plays an important role in oocyte maturation [7, 14, 15]. In humans, the level of immunoreactive PACAP in the follicular fluid of hyperstimulated women is correlated with the number of retrieved oocytes [16]. PACAP also plays a role in the muscle contraction of the vaginal wall as well as that of the uterus and uterine tube [17–19]. Decreased immunoreactivity was shown in vaginal wall diseases and the plasticity of the PACAPergic system was demonstrated after vaginal reconstructive surgery [20, 21]. The PACAPergic innervation of the female genital tract was also described and has been associated with nerves originating from the paracervical ganglia [22, 23].

During pregnancy, the serum level of PACAP increases in the third trimester in healthy pregnant women and it markedly decreases during delivery, reaching pre-birth levels 3 days after delivery [24]. Winters and colleagues indicated a possible difference between cesarian and vaginal births regarding PACAP levels in the fetal cord blood [25]. In pregnant rats, Papka et al. [26] detected PACAP immunoreactivity in cervical nerves, lumbosacral dorsal root ganglia and in the spinal cord. Immunoreactivity showed changes during pregnancy, indicating that sensory nerve-derived PACAP is involved in the innervation of the cervix and may play a role in cervical ripening [26]. The role of PACAP in reproduction and offspring care does not seem to finish with birth: PACAP plays a complex regulatory role in breastfeeding. The action of PACAP on prolactin synthesis and release is complex and influenced by several factors [27, 28]. The high concentrations of PACAP in the milk suggest that the peptide plays a role in the development of the newborn [29–31]. Equally possible function of PACAP in the milk is a local regulatory action of milk production and/or mammary gland development [32, 33]. (This is reviewed elsewhere in this book by Tamas and co-workers 49.). In addition, PACAP was implicated to play a role in maternal behavior as showed by the decreased maternal crouching behavior of PACAP knockout mice [34].

It seems that placental functions of the neuropeptide are somewhat neglected in spite of the findings of Isaac and Sherwood [35], who described that the reproductive rate of PACAP knockout mice is lower due to implantation insufficiency, clearly indicating a placental role of endogenous PACAP. The present review briefly summarizes the occurrence and actions of PACAP and its receptors in the placenta.

Occurrence of PACAP and PACAP Receptors in the Placenta

Occurrence of PACAP and its receptors are summarized in a schematic drawing (Fig. 23.1). The gene encoding VPAC receptors was found to be weakly expressed in human placenta at relative prevalent levels comparable to that in the testis, kidney and thymus [36]. Another study also confirmed these findings [37].

Subsequent studies gave further insight into the occurrence and distribution of PACAP and its receptors in the placenta. Radioimmunoassay and immunocytochemistry first confirmed the expression of PACAP27 and PACAP38 in human placentas [38]. PACAP levels in the placenta were compared to those in the isthmic region of the uterus and in the umbilical cord. Both forms of PACAP could be detected in the examined specimens of the uteroplacental unit. PACAP38 concentration was higher than PACAP27 levels in all examined regions. Uterus and placenta showed similar levels of immunoreactivity, while intensity in the umbilical cord was much weaker [38]. These authors found no immunoreactive nerve fibers in the placenta or umbilical cord, immunoreactive nerve fibers were only present in the uterus, with isthmic region and nonpregnant myometrium showing stronger immunoreactivity than pregnant uterus. PACAP immunoreactivity by radioimmunoassay was confirmed later by Brubel et al. [39]: both forms of the peptide could be detected with PACAP38

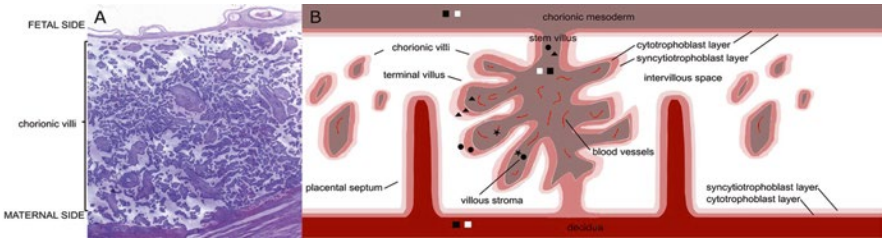

Fig. 23.1 Distribution of PACAP and PACAP receptors in the human placenta. (**A**). Representative photomicrograph of the human placenta indicating the parts depicted in the (**B**) schematic illustration of the human placenta. *Star:* PACAP38 immunohistochemistry, *filled circle:* PACAP mRNA, *filled triangle:* PAC1 mRNA, *filled square:* PACAP38 radioimmunoassay, *open square:* PACAP27 radioimmunoassay. Exact localization of VPAC receptor is not known

showing stronger immunoreactivity, in agreement with the above earlier observations. Furthermore, it was also found that different parts of the human placenta contained similar levels of PACAPs, such as central maternal, peripheral maternal, central fetal, and peripheral fetal parts. The umbilical cord showed very weak immunoreactivity. In addition, Brubel et al. [39] also compared the level of immunoreactivity in the 1st trimester samples and full-term placentas. Markedly stronger immunoreactivity for PACAP38 was found in full-term placentas on both the maternal and fetal sides. In contrast, PACAP27-immunoreactivity only increased on the maternal side, while it did not change on the fetal side towards the end of pregnancy.

Scaldaferri and collagues [40] studied PACAP and PAC1 receptor expression by means of Northern blot analysis, polymerase chain reaction (PCR) and immunohistochemistry. The authors detected the presence of PACAP and PAC1 receptor in both rat and full-term human placentas. In human placentas, strong immunohistochemical staining was observed in stromal cells surrounding blood vessels and weaker signal was detected in vessel walls in stem villi. In terminal villi, stromal cells expressed PACAP38 immunoreactivity. In stem villi, the stromal immunoreactivity was restricted to the periphery, while this spatial distribution pattern was lacking in terminal villi, where immunostaining was dispersed throughout the stroma. In rat placentas, several immunostained cells were observed in the labyrinth and in the villous-like structures of the intraplacental yolk sac, structures derived from yolk sac extensions into placental disc during late pregnancy [40].

Isoforms of the PAC1 receptor were also studied by RT-PCR. Different isoform expression was revealed in rat and human placentas. Rat placenta was shown to express 3 isoforms: the short, hip or hop variant and the hip-hop variant. In contrast, human placenta only expressed the SV2 form, homologous to the rat hop form. Radioligand receptor binding assay revealed that the relative potencies of PACAP-related peptides were PACAP27, PACAP38 with comparable strong binding (almost equipotent, PACAP27 slightly stronger) and VIP with weaker binding (10 times less potent). Growth hormone releasing hormone and unrelated peptides, such as beta-endorphin and corticotropin-releasing hormone, did not bind to the receptor [40].

PACAP and PAC1 receptor mRNAs were investigated in rat placenta also by Koh and coworkers [41]. Rat placenta consists of decidua basalis, junctional and labyrinth zones. Expression of PACAP and PAC1 receptor mRNA was detected by in situ hybridization in decidual cells, and in chorionic vessels and stromal cells of the labyrinth zone [41]. In decidual cells, signals were strongest on day 13.5 of gestation, then decreased with more advanced stages. No signal could be detected in the junctional zone. In contrast, signals were gradually increasing with advancing pregnancy in the labyrinth zone, in the branching villi, stem villi and also in chorionic vessels.

Koh et al. [42] investigated the expression of PACAP and PAC1 receptor mRNA from human legal abortions of 6–7 weeks, from induced abortions of 14–24 weeks (second trimester) and term placentas by cesarian section or normal vaginal delivery. In situ hybridization revealed expression of PACAP and PAC1 receptor mRNAs in stem villi and terminal villi. In 7- and 14-week-old samples, PACAP mRNA was detected in stroma cells surrounding blood vessels within stem villi, with moderate expression level, while stronger expression of PACAP mRNA was found at later stages [42]. PACAP mRNA was only weakly expressed in cytotrophoblast and syncytiotrophoblast cells. PAC1 receptor expression was detected in the same areas: stronger expression was described in stroma cells of the villi, while weaker expression in the trophoblast cells. Similar pattern of VIP immunoreactivity was detected by Marzioni and coworkers [43]. Immunostaining was present in both trophoblast layers and in the endothelium of the fetal vessels.

The gradual increase of the mRNA expression for both PACAP and its specific receptor implies a role of PACAP in placental growth. The findings with RIA also confirmed these increasing levels in late placentas compared to early placentas [4].

Expression of PACAP in Pathological Pregnancies

Butadiene diepoxide is a reactive metabolite of 1,3-butadiene that is an important industrial chemical and causes a dose-dependent inhibition of deciduoma development in rats [44, 45]. Placental expression of PACAP mRNA significantly decreased in rats pretreated with 1,3-butadiene, a chemical toxin for reproduction in rats [44, 46]. The decrease was more drastic on gestation day 12 (63 %) than on day 9 (48 %).

Our recent preliminary investigations have focused on the levels of PACAP38 and PACAP27 in cases of different pathological situations. These measurements were done in order to show possible changes of PACAP expression caused by maternal smoking during pregnancy or fetal distress or hypoxia leading to presence of meconium in the amniotic fluid. Human placentas were collected from full-term placentas. Samples were taken from the chorionic villi (fetal side), the decidua (maternal side) and the umbilical cord. Four different groups were examined: (1) normal pregnancy and birth; (2) amniotic fluid with meconium—premature birth (36–38 weeks); (3) premature birth (31–32 weeks) with smoking during pregnancy; (4) post term birth with smoking during pregnancy ($n = 3$ in all groups).

The procedure used was in accordance with protocols approved by the ethical committee (no. 2784,3117, University of Pecs; 8-28/92 009-10 I 8EKU, ETT TUKEB, Ministry of Health, Hungary). Tissue samples were weighed and homogenized in ice-cold distilled water. The homogenate was centrifuged (12,000 rpm, 4 °C, 30 min), and the supernatant was further processed for RIA analysis of PACAP38 and PACAP27 contents, as previously described [47, 48].

Briefly, the conditions were as follows: antisera: PACAP38 "88111-3" (working dilution, 1:10,000) and PACAP27 "88123" (dilution: 1:45,000); tracer: mono-125I-labelled ovine PACAP24-38 and mono-125I-labelled ovine PACAP27 prepared in our laboratory (5000 cpm/tube); standard: ovine PACAP38 and PACAP27 ranging from 0 to 1000 fmol/ml; buffer: assay prepared in 1 ml of 0.05 mol/l (pH 7.4) phosphate buffer containing 0.1 mol/l sodium chloride, 0.25 % (wt/vol) bovine serum albumin, and 0.05 % (wt/vol) sodium azide; incubation time: 48–72 h of incubation at 4 °C; separation solution: charcoal/dextran/milk powder (10:1:0.2 g in 100 ml of distilled water). Results are given as fmol/mg PACAP38-like immunoreactivity and PACAP27-like immunoreactivity in the tissue samples. Differences between PACAP contents were assessed by ANOVA test.

As sample sizes were low, no definite statistical comparison was possible, but some conclusions on tendencies can be drawn based on these preliminary results. We found detectable differences in levels of PACAP38 between pathological and physiological pregnancies suggesting that PACAP expression may be disturbed or upregulated during pathological events related to pregnancy (Fig. 23.2). PACAP27 levels did not show pronounced alterations in any examined condition. In accordance with previous results, levels of PACAP27 were significantly lower than PACAP38 levels in each sample. Under hypoxic condition, as indicated by the presence of meconium in the amniotic fluid, PACAP levels did not change in most samples. Only a slight decrease was observed in the chorionic villi and in the umbilical cord. Samples from premature births of smoking mothers showed marked increases in all regions examined except for the central decidua, where a slight decrease was observed. Decreases were also detected from samples derived from post term births of smoking mothers. Although our results are preliminary from limited number of clinical cases, some tendencies are promising as it seems that PACAP levels change in some pathological conditions. PACAP alterations have been observed in several diseases in both tissue samples and body fluids including plasma, cerebrospinal fluid and follicular fluid [16, 49]. Recent studies have shown that PACAP levels were lower in lung cancer, colon and kidney tumor samples compared to healthy tissue, while higher in prostate cancer samples compared with samples from benign prostatic hyperplasia [50, 51]. As PACAP is indicated as a potential biomarker for various conditions by several studies, it would be important to conduct a clinical study including enough pathological placenta samples to draw final conclusions (clinical review: see Reglodi et al. in this book, chapter 2.).

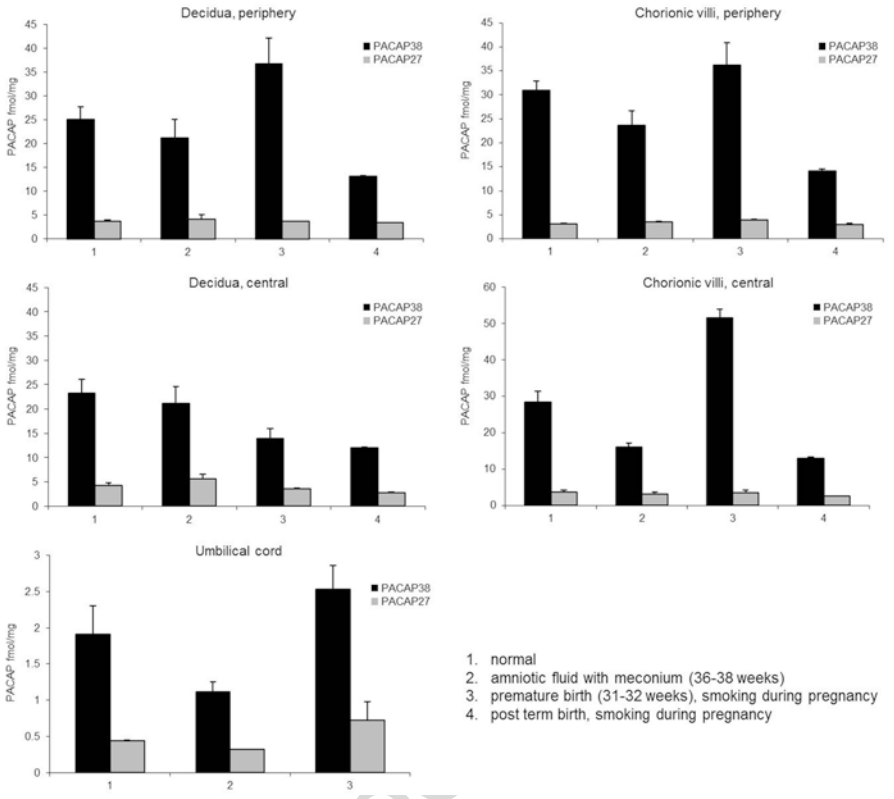

Fig. 23.2 Levels of PACAP27 and PACAP38 in placentas from healthy and pathological pregnancies. Results are given as mean fmol/mg tissue PACAP ± SEM. Differences between levels of PACAP38 and PACAP27-like immunoreactivities were significant in all cases

Effects of PACAP in Vessels of the Placenta

Effects of PACAP on different components and cells of the placenta are summarized in Table 23.1. Steenstrup et al. [38] investigated the effects of PACAP on vessels and smooth muscle contractility in the uteroplacental unit. They found that preincubation of the vessels with PACAPs and VIP produced a significant and concentration-dependent inhibition of the norepinephrine-induced contraction on the intramyometrial and stem villus arteries. The high concentration needed for significant relaxation indicates that the local release of the peptides is necessary to achieve this effect in vivo. These results show that PACAP causes relaxation of the placental vessels. In contrast, no effect was observed on either the amplitude, tone or frequency of strips of spontaneously contracted myometrial smooth muscle obtained from pregnant women [38]. These observations indicate that PACAP may be involved in the regulation of the uteroplacental blood flow. The time-related localization of endometrial/

t1.1 **Table 23.1** Summary of the effects of PACAP in the placenta

t1.2 t1.3 t1.4 Cells/region of placenta	Effect	PACAP concentration (M)	Reference
t1.5 Stem villous arteries	Relaxation	10^{-10}–10^{-6}	[38]
t1.6 Intramyometrial arteries	Relaxation	10^{-10}–10^{-6}	[38]
t1.7 t1.8 JEG3 choriocarcinoma cells	cAMP increase, IL-6 secretion, alpha-subunit gene transcription	10^{-7}	[52]
t1.9 t1.10 t1.11 JEG3 choriocarcinoma cells	No influence on survival of cells exposed to oxidative stress	10^{-7}	Horvath et al. unpublished observation
t1.12 t1.13 t1.14 JAR choriocarcinoma cells	Decreased survival in cells exposed to oxidative stress or hypoxia	10^{-7}	[53]
t1.15 t1.16 t1.17 JAR choriocarcinoma cells	No influence on survival of cells exposed to LPS, ethanol, methotrexate	10^{-9}–10^{-7}	[39, 53]
t1.18 t1.19 t1.20 t1.21 t1.22 t1.23 JAR choriocarcinoma cells	Phosphorylation of ERK1/2 and JNK↑; Akt, GSK-3β, and p38 MAPK↓, Bax expression↓; in cells exposed to oxidative stress, PACAP decreased phosphorylation of all these	10^{-7}	[53]
t1.24 t1.25 JAR choriocarcinoma cells	Agonistic effects of PACAP6-38 on signaling	10^{-7}	[62]
t1.26 t1.27 t1.28 HTR-8/SVneo nontumorous primary trophoblast cells	Pretreatment increased survival in oxidative stress, co-treatment no effect	10^{-8}–10^{-7}	[61]
t1.29 t1.30 HTR-8/SVneo nontumorous primary trophoblast cells	No effect on invasion	10^{-7}	[61]
t1.31 t1.32 t1.33 t1.34 t1.35 t1.36 HTR-8/SVneo nontumorous primary trophoblast cells	Reduced levels of angiogenic factors active A, ADAMTS-1, angiogenin, angiopoietin-1, endocrine gland-derived vascular endothelial growth factor, and endoglin	10^{-6}	[61]
t1.37 t1.38 t1.39 t1.40 HIPEC65 proliferative extravillous cytotrophoblast cells	Induced proliferation, but no effect on methotrexate-induced cell death decreased invasion	10^{-7}	[61]
t1.41 t1.42 t1.43 t1.44 Decidual and peripheral mononuclear cells from early pregnancies	No effect on secreted angiogenic molecules or inflammatory cytokine production	10^{-6}	[61]

uterine PACAP was described by Spencer et al. [45]. PACAP mRNA pattern showed similarity with that of the progesterone receptor during decidualization, but it was consistently lower during gestation. Furthermore, both uterine and placental mRNA expression pattern of decidual prolactin-related protein corresponded to PACAP and progesterone receptor mRNA levels. They suggest that PACAP could be important in facilitating endometrial blood flow and increase availability of metabolic substrates to the developing deciduoma or embryo [45].

Effects on Hormonal Secretion of the Placenta

Using JEG3 choriocarcinoma cells PACAP was found to induce a 12-fold increase in cAMP secretion [52]. This action of PACAP was rapid, cAMP increase started already after 30 min. cAMP in the placenta is known to stimulate alpha-subunit expression. Alpha-subunit of the hypophyseal hormones LH, FSH, and TSH is also present in the placental hormone human chorionic gonadotropin. PACAP38 was found to positively regulate alpha-gene transcription in JEG3 cells, with maximal effect at 100 nM concentration. The time course of this effect showed that PACAP effect started after 8 h. Similar effects were observed with the homolog peptide, VIP, but the effects of VIP an alpha-gene transcription started only after 24 h [52]. These findings show that PACAP may be involved in placental hormone secretion, and the similar effects of PACAP and VIP suggest that these effects are mediated by the shared VPAC receptors.

Effects of PACAP on Survival of Trophoblast Cells

Effects of PACAP on trophoblast cell survival were studied in JAR human choriocarcinoma cells [53]. PACAP treatment alone did not influence the survival rate. Cells exposed to oxidative stress induced by H_2O_2 showed decreased survival rate, which was further decreased by PACAP. A similar effect was observed in cells undergoing chemically induced hypoxia by $CoCl_2$. No effects on survival were observed in cells exposed to lipopolysaccharide (LPS), methotrexate or ethanol [39, 53]. These findings were contradicting the general survival-promoting effect of PACAP observed in many different cell lines and tissues both in vitro and in vivo [54]. Examining the signaling pathway revealed that PACAP treatment alone slightly increased phosphorylation of ERK1/2 (extracellular signal-regulated kinase) and JNK (c-Jun N-terminal kinase) but decreased that of Akt (protein kinase B), MAPK (mitogen-activated protein kinase), GSK-3β (glycogen synthase kinase 3 beta) and the expression of Bax. Oxidative stress alone increased phosphorylation of JNK and slightly decreased that of Akt, ERK, and GSK-3β, while no changes were observed in the expression of

phospho-p38 and Bax. In cells exposed to oxidative stress, PACAP treatment decreased phosphorylation of all of these examined signaling molecules compared to H_2O_2-treated controls. These results indicate that PACAP sensitizes the cells to some stressors, like oxidative stress and in vitro hypoxia, while it does not affect the deleterious effects of other stressors [53]. The reason for this is not known at the moment, and may or may not reflect the physiological role of the peptide, since these experiments were performed in choriocarcinoma cells, in which the receptor expression and signaling induced by PACAP may be significantly altered.

Similar results were described in retinoblastoma cells, where PACAP treatment induced cell death [55] in spite of the well-known protective effects of PACAP in the retina [56, 57]. PACAP also inhibited the growth of the neuronal tumor medulloblastoma in spite of the well-known neuroprotective effects of the peptide [58–60]. An equally possible explanation is that the sensitizing effect of PACAP to stressors such as hypoxia and oxidative stress may be involved in the adaptation promoting effect of PACAP in pregnancy and under pathological conditions. The first explanation is supported by later findings in non-tumorous primary trophoblast cells (HTR-8/Svneo cells), where PACAP pretreatment led to a significant increase in survival measured by MTT test in cells exposed to oxidative stress by H_2O_2, while co-treatment had no effect [61]. However, PACAP treatment did not influence the cell death-inducing effect of methotrexate in HIPEC65 proliferative extravillous cytotrophoblast cell line, but induced proliferation of these cells when treated alone with PACAP [61]. These results show that the effects of PACAP on proliferation and survival of trophoblast cells depend on the type of stressor, the timing of treatment and the type of cell.

Another interesting finding regarding survival and signaling effects in this cell line was that the generally accepted PAC1/VPAC2 antagonist PACAP6-38 exerted agonistic effects in JAR cells [62]. This does not seem to be specific for this cell line, since agonistic effects were found for example on rat trachea neuropeptide release, retinoblastoma cell survival and cartilage and bone development [55, 62–64]. The reason for this might be the expression of a yet unknown splice variant of the receptor or the tumorous nature of the JAR cells.

Effects of PACAP on Invasion of Trophoblast Cells

PACAP treatment did not influence the invasion of HTR-8/Svneo human first trimester extravillous primary trophoblast cells, while it decreased the invasion of HIPEC invasive, proliferative extravillous cytotrophoblast cells [61].

Effects of PACAP on Trophoblast Angiogenesis

Effects of PACAP on angiogenic factors were investigated in HTR-8/Svneo cells using an angiogenesis array method. Levels of several angiogenic markers were markedly decreased in the cell culture supernatant after 24 h of PACAP treatment.

Secreted levels of active A, ADAMTS-1, angiogenin, angiopoietin-1, endocrine gland-derived vascular endothelial growth factor, and endoglin were reduced [61]. In human peripheral blood and decidual mononuclear cells obtained from healthy pregnant women undergoing elective termination of apparently normal pregnancies no effect on levels of secreted angiogenic molecules was found. Similarly, PACAP treatment had no effect on the inflammatory cytokine production of these cells [61].

Observations in PACAP Knockout Mice, Effects on Implantation

It has been reported several times that the reproductive rate of PACAP and PAC1 receptor knockout mice is lower than that of wild types [34, 65]. The reason for this is not elucidated yet, several mechanisms may be responsible for this effect. For example, PACAP is involved in spermatogenesis and sperm motility [4], in steroid hormone synthesis, in ovarian folliculogenesis, and in reproductive behavior [34, 35]. The small litter can also be due to the premature intrauterine death and early postnatal death due to defects in breathing and temperature regulation [66, 67]. In addition to this multifactorial mechanism, it seems that placental defects are also partially responsible for the lower reproductive rate of PACAP knockout mice. Isaac and Sherwood [35] observed that while the puberty onset, estrous cycle and seminal plugs of PACAP knockout mice were normal, significantly fewer PACAP null females gave birth following mating than wild types. The authors found no defect in ovulation, ovarian histology or fertilization of released eggs, only 13 % had implanted embryos 6.5 days after mating compared to 81 % in wild types. Levels of prolactin and progesterone were significantly lower in PACAP knockout females. These observations suggest that impaired implantation is involved in the observed decreased fertility, the details of which need further clarification [35].

In summary, we give a brief review of data supporting a role of PACAP in normal and pathological pregnancies. The currently available experimental data are worth to be further investigated to elucidate the exact role in the placenta and evaluate the potential biomarker value of PACAP in reproductive pathology.

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