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Original article

Pituitary adenylate cyclase-activating peptide-27 (PACAP-27) is co-stored with galanin, substance P and corticotropin releasing factor (CRF) in intrapancreatic ganglia of the sheep

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Abstract

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide existing in two variant forms (of either 27 or 38 residues), widely present in numerous organs and evoking multiple effects both in the central and peripheral nervous systems. The present study was undertaken to evaluate the distribution pattern of PACAP-27 expression in the ovine pancreas. Using double immunohistochemical stainings co-localizations of PACAP-27 with galanin, SP or CRF were studied in intrapancreatic neurons. In intrapancreatic ganglia, immunoreactivty to PACAP-27 was found in 87.6 ± 5.4% of PGP 9.5-positive intrapancreatic neurons but not in intraganglionic nerve fibres. Numerous PACAP-27-immunoreactive nerve terminals were also observed between pancreatic acini and around small arterioles. No immunoreactivity to PACAP-27 was found in the endocrine pancreas. In 42.9 ± 6.2% of PACAP-27-immunoreactive intrapancreatic neurons the expression of galanin was also found. Statistically lower subpopulation (12.4 ± 4.0%) of intrapancreatic neurons exhibited simultaneously the immunoreactivity to PACAP-27 and SP. The expression of CRF was detected in the relatively smallest group (3.2 ± 1.4%) of PACAP-27-positive intrapancreatic neurons. The present results suggest that in the ovine pancreas PACAP-27 may play an important role as mediator of pancreatic functions. In PACAP-related pancreatic activities, a modulatory role of galanin, SP and to a lower extend of CRF is also likely.

Key words: pituitary adenylate cyclase-activating peptide-27, neuropeptides, sheep, pancreas, intrapancreatic neurons

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Introduction

Pituitary adenylate cyclase-activating peptide (PACAP) is a member of a secretin/glucagon/vasoactive intestinal peptide (VIP) superfamily which consists of nine biologically active substances (Vaudry et al. 2009). The discovery of PACAP from extracts of the sheep hypothalamus was made by the ability to increase adenylate cyclase in the rat anterior pituitary cell cultures (Miyata et al. 1989). Alternative processing of PACAP precursor results in formation of two biologically active PACAP amidated isoforms consisting of either 38 or 27 amino acids residues, respectively (Miyata et al. 1990). Molecular studies revealed strong similarities between PACAP and VIP precursors what explains why VIP and both PACAP isoforms elicit its action via common class B G-protein coupled receptors. So far, PACAP-specific PAC1 receptors and two common for VIP and PACAP: VPAC1 and VPAC2 receptors have been characterized and cloned (Svoboda et al. 1993, He et al. 2014). Since the discovery in 1989, PACAP has been an object of intense anatomical, physiological and pharmacological studies. Using immunohistochemical and radioimmunoassay techniques, the expression of PACAP and its receptors has been found in numerous mammalian tissues including neurons of the central and peripheral nervous system (Masuo et al. 1991, Ghatei et al. 1993, Csati et al. 2012, Rytel et al. 2014) as well as enteric (Kirchgessner and Liu 2001, Miampamba et al. 2002) and intramural ganglia of the pancreas (Hannibal and Fahrenkrug 2000) and heart (Girard et al. 2007). At the central level, PACAP may exert a variety of functions like modulation of hypothalamic-pituitary hormones release (Kanasaki et al. 2015), food consumption and water drinking (Puig de Parada et al. 1995, Mounien et al. 2009), neuronal differentiation, neuroprotection (Manecka et al. 2013) and many more, whereas its role at the periphery is relatively less understood. Additionally, functional studies have revealed that the biological effect of PACAP-27 and PACAP-38 is different which may be correlated to the fact that in numerous tissues PACAP-38 activates intracellular signaling mechanisms that differ from those employed by PACAP-27 (Martínez-Fuentes et al. 1998). For example, in the rat small intestine PACAP-27 evoked potent secretory response not observed with PACAP-38 (Cox 1992).

In mammals, the pancreas receives unique neuronal input from at least four (or even five) different sources. Pancreas projecting efferent sympathetic neurons originate from the celiac ganglion (Sharkey et al. 1984), whereas autonomic parasympathetic supply is provided by dorsal motor and ambiguous nuclei of the vagus (Luiten et al. 1984). Sensory impulses from the pancreas are conveyed via afferent fibres that have their cell bodies in vagal nodose ganglia as well as in Th_6-L_2 dorsal root ganglia (Sharkey et al. 1984). Additionally, local pancreatic reflexes are controlled by enteric ganglia located in the stomach and duodenum (Kirchgessner and Gershon 1990). The last but not the least source of the pancreatic innervation is pancreatic ganglia scattered throughout the pancreas parenchyma (Anglade 1987). Thus, the activity of the pancreas is neuronally controlled at multiple levels and it is believed that direct synchronization and modulation of autonomic impulses are managed by intrapancreatic ganglia. In the past two decades, a number of researchers have sought to determine PACAP-38-ergic expression pattern in the pancreas/intrapancreatic ganglia of mammals with the special emphasis to rodents (Filipsson et al. 1988, Fridolf et al. 1992, Hannibal and Fahrenkrug 2000) and some species of domestic animals (Tornze et al. 1996, Love and Szebeni 1999). Interestingly, in these studies the sheep has been only occasionally included as an experimental model (Koves et al. 1993) which is obviously a basic knowledge gap in the veterinary neuroscience field. Additionally, the innervation of the pancreas of the sheep seems to be especially interesting also from the comparative point of view. This is due to the fact that it has been shown that some aspects of the innervation of the ovine exocrine and endocrine pancreas as well as the chemical coding of intrapancreatic neurons exhibit species-specific peculiarities (Arciszewski 2007, Arciszewski and Zacharko-Siembida 2007a,b). Therefore, in continuation of the prior studies on the ovine pancreas innervation, the present immunofluorescence study aimed 1) to examine the distribution pattern of PACAP-27 in neuronal elements of the ovine pancreas, and 2) to determine the co-localization of PACAP-27 with galanin, substance P (SP) and corticotropin releasing factor (CRF) in intrapancreatic neurons.

Material and Methods

Animals and tissue sampling

All experimental procedures were conducted in accordance with the Polish law on animal experiments and approved by the local committee of ethics at the University of Life Sciences, Lublin, Poland. Five sexually mature sheep of both sexes (weighing ca. 35-45 kg) were used in the study. Following the sedation with xylazine (Rometar, Spofa Prague, Czech Republic; 0.4 mg/kg b.w., i.m.) the animals were killed with an overdose of sodium pentobarbital (Pentobarbitalnatrium, Apoteket, Sweden; 35 mg/kg b.w., i.v.). The abdomen was opened with a midline incision and samples (approx. 1 cm^3) of the body and left and right lobe of the

pancreas were collected. The tissues were washed with cold (4°C) 0.01M phosphate buffer-saline (PBS) and immediately immersed with a mixture of paraformaldehyde and picric acid (Stefanini's fixative) for 3 days. Next the material was rinsed with PBS, put in 16% sucrose solution (4°C) and kept until they sunk into the bottom of the container. Finally, the pancreas samples were embedded in optimum cutting temperature (O.C.T.) compound and frozen in dry ice. Ten µm thick serial cryostat sections were cut. Every fifth section was mounted on adhesion glass slides (SuperFrost Plus, Menzel GmbH & CoKG, Germany) and stored at -70°C until further used.

Double immunofluorescence

In order to visualize the presence of PACAP-27 in intrapancreatic neurons double immunohistochemical stainings utilizing guinea-pig anti-PACAP-27 sera (1:400, Bachem, Switzerland; T-5039) and mouse antibodies raised against pan-neuronal marker PGP 9.5 (1:100, Abcam, UK; ab 8189) were applied. In order to evaluate whether in the pancreas of the sheep PACAP-27 is co-expressed with other biologically active substances double immunohistochemical stainings were also made according to the procedure described elsewhere (Arciszewski et al. 2011). Briefly, following drying for 15 minutes (at room temperature; RT) the sections were 3 times washed (10 minutes each) with a blocking/permeabilizing solution composed of PBS, 10% normal goat serum, 0.25% Triton X-100 and 1% bovine serum albumin (Sigma-Aldrich). For an overnight incubation (RT) with a mixture of primary antibodies the sections were placed in a humidified box. Guinea-pig anti-PACAP-27 sera were mixed with one of the following rabbit sera: anti-galanin (1:1200, AbD Serotec, UK; 4600-5004), anti-SP (1:300, Enzo Life Sciences, UK; SA1270) or anti-CRF (1:100, Sigma-Aldrich, Germany; C5348). Next day, after pouring off the excess of primary antisera, the slides were again washed with three changes of PBS (10 min) and incubated (1h, RT) with a mixture of species-specific fluorochrome-conjugated secondary antisera. For visualization of antigen-antibody complexes FITC-conjugated goat anti-guinea-pig IgG (1:400; MP Biomedicals, USA) were combined with Texas Red-conjugated goat anti-rabbit IgG (1:400; MP Biomedicals, USA) or Texas Red-conjugated goat anti-mouse IgG (1:400; MP Biomedicals, USA). Following the final washing with PBS, the stained slides were coverslipped with phosphate-buffered glycerol (pH=8.2) and examined under an epifluorescent microscope equipped with a digital camera. For examination of FITC- and/or Texas Red-fluorescent neuronal elements, appropriate filters with 470-490 nm (excitation/emission wavelength) and 545-580 nm (excitation/emission wavelength) were used. For immunohistochemical negative control purposes, the sections were incubated with non-immune sera instead of primary antibodies or with primary antibodies that had been preincubated overnight with an excess of the synthetic peptide. In control sections no positive immunostaining was observed.

Semi-quantification, cell counting and statistical analysis

The expression of PACAP-27 in the particular parts of the ovine pancreas was assessed visually according to the following semi-quantitative scale: numerous, moderate, single, absent. The same scale was also applied for co-localization studies of PACAP-27-positve nerve fibres. In each animal, a random sample of at least 300 PACAP-27-positive intrapancreatic neurons was studied in each anatomical portion of the pancreas. The proportion of PACAP-27-expressing intrapancreatic neurons was presented as a percentage relative to the total number of neurons analyzed. The subpopulations of PACAP-27-positive intrapancreatic neurons co-expressing galanin, SP or CRF were calculated in relation to the total number of PACAP-27-IR perikarya. All numerical data are expressed as mean ± S.D. In order to test differences between different subpopulations of PACAP-27-expressing intrapancreatic neurons one-way ANOVA test following Tukey's post-hoc test were used. The data were considered statistically significant when $p<0.05$.

Results

Immunoreactivity to PACAP-27 was detected in the body as well as the right and left lobes of the ovine pancreas. No differences in PACAP-27 innervation between anatomical parts of the pancreas were found. Also the sex of animals had no influence on the distribution patterns of PACAP-27 in either pancreatic neurons or nerve fibres. As many as 87.6 ± 5.4% (n=5) of PGP 9.5-immunoreactive (IR) intrapancreatic neurons showed the presence of PACAP-27, whereas none of ganglionic nerve fibres exhibited PACAP-27 expression (Fig. 1A). The pancreatic acini were richly supplied with PACAP-27-IR nerve fibres (Fig. 1B), whereas interlobular ducts showed no presence of PACAP-27-positive nerve elements. Moderate numbers of PACAP-27-expressing nerve fibres were also detected around small arterioles (Fig. 1C) located in connective tissue septa. No PACAP-27 immunoreactivity was detected in the endocrine pancreatic tissue.

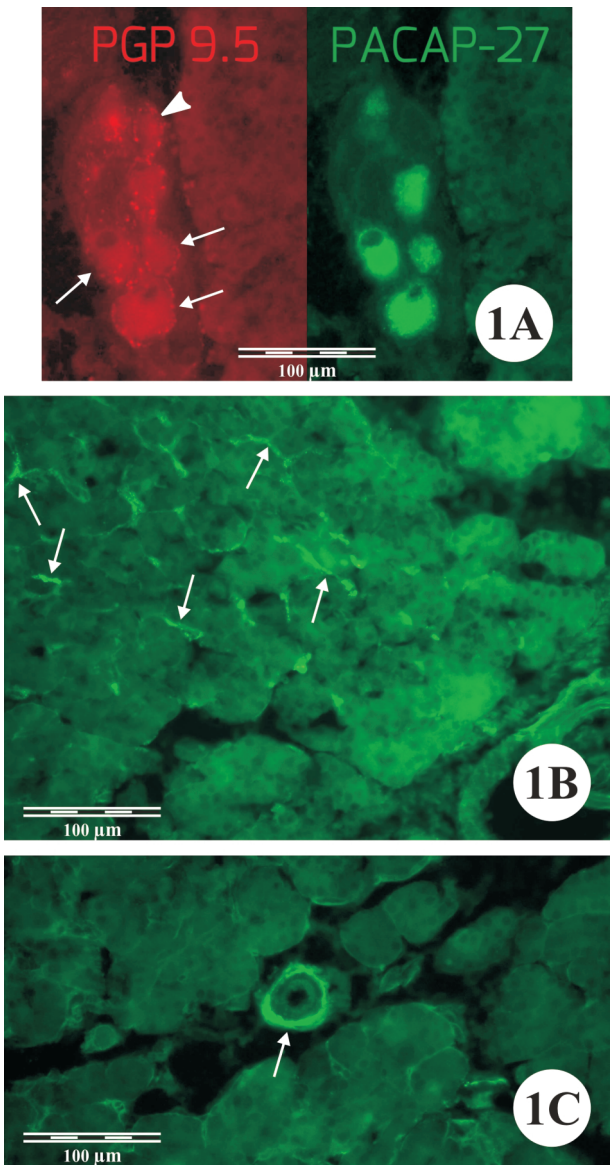

Fig. 1. Immunoreactivity to PACAP-27 in the pancreas of the sheep. In intrapancreatic ganglia (Fig. 1A) the expression of PACAP-27 was observed in very numerous PGP 9.5-IR intrapancreatic neurons (arrows) but not in ganglionic nerve fibres; the arrowhead marks PGP 9.5-IR neuron lacking PACAP-27. Arrows in Fig. 1B indicate PACAP-27-positive nerve fibres supplying the exocrine pancreas. In Fig. 1C PACAP-27-IR nerve fibres supplying pancreatic small arterioles are marked with the arrow.

PACAP-27/galanin. Amongst PACAP-27-expressing intrapancreatic neurons as many as 42.9 ± 6.2% (n=5) showed the simultaneous presence of galanin (Fig. 2A). In general, in virtually all galanin-IR intrapancreatic neurons the expression of PACAP-27 was shown (no galanin-positive/PACAP-27 negative perikarya were detected). Mainly varicose galanin-IR/PACAP-27 negative intraganglionic nerve fibres frequently encircled both PACAP-27-IR/galanin-IR as well as PACAP-27-positive/galanin-negative intrapancreatic neurons. Co-localization of PACAP-27 and galanin was also detected in moderate numbers of nerve terminals supplying small arterioles as well as pancreatic acini (however single galanin-IR nerve fibres lacking PACAP-27 were also found).

PACAP-27/SP. In the vast majority of PACAP-27-expressing intrapancreatic neurons no immunoreactivity to SP was detected. The co-existence of PACAP-27 and SP was found only in a small subpopulation (12.4 ± 4.0%; n=5) of intrapancreatic neurons (Fig. 2B). The subpopulation of PACAP-27-IR/SP-IR neurons was statistically lower ($P<0.05$) when compared to PACAP-27-IR/galanin-IR perikarya. In intrapancreatic ganglia varicose SP-positive/ /PACAP-27-negative nerve fibres running between PACAP-27-IR neurons were frequently found. In general, SP-IR nerve fibres did not form „basket-like" formations around intrapancreatic neurons. The co-localization of PACAP-27 and SP was also found in single acini-supplying nerve fibres (but not around small arterioles).

PACAP-27/CRF. The expression of CRF was found in extremely small population (3.2 ± 1.4%; n=5) of PACAP-27-IR intrapancreatic neurons (Fig. 2C). The subpopulation of PACAP-27-IR/CRF-IR neurons was statistically different ($P<0.05$) in relation to PACAP-27-IR/galanin-IR as well as PACAP-27-IR/ /SP-IR neuronal groups. In intrapancreatic ganglia CRF-IR nerve fibres displaying no immunoreactivity to PACAP-27 were frequently found. In general varicose CRF-positive nerve fibres very closely encircled PACAP-27-expressing intrapancreatic neurons (Fig. 2C). No co-expression of PACAP-27 and CRF was detected in pancreatic small arterioles as well as between acini.

Discussion

In general, described in the present study distribution pattern of PACAP-27 immunoreactivity in the pancreas of the sheep partially resembles PACAP-38 innervation pattern (Koves et al. 1993), with a substantial exception that no presence of PACAP-27-positive nerve fibres was found in the endocrine pancreas. Moreover, the presented herein expression pattern of PACAP-27 in the ovine pancreatic tissue is in line with previous immunohistochemical studies of the porcine pancreas (Tornre et al. 1996) but substantially differs from that in the rat (Hannibal and Fahrenkrug 2000), mouse (Fridolf et al. 1992) or rabbit (Love and Szebeni 1999). In previous functional studies utilizing intravenous infusion of either PACAP-27 or PACAP-38 it has been found that both PACAP-27 and PACAP-38 increased the exocrine activity of the ovine pancreas in

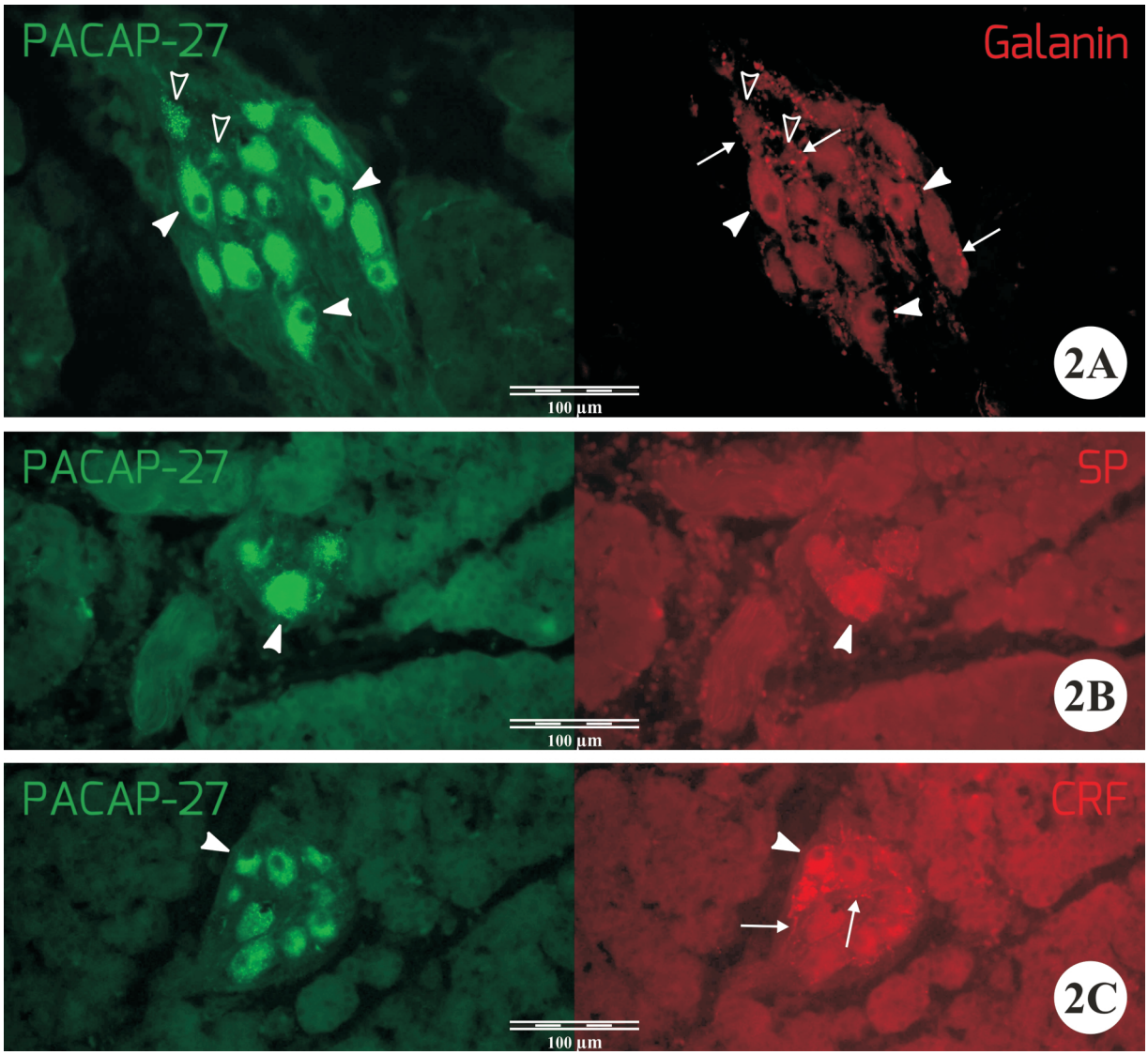

Fig. 2. Paired micrographs showing different subpopulations of PACAP-27-IR intrapancreatic neurons found in the ovine pancreas. In Fig. 2A numerous neurons co-expressing PACAP-27 and galanin are marked with arrowheads, whereas galanin-positive nerve fibres encircling PACAP-IR perikarya are indicated with arrows; hollow arrowheads mark PACAP-27-IR neurons lacking galanin. Fig. 2B presents PACAP-27-IR/SP-IR neuron (arrowhead) found in the pancreas of the sheep. Fig. 2C. Amongst numerous PACAP-27-positve intrapancreatic neurons only one (arrowhead) is additionally positive for CRF; note the presence of CRF-positive nerve fibres (arrows) running in a close vicinity to PACAP-27-IR neuronal somata.

dose-dependent manner, and the effect of PACAP-27 was more potent when compared to PACAP-38 (Onaga et al. 1996). In the latter study it has been also shown that intravenous infusion of PACAP-38 (no data concerning PACAP-27 are available) did not influence the plasma concentration of insulin, glucagon and glucose (Onaga et al. 1996) what indicates no effect of PACAP-38 on the ovine endocrine pancreas activity. The role of both PACAP-27 and PACAP-38 in the regulation of the pancreatic blood flow has been also experimentally determined in several animal species (Naruse et al. 1998, Yamaguchi et al. 2003). Interestingly in the present experiment we have found for the first time, that PACAP-27 is very widely expressed in the ovine intrapancreatic neurons (but not in ganglionic nerve fibres) and PACAP-27-IR neurons co-expressed galanin, SP and CRF (however to a various degree). The most numerous subpopulation of PACAP-27-IR intrapancreatic neurons was that co-expressing galanin. In recent studies it has been well documented that galanin widely present in the ruminants' pancreas (Baltazar et al. 2001) possesses a potent inhibitory action on pancreatic insulin as well as pancreatic juice secretion (Runzi et al. 1992). As outlined above, PACAP-27 in the mammalian pancreas should be regarded as stimulatory neurotransmitter and the co-localization of two peptides showing opposite actions in one intrapancreatic neuron is intriguing

from the functional point of view. Since in the present study we found numbers of PACAP-27-IR/galanin-IR nerve endings located between pancreatic acini and small arterioles, it is justified to speculate that galanin may functionally counteract the secretory or vasoconstrictory effect of PACAP-27. On the other hand, PACAP is also known for its neuroprotective and neuritogenic actions (Zhang et al. 1995, Tsuchida et al. 2014), and in a series of experiments a similar role of galanin has been also shown in relation to neurons of the central, peripheral and enteric nervous system (Hobson et al. 2010). Taking into account the above, it is also likely that both neuropeptides may, at least to some degree, participate and complement each other in innate pancreatic defense system coordinating neuronal response to noxious stimuli. So far, the co-expression of PACAP and galanin was observed in the rat sympathetic cranial cervical ganglion neurons (Moller et al. 1997) and both peptides were found to promote neuronal sprouting in axotomized rat cranial motor neurons (Suarez et al. 2006). Finally, since in the present study numerous varicose galanin-positive/ /PACAP-27-negative nerve terminals encircling PACAP-27-IR neurons were found it is possible that in the ovine pancreas galanin also acts as neuromodulator controlling the neuronal release of PACAP-27 from intrapancreatic neurons. Synaptic interactions between both neuropeptides have been previously described at the central level (Kozicz and Arimura 2000). In approx. 10% population of PACAP-27-IR intrapancreatic neurons co-expression with SP was observed. Since PACAP-27-IR/SP-IR nerve terminals were found around acini only we speculate that they may originate from local intrapancreatic ganglia. In the pancreas of the mammals, SP acting via NK1 receptor has been recognized as a potent stimulator of both endocrine and exocrine function (Schmidt et al. 2000). Till now not much is known whether and how PACAP and SP functionally interact in the pancreas so analogies to other ganglia would be of interest. At the periphery, the co-localization of SP and PACAP is frequently noted in 40-50% of sensory neurons (Rytel et al. 2014). In the rat model of neurogenic inflammation, PACAP-38 released from afferent nerve terminals has been found to inhibit outflow of SP, somatostatin and calcitonin-gene related peptide (Nemeth et al. 2006). In the guinea-pig, PACAP increased airway plasma extravasation evoked by SP as well as inhibited the release of SP from non-adrenergic non-cholinergic nerve terminals (Shigyo et al. 1998). However the exact functions of both peptides in the ovine pancreatic physiology and pathophysiology need to be further explored with the use of experimental protocols.

In the present study, we have also shown the co-expression of PACAP-27 and CRF in a very small subset of intrapancreatic neurons. Interestingly, throughout the ovine pancreas we were unable to localize PACAP-27-IR/CRF-IR nerve fibres and at the moment no reasonable explanation of this finding can be proposed. The presence of numerous mainly varicose CRF-positive nerve terminals has been however found around PACAP-27-positive neurons what may further suggest the existence of functional synaptic co-operation between those two neuropeptides. Similar relationship was found in the bed nucleus of the stria terminalis, where PACAP-containing nerve terminals formed functional synapses with CRF-positive neurons (Kozicz et al. 1997). Little is known about the role of CRF in the pancreas physiology and the findings are sometimes conflicting. So far, it has been documented that in the rat pancreas CRF acting directly on beta cells stimulates insulin secretion (Torres-Aleman et al. 1984), whereas in other studies such effect was not observed (Moltz and Fawcett 1985). In human pancreas CRF was found to stimulate the exocrine (but not endocrine) activity (Lytras et al. 1984). In the mouse model, CRF released in response to stress was able to inhibit pancreatic exocrine secretion (Lenz et al. 1992). Examples and underlying mechanisms of relationships between CRF and PACAP in peripheral organs are barely known and understood. Among others, in hypophysectomized calves, after the intraaortic infusion of PACAP an increase of CRF in adrenal medulla was noted (Edwards and Jones 1994). At the central level, CRF was found to mediate inhibitory effect on female rat ovulation evoked by intracerebroventricular administration of PACAP (Kántor et al. 2000). Additionally, CRF is believed to act centrally as mediator of PACAP-stimulated anxiogenic and pro-depressant effects (Dore et al. 2013). Whether CRF has any influence on PACAP-27-mediated ovine pancreatic activities needs to be further elucidated.

In conclusion, the present study revealed that galanin, SP and CRF are co-expressed with PACAP-27 in intrapancreatic neurons of the sheep. We believe that the results obtained should provide a valuable basis for future functional experiments.

References

Anglade P (**1987**) Ultrastructural study of acetylcholinesterase activity in the intrapancreatic ganglia of the rat. Cell Mol Biol 33: 63-67.

Arciszewski MB (**2007**) Expression of neuronal nitric oxide synthase in the pancreas of the sheep. Anat Histol Embryol 36: 375-381.

Arciszewski MB, Zacharko-Siembida A (**2007a**) A co-localization study on the ovine pancreas innervation. Ann Anat 189: 157-167.

Arciszewski MB, Zacharko-Siembida A (**2007b**) Cholinergic

innervation of the pancreas in the sheep. Acta Biol Hung 58: 151-161.

Arciszewski MB, Stefaniak M, Zacharko-Siembida A, Całka J (**2011**) Aquaporin 1 water channel is expressed on submucosal but not myenteric neurons from the ovine duodenum. Ann Anat 193: 81-85.

Baltazar ET, Kitamura N, Sasaki M, Cottrell DF, Boloron HM, Yamada J (**2001**) Galanin-like immunoreactive neural elements in domestic ruminant pancreas. J Vet Med Sci 63: 841-848.

Cox HM (**1992**) Pituitary adenylate cyclase activating polypeptides, PACAP-27 and PACAP-38: stimulators of electrogenic ion secretion in the rat small intestine. Br J Pharmacol 106: 498-502.

Csati A, Tajti J, Kuris A, Tuka B, Edvinsson L, Warfvinge K (**2012**) Distribution of vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide, nitric oxide synthase, and their receptors in human and rat sphenopalatine ganglion. Neuroscience 202: 158-168.

Dore R, Iemolo A, Smith KL, Wang X, Cottone P, Sabino V (**2013**) CRF mediates the anxiogenic and anti-rewarding, but not the anorectic effects of PACAP. Neuropsychopharmacology 38: 2160-2169.

Edwards AV, Jones CT (**1994**) Adrenal responses to the peptide PACAP in conscious functionally hypophysectomized calves. Am J Physiol 266: E870-E876.

Filipsson K, Sundler F, Hannibal J, Ahren B (**1998**) PACAP and PACAP receptors in insulin producing tissues: localization and effects. Regul Pept 74: 167-175.

Fridolf T, Sundler F, Ahren B (**1992**) Pituitary adenylate cyclase-activating polypeptide (PACAP): occurrence in rodent pancreas and effects on insulin and glucagon secretion in the mouse. Cell Tissue Res 269: 275-279.

Ghatei MA, Takahashi K, Suzuki Y, Gardiner J, Jones PM, Bloom SR (**1993**) Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. J Endocrinol 136: 159-166.

Girard BM, Young BA, Buttolph TR, White SL, Parsons RL (**2007**) Regulation of neuronal pituitary adenylate cyclase-activating polypeptide expression during culture of guinea-pig cardiac ganglia. Neuroscience 146: 584-593.

Hannibal J, Fahrenkrug J (**2000**) Pituitary adenylate cyclase-activating polypeptide in intrinsic and extrinsic nerves of the rat pancreas. Cell Tissue Res 299: 59-70.

He X, Meng F, Wang Y, Li J (**2014**) Molecular cloning and characterization of two pig vasoactive intestinal polypeptide receptors (VPAC1-R and VPAC2-R). DNA Cell Biol 33: 259-270.

Hobson SA, Bacon A, Elliot-Hunt CR, Holmes FE, Kerr NC, Pope R, Vanderplank P, Wynick D (**2010**) Galanin acts as a trophic factor to the central and peripheral nervous systems. EXS 102: 25-38.

Kanasaki H, Oride A, Kyo S (**2015**) Role of pituitary adenylate cyclase-activating polypeptide in modulating hypothalamus-pituitary neuroendocrine functions in mouse cell models. J Neuroendocrinol 27: 1-7.

Kántor O, Molnár J, Heinzelmann A, Fürst Z, Arimura A, Köves K (**2000**) The inhibitory effect of PACAP38 on ovulation is mediated by CRF and endogenous opioids. Ann N Y Acad Sci 921: 405-409.

Kirchgessner AL, Gershon MD (**1990**) Innervation of the pancreas by neurons in the gut. J Neurosci 10: 1626-1642.

Kirchgessner AL, Liu MT (**2001**) Pituitary adenylate cyclase activating peptide (PACAP) in the enteropancreatic innervation. Anat Rec 262: 91-100.

Köves K, Arimura A, Vigh S, Somogyvári-Vigh A, Miller J (**1993**) Immunohistochemical localization of PACAP in the ovine digestive system. Peptides 14: 449-455.

Kozicz T, Vigh S, Arimura A (**1997**) Axon terminals containing PACAP- and VIP-immunoreactivity form synapses with CRF-immunoreactive neurons in the dorsolateral division of the bed nucleus of the stria terminalis in the rat. Brain Res 767: 109-119.

Kozicz T, Arimura A (**2000**) Synaptic interaction between galanin immunoreactive neurons and axon terminals immunopositive for VIP and PACAP in the bed nucleus of the stria terminalis in the rat. Ann N Y Acad Sci 921: 327-332.

Lenz HJ, Messmer B, Zimmerman FG (**1992**) Noradrenergic inhibition of canine gallbladder contraction and murine pancreatic secretion during stress by corticotropin-releasing factor. J Clin Invest 89: 437-443.

Love JA, Szebeni K (**1999**) Morphology and histochemistry of the rabbit pancreatic innervation. Pancreas 18: 53-64.

Luiten PG, ter Horst GJ, Koopmans SJ, Rietberg M, Steffens AB (**1984**) Preganglionic innervation of the pancreas islet cells in the rat. J Auton Nerv Syst 10: 27-42.

Lytras N, Grossman A, Rees LH, Schally AV, Bloom SR, Besser GM (**1984**) Corticotrophin releasing factor: effects on circulating gut and pancreatic peptides in man. Clin Endocrinol (Oxf) 20: 725-729.

Manecka DL, Mahmood SF, Grumolato L, Lihrmann I, Anouar Y (**2013**) Pituitary adenylate cyclase-activating polypeptide (PACAP) promotes both survival and neuritogenesis in PC12 cells through activation of nuclear factor ęB (NF-ęB) pathway: involvement of extracellular signal-regulated kinase (ERK), calcium, and c-REL. J Biol Chem 288: 14936-14948.

Martínez-Fuentes AJ, Castaño JP, Gracia-Navarro F, Malagón MM (**1998**) Pituitary adenylate cyclase-activating polypeptide (PACAP) 38 and PACAP27 activate common and distinct intracellular signaling pathways to stimulate growth hormone secretion from porcine somatotropes. Endocrinology 139: 5116-5124.

Masuo Y, Ohtaki T, Masuda Y, Nagai Y, Suno M, Tsuda M, Fujino M (**1991**) Autoradiographic distribution of pituitary adenylate cyclase activating polypeptide (PACAP) binding sites in the rat brain. Neurosci Lett 126: 103-106.

Miampamba M, Germano PM, Arli S, Wong HH, Scott D, Tache Y, Pisegna JR (**2002**) Expression of pituitary adenylate cyclase-activating polypeptide and PACAP type 1 receptor in the rat gastric and colonic myenteric neurons. Regul Pept 105: 145-154.

Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH (**1989**) Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun 164: 567-574.

Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N, Arimura A (**1990**) Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the

pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). Biochem Biophys Res Commun 170: 643-648.

Moller K, Reimer M, Ekblad E, Hannibal J, Fahrenkrug J, Kanje M, Sundler F (**1997**) The effects of axotomy and preganglionic denervation on the expression of pituitary adenylate cyclase activating peptide (PACAP), galanin and PACAP type 1 receptors in the rat superior cervical ganglion. Brain Res 775: 166-182.

Moltz JH, Fawcett CP (**1985**) Corticotropin-releasing factor: its action on the islets of Langerhans. Endocr Res 11: 87-93.

Mounien L, Do Rego JC, Bizet P, Boutelet I, Gourcerol G, Fournier A, Brabet P, Costentin J, Vaudry H, Jégou S (**2009**) Pituitary adenylate cyclase-activating polypeptide inhibits food intake in mice through activation of the hypothalamic melanocortin system. Neuropsychopharmacology 34: 424-435.

Naruse S, Ito O, Kitagawa M, Ishiguro H, Nakajima M, Hayakawa T (**1998**) Effects of PACAP/VIP/secretin on pancreatic and gastrointestinal blood flow in conscious dogs. Ann N Y Acad Sci 865: 463-465.

Németh J, Reglödi D, Pozsgai G, Szabó A, Elekes K, Pintér E, Szolcsányi J, Helyes Z (**2006**) Effect of pituitary adenylate cyclase activating polypeptide-38 on sensory neuropeptide release and neurogenic inflammation in rats and mice. Neuroscience 143: 223-230.

Onaga T, Uchida M, Kimura M, Miyazaki M, Mineo H, Kato S, Zabielski R (**1996**) Effect of pituitary adenylate cyclase-activating polypeptide on exocrine and endocrine secretion in the ovine pancreas. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 115: 185-193.

Puig de Parada M, Parada MA, Hernández L (**1995**) Dipsogenic effect of pituitary adenylate cyclase activating polypeptide (PACAP38) injected into the lateral hypothalamus. Brain Res 696: 254-257.

Rünzi M, Müller MK, Schmid P, von Schönfeld J, Goebell H (**1992**) Stimulatory and inhibitory effects of galanin on exocrine and endocrine rat pancreas. Pancreas 7: 619-623.

Rytel L, Palus K, Całka J (**2014**) Co-expression of PACAP with VIP, SP and CGRP in the porcine nodose ganglion sensory neurons. Anat Histol Embryol 44: 86-91.

Shigyo M, Aizawa H, Inoue H, Matsumoto K, Takata S, Hara N (**1998**) Pituitary adenylate cyclase activating peptide regulates neurally mediated airway responses. Eur Respir J 12: 64-70.

Schmidt PT, Tornøe K, Poulsen SS, Rasmussen TN, Holst JJ (**2000**) Tachykinins in the porcine pancreas: potent exocrine and endocrine effects via NK-1 receptors. Pancreas 20: 241-247.

Sharkey KA, Williams RG, Dockray GJ (**1984**) Sensory substance P innervation of the stomach and pancreas. Demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. Gastroenterology 87: 914-921.

Suarez V, Guntinas-Lichius O, Streppel M, Ingorokva S, Grosheva M, Neiss WF, Angelov DN, Klimaschewski L (**2006**) The axotomy-induced neuropeptides galanin and pituitary adenylate cyclase-activating peptide promote axonal sprouting of primary afferent and cranial motor neurones. Eur J Neurosci 24: 1555-1564.

Svoboda M, Tastenoy M, Ciccarelli E, Stiévenart M, Christophe J (**1993**) Cloning of a splice variant of the pituitary adenylate cyclase-activating polypeptide (PACAP) type I receptor. Biochem Biophys Res Commun 195: 881-888.

Tornze K, Hannibal J, Giezemann M, Schmidt P, Holst JJ (**1996**) PACAP 1-27 and 1-38 in the porcine pancreas: occurrence, localization, and effects. Ann N Y Acad Sci 805: 521-535.

Torres-Aleman I, Mason-Garcia M, Schally AV (**1984**) Stimulation of insulin secretion by corticotropin-releasing factor (CRF) in anesthetized rats. Peptides 5: 541-546.

Tsuchida M, Nakamachi T, Sugiyama K, Tsuchikawa D, Watanabe J, Hori M, Yoshikawa A, Imai N, Kagami N, Matkovits A, Atsumi T, Shioda S (**2014**) PACAP stimulates functional recovery after spinal cord injury through axonal regeneration. J Mol Neurosci 54: 380-387.

Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, Fournier A, Chow BK, Hashimoto H, Galas L, Vaudry H (**2009**) Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 61: 283-357.

Yamaguchi N, Minassian TR, Yamaguchi S (**2003**) Effects of PACAP(1-27) on the canine endocrine pancreas in vivo: interaction with cholinergic mechanism. Can J Physiol Pharmacol 81: 720-729.

Zhang Q, Shi TJ, Ji RR, Zhang YZ, Sundler F, Hannibal J, Fahrenkrug J, Hokfelt T (**1995**) Expression of pituitary adenylate cyclase-activating polypeptide in dorsal root ganglia following axotomy: time course and coexistence. Brain Res 705: 149-158.