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Short communication

Effect of PACAP treatment on kidney morphology and cytokine expression in rat diabetic nephropathy

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ABSTRACT

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide, exerting diverse effects. One of its frequently examined functions is cell protection, which is achieved mainly via inhibiting apoptotic, inflammatory and oxidative processes. All its three receptors (PAC1, VPAC1, VPAC2) are expressed in the kidney and PACAP has been shown to have protective effects against different renal pathologies. Diabetic nephropathy is the leading cause of end stage renal disease. The aim of the present study was to investigate the possible ameliorative effect of PACAP in streptozotocin-induced diabetic nephropathy and to evaluate its anti-inflammatory effect in this model. Diabetes was induced by a single intravenous injection of streptozotocin (65 mg/kg) in male Wistar rats. PACAP-treated animals were administered ip. 20 μg PACAP every second day, while untreated animals were given vehicle. Kidneys were removed after 8-weeks survival. Besides the complex histological analysis (glomerular PAS positive area/glomerulus area, tubular damage, arteriolar hyalinosis), expression of several cytokines was evaluated by cytokine array and Luminex assay. Histological analysis revealed severe diabetic changes in kidneys of control diabetic animals (glomerular PAS-positive area expansion, tubular damage, Armanni-Ebstein phenomenon). PACAP treatment significantly diminished the damage. Diabetic kidneys showed significant cytokine activation compared to their healthy controls. PACAP was effective in downregulation of several cytokines including CINC-1, TIMP-1, LIX, MIG, s-ICAM. To conclude, PACAP is effective in ameliorating diabetic nephropathy at least partly through its well-known anti-inflammatory effect. These results raise the opportunity for the use of PACAP as a possible therapeutic or preventive method in treating the complications of diabetes.

1. Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective neuropeptide, which exists in two forms with 38 and 27 amino acid residues: PACAP-38 and -27, respectively [42]. PACAP acts through three G protein-coupled receptors: the specific PAC1 and the non-specific VPAC1 and VPAC2 receptors. Highest levels of PACAP occur in the central nervous system and endocrine glands [42]. However, lower expression has also been shown in the gastrointestinal, respiratory, cardiovascular and urogenital systems [13,42]. Based on the widespread distribution, PACAP has been found to have diverse effects in the nervous system and peripheral organs. One of the early described effects of PACAP is its strong neuroprotective potential. Several studies have proven that PACAP is a general cytoprotective peptide, exerting cell-survival promoting effects in numerous tissues and cells, including endothelial cells, lymphocytes, liver, lungs and ovary [11,28,45,47].

Actions of PACAP in the kidney are less known. PACAP and its receptors (PAC1, VPAC1 and VPAC2) are all expressed in the human, rat and chicken renal tissues [27]. Some effects have also been described in the kidney, such as vasodilation and renin secretion [27]. However, a large body of experimental data supports the protective effects of PACAP in the kidney. It is effective against hydrogen peroxide-induced oxidative stress in vitro [15]. In vivo, PACAP is protective against ischemia/reperfusion injury and improves the survival rate of rats undergone renal vessel clamping [17,38]. PACAP is also renoprotective against myeloma nephropathy, subsequently even confirmed in a single patient study [2,20].

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Similar nephroprotective effect can be observed in cyclosporine A and gentamicin-induced injuries [2,18,20,27].

Based on these data, the nephroprotective effects of PACAP are well-established. However, little is known about the relationship between PACAP and one of the most common nephropathies, the diabetic nephropathy. Among all the classical diabetic complications (retino-, nephro- and neuropathy), diabetic nephropathy causes the highest increase in morbidity and mortality. Diabetic nephropathy affects approximately 30–40% of all the diabetic patients and it is the leading cause of chronic kidney disease and end stage renal failure. Patients suffering from diabetic nephropathy show 15–20-fold higher mortality rate compared to healthy subjects. The disease is characterized by both functional and structural changes of the kidney. Glomerular hypertrophy, glomerular basement membrane thickening, apoptosis and desquamation of the podocytes are already present in the early stage of the disease. Mesangial matrix expansion, diffuse and nodular glomerulosclerosis, tubulointerstitial fibrosis, tubular atrophy and glycogen-accumulation also develop with the progression of the disease (Armanni-Ebstein phenomenon) [40,48]. In rats, diabetic nephropathy can be mimicked by streptozotocin-induced pancreatic beta cell loss [41]. An earlier study has described protective effects of PACAP in early diabetic nephropathy [20] and we have recently proven that PACAP is protective in another common complication of diabetes, the diabetic retinopathy [36]. The aim of the present study was to investigate the protective potential of in vivo PACAP treatment in 8 week diabetes-induced nephropathy and to study the involvement of anti-inflammatory pathways by using cytokine measurements.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 19$) weighing 250–300 g were housed under light/dark cycles of 12:12 h and received normal rat chow and drinking water ad libitum. Rats were randomly divided in 4 groups: (1) untreated control (saline i.v. and i.p., $n = 4$); (2) PACAP-treated control (saline i.v. and 20 μg PACAP1-38 i.p. every second day, $n = 4$); (3) untreated diabetic (65 mg/kg streptozotocin (Sigma, Hungary) i.v. and saline i.p., $n = 6$); (4) PACAP-treated diabetic (65 mg/kg streptozotocin i.v. and 20 μg PACAP1-38 i.p. every second day, $n = 5$). Rats were weighed and their blood glucose concentration was measured weekly by blood glucose monitor (Accu-Check Active, Roche, Hungary). Animals were considered diabetic in cases they showed elevated blood glucose levels (>11 mmol/l) and the classical symptoms of diabetes including polyuria, polydipsia, polyphagia and excessive weight loss. After 8 weeks, animals were sacrificed with an overdose of anesthetics and kidneys were removed and weighed. Experimental procedures were carried out in accordance with approved protocols (University of Pecs; BA02/2000-15024/2011).

2.2. Renal histology and morphometric analysis

Kidneys were fixed in 10% formalin, embedded in paraffin and 5 μm thin sections were cut with microtome. Sections were stained with periodic acid-Schiff base (PAS) or with hematoxylin–eosin and digital photos were taken. Diastase digested PAS reaction after absolute alcohol fixation was also performed to prove the glycogen content of tubular granules. Renal histological analysis was carried out by a nephropathologist blinded to the treatment groups (PD). At least 10 glomeruli on each slide were examined for determination of glomerular changes [40]. The degree of accumulated glycogen granules (Armanni-Ebstein phenomenon) was evaluated as an indicator of tubulointerstitial lesions. Analysis was carried out at 400× magnification. Magenta staining of intraglomerular PAS positive area shows the mesangial matrix and basal membrane, while tubular PAS positive granules, Armanni-Ebstein lesions are the sign of tubular damage. Adobe Photoshop program was used to mark the glomerulus manually, then it was converted to black, while the remaining area to white. The proper magenta of intraglomerular PAS positive area and tubular glycogen granules were also marked and converted to black with the same method. Finally in every picture the "area of interest" was converted to black with the remaining area becoming white, thus creating black and white pictures. Black areas in the vision fields were measured using Scion Image program. Finally, the intraglomerular PAS positive area was expressed against the area of the same glomerulus, while glycogen granules against the vision field. Arteriolar hyalinosis, a lack of smooth muscle in the arteriolar wall, was evaluated on hematoxylin-eosin sections according to the following score: "0" arteriole without any sign of hyalinosis, "1", "2", "3", "4" with hyalinosis affecting ¼, ½, ¾ or the entire cross section of the arteriole, respectively.

2.3. Cytokine measurements

The semiquantitative cytokine array was performed from kidney homogenates using Rat Cytokine Array kit (R&D Systems; Biomedica Hungaria, Hungary). These arrays are based on binding between sample proteins and carefully selected captured antibodies spotted on nitrocellulose membranes. We examined tissue samples from all the 4 groups: control, PACAP-treated control, diabetic and PACAP-treated diabetic groups. The array was performed as described by the manufacturer, similarly to our previous study [25]. Briefly, kidney samples were homogenized in PBS with protease inhibitor. Triton X-100 was added to the final concentrations of 1%. After blocking the array membranes for 1 h and adding the reconstituted detection antibody cocktail for another 1 h at room temperature, the membranes were incubated with 1 ml of tissue homogenates at 2–8 °C overnight on a rocking platform. After washing with buffer three times and addition of horseradish peroxidase-conjugated streptavidin to each membrane, we exposed them to a chemiluminescent detection reagent (Amersham Biosciences, Hungary), then side up to an X-ray film cassette. The array was run in duplicate sets. Results are represented in pixel density and are compared in a semiquantitative fashion.

Luminex Multiplex Immunoassay was performed as described previously [25]. Briefly, soluble intercellular adhesion molecule-1 (sICAM1) and L-selectin levels were determinated using customized Flurokine MAP Rat Base Kit (R&D Systems). The experiment was performed according to the manufacturer's instructions (R&D Systems). Following previous optimizations, all samples were tested undiluted in a blind-fashion. Luminex100 device was used for the immunoassay and Luminex 100 IS software for the analysis of bead median fluorescence intensity. Samples were homogenized with RPMI-1640 (GIBCO) containing 1% protease inhibitor cocktail, samples were used in 20 mg/ml concentrations. All the tests were run in duplicate. 50 μl volume of each sample, control, or standard was added to a 96-well plate (provided with the kit) containing 50 μl of antibody-coated fluorescent beads. Biotinylated secondary and streptavidin-PE antibodies were added to the plate with alternate incubation and washing steps. After the last washing step, 100 μl of the buffer was added to the wells; the plate was incubated and read on the Luminex100 array reader, using a four-PL regression curve to plot the standard curve. Data were subsequently analyzed using the Luminex100 manager software. Results are given in pg L-selectin or s-ICAM/g wet tissue.

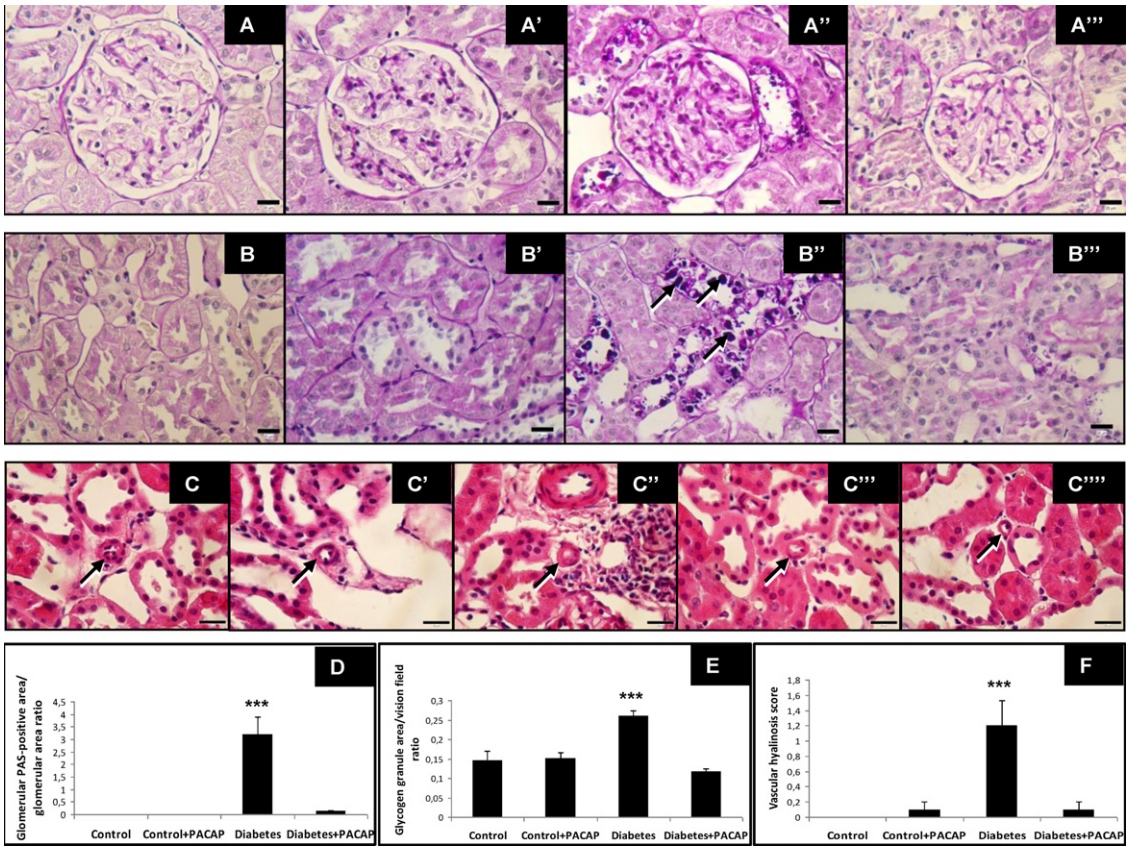

Fig. 1. Histological analysis of control and diabetic kidneys. Representative histological sections of glomeruli (row A) and renal tubules (B). Sections of control (A and B), PACAP-treated control (A′ and B′), diabetic (A″ and B″), PACAP-treated diabetic (A‴ and B‴) animals. Control and PACAP-treated control animals showed normal glomerular and tubular structure. Diabetic kidneys showed severe intraglomerular PAS positive area expansion and glomerular damage (A″) and severe tubular atrophy and deposition of glycogen granules, called Armanni-Ebstein phenomenon (B″, arrows). PACAP-treatment was preventive against diabetic changes, milder damage was observed in these sections (A‴ and B‴). Periodic acid-Schiff (PAS) reaction. Calibration mark: 20 μm. Representative histological sections of arterioles with a severity score of 0, 1, 2, 3, 4 (C, C′, C″, C‴, C⁗ respectively). Hematoxylin–eosin staining. Calibration mark: 20 μm. PAS positive area per glomerular area ratio (D) ***$p < 0.0001$ vs. all other groups (control, control + PACAP, diabetes + PACAP groups). Glycogen granules (Armanni-Ebstein phenomenon) per vision field ratio (E) ***$p < 0.0001$ vs. all other groups (control, control + PACAP, diabetes + PACAP groups). Vascular hyalinosis score (F). Significant hyalinosis was observed in diabetic animals, while in the PACAP-treated diabetic animals there were no signs of the PAS positive arteriolar wall thickening. ***$p < 0.0001$ diabetes vs all other groups (control, control + PACAP, diabetes + PACAP groups).

2.4. Statistical analysis

Statistical analysis was performed by Microsoft Office Excel and GraphPad software. Repeated measures analysis of variance (ANOVA) with Bonferroni correction was used to detect significant differences between groups. p-Value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Body weight, total kidney weight to body weight ratio and blood glucose levels

The body weight of the animals after 8 weeks of treatments was 365 ± 29 g in the intact controls and 351 ± 31 g in the PACAP-treated group. Diabetic animals showed a significant decrease in their weight: we measured 230 ± 17 g in the diabetic and 260 ± 19 g in the PACAP-treated diabetic group. However, the slight difference between diabetic and PACAP-treated diabetic rats did not result to be statistically significant. PACAP treatment alone did not result in significant change in kidney/body weight (6.44 ± 0.13) compared to the untreated control kidneys (6.68 ± 0.09). In diabetic animals, we could observe a significant increase in this ratio (13.80 ± 1.36), similarly to observations by others [6]. PACAP treatment in diabetic animals slightly decreased this ratio, however, this was not statistically significant (12.33 ± 0.37). There was no difference in the weekly blood sugar level in the intact control and PACAP-treated groups or between the diabetic control and PACAP-treated groups. At the end of the experiment (at week 8) the blood glucose level was 7.85 ± 0.42 and 7.55 ± 0.36 mmol/L in the intact control and PACAP-treated intact animals, respectively. In the diabetic animals, these values were 30.13 ± 1.75 mmol/L in the diabetic rats and 32.42 ± 0.69 mmol/L in the PACAP-treated diabetic animals.

3.2. PACAP attenuated diabetic nephropathy: renal histology and morphometric analysis

In control animals, normal glomerular structure was found without any tubular or glomerular injury and PACAP treatment for 8 weeks did not cause any changes. Kidneys from diabetic animals showed signs of severe nephropathy, with mesangial matrix expansion and abundant glycogen granules (Fig. 1A–D). A hallmark of diabetic changes indicating the progression of the mesangial changes in diabetic nephropathy is the ratio of intraglomerular PAS positive area per glomerular area. This significantly increased in the diabetic rats, while PACAP treatment could effectively counteract this lesion: PACAP-treated diabetic animals showed no significant difference compared with intact control kidneys (Fig. 1A and D). Another very important characteristics of diabetic nephropathy is the appearance of glycogen deposits in the tubules, also called Armanni-Ebstein phenomenon [48]. This feature was completely missing from control kidneys, while the presence of

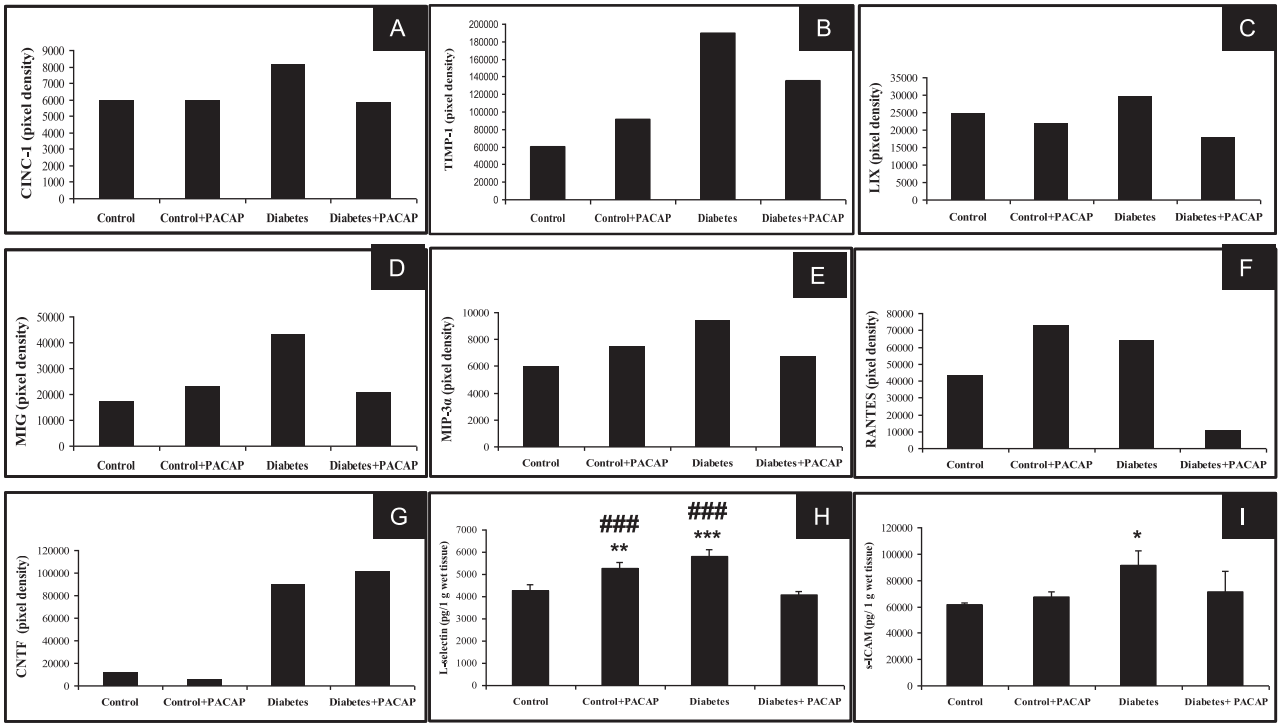

Fig. 2. Data of the semiquantitative cytokine array of CINC-1, TIMP-1, LIX, MIG, RANTES, MIP-3α, CNTF (A)–(G). Data are presented as mean pixel density. Measurement of L-selectin and s-ICAM by Luminex assay (H) and (I). Data are presented as mean pg/g wet tissue ± SEM. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$ vs. control, $^{\#\#\#}p < 0.0001$ vs. diabetes + PACAP.

glycogen deposits markedly increased in diabetic rats. In contrast, the amount of glycogen deposits was significantly lower in the PACAP-treated diabetic group than in the untreated diabetic group (Fig. 1B and E). A characteristic feature of microangiopathy is vascular hyalinosis, which is sparingly present in the control groups. The degree of arteriolar hyalinosis was significantly higher in the diabetic group compared to control animals, while PACAP treatment was able to completely prevent the development of hyalinosis (Fig. 1C and F).

3.3. Cytokine measurements

PACAP treatment had no effect on the level of numerous cytokines in control animals, however, it elevated the expression of a few cytokines: tissue inhibitor of metalloproteinase 1 (TIMP-1), monokine induced by gamma interferon (MIG/CXCL9), macrophage inflammatory protein 3α (MIP-3α), regulated and normal T cell expressed and secreted (RANTES, CCL5), L-selectin (CD62L/LECAM-1) and decreased lipopolysaccharide-induced CXC chemokine (LIX/CXCL5) and ciliary neurotrophic factor (CNTF) levels. Diabetes strongly increased the expression of cytokine induced neutrophil chemokine (CINC-1), TIMP-1, LIX, MIG, MIP-3α, RANTES, CNTF and L-selectin. Treatment with 20 μg PACAP markedly decreased the above-mentioned cytokines and chemokines, with some of them reaching control levels (Fig. 2A–I). No changes were observed in other spots: Fractalkine, thymus chemokine and interleukins (data not shown).

4. Discussion

In the present study we showed that in vivo PACAP treatment is protective in diabetic nephropathy and this ameliorative effect is at least partly due to the anti-inflammatory effect of PACAP. Our observations complete the list of kidney pathologies against which PACAP has protective effects. Among others, it is effective against oxidative stress, gentamicin-induced nephropathy, myeloma and ischemia-induced injuries and cyclosporine A-induced lesion [2,15,18,20,38]. An earlier study has even mentioned that PACAP has protective effects in early (2 weeks) diabetic nephropathy in rats [20]. Our present results, with prolonged survival time, are thus in accordance with these observations and complete these findings with detailed histological analysis and cytokine measurements. Furthermore, we applied long-term PACAP administration for the first time in the treatment of renal diseases, proving that PACAP treatment can be effective even in chronic therapy.

Our study showed that PACAP markedly attenuated the characteristic pathological alterations observed in diabetic rats without altering the blood glucose levels. Increased intraglomerular PAS positive area is a reliable indicator of basement membrane thickening and glomerular mesangial matrix expansion, which are characteristic for diabetic glomerulopathy [40]. In the present study we found significant expansion of glomerular PAS-positive area caused by hyperglycemia after a single streptozotocin injection similarly to others [22]. Hyperglycemia stimulates the pathological accumulation of the otherwise physiological components in the extracellular matrix, like laminin, fibronectin, collagene IV through overexpression of TGF-β1 [12]. Changes in the tubulointerstitium may possess even higher importance in the progression of diabetic kidney disease [39]. Tubular vacuolation on autopsy is considered to be pathognomic to death due to diabetic coma. In our study, tubular damage was evaluated based on the amount of glycogen granules within the proximal tubules, called Armanni-Ebstein phenomenon [19,48]. Arteriolar hyalinosis occurs in both afferent and efferent arterioles in the diabetic kidney, however, efferent arteriolar hyalinosis is considered to be characteristic for diabetic nephropathy [35]. Kidneys of PACAP-treated diabetic animals showed significantly milder glomerular, tubular and vascular changes.

As far as the protective mechanism is concerned, we investigated the anti-inflammatory effects of PACAP in the present study.

PACAP is well-known for its anti-inflammatory actions also in some of the above-mentioned renal pathological conditions [27]. Recent studies indicate that inflammation is one of the key mechanisms in the development of diabetic nephropathy in addition to hyperglycemia and hemodynamic factors [24]. Most renal cells, such as endothelial, mesangial, epithelial cells and podocytes become activated and produce proinflammatory cytokines, chemokines and adhesion molecules upon injury. These chemoattractant molecules induce infiltration of several immune cells, like monocytes, neutrophils and lymphocytes into the kidney, leading to tubulointerstitial fibrosis, glomerulosclerosis, tubular atrophy and vascular damage [1,46]. PACAP has been found to alter cytokine expression in numerous studies. We have described earlier that PACAP counteracted several changes in cytokine and chemokine expression induced by intestinal and kidney ischemia/reperfusion and retinal ischemia [15,25,37]. In the present study we found that PACAP reduced the diabetes-induced elevation of CINC-1, TIMP-1, LIX, MIG, MIP-3α, RANTES, L-selectin and s-ICAM. All these factors play a potential role in diabetic nephropathy. For example, CINC-1, one of the major neutrophil chemoattractants stimulated by oxidative stress and ischemia/reperfusion, is also present in diabetic nephropathy [7,23]. Levels of TIMP-1, LIX, MIG and s-ICAM, are also elevated in the urine and serum of diabetic nephropathy patients [4,5,14,16]. MIP-3α mRNA and protein were found to be upregulated by hyperglycemia both in vivo and in vitro and elevated urinary MIP-3α after transplantation might contribute to allograft rejection [26,43]. The effects of PACAP on the injury-induced upregulated expression of inflammatory cytokines seem to be a more general phenomenon, since we found similar effects in the retina and intestinal tissue [25,37].

We found an interesting pattern of RANTES and L-selectin expression in our treated groups. PACAP alone increased the expression of both, while it descreased their levels in diabetic animals. The effect of PACAP on some cytokines and chemokines is contradictory and both stimulating and inhibiting effects have been described depending on the cell type injury [9,37]. Earlier studies have shown upregulated RANTES in diabetic kidneys, correlating with proteinuria and expression of NFκB, suggestive of progressive diabetic nephropathy. Earlier studies have found that PACAP can induce the secretion of RANTES in astrocytes and cortical neurons [3,31], most probably associated with increased neuroprotection. However, in other studies, PACAP reduced the elevated expression of RANTES in the retina and in microglial cells [9,37]. The exact role of PACAP in renal RANTES expression needs to be further investigated, but the finding that PACAP could reduce the diabetes-induced elevation suggests a nephroprotective action. A rapid increase in L-selectin, a cell adhesion molecule responsible for the renal mononuclear infiltration, has been described after renal ischemia/reperfusion injury [10,32,34]. The finding that PACAP reduced the diabetes-induced elevation of L-selectin suggests a protective effect, but the initial stimulatory effect of PACAP in control animals needs to be further clarified. In contrast to other cytokines, PACAP stimulated the expression of the trophic factor CNTF in diabetic kidneys, indicating another possible protective mechanism similarly to earlier descriptions [29].

In summary, our present study showed the protective effects of in vivo PACAP treatment in diabetic nephropathy by involving anti-inflammatory actions. Our study further supports the implication that PACAP might have protective potential in diabetes and its complications. PACAP is able to increase pancreatic beta cell proliferation, enhance insulin secretion and it has been shown to be protective in two major diabetic complications: retinopathy and nephropathy [30,33,36,44]. Thus, PACAP is a good candidate for therapeutic interventions in diabetes at multiple levels. One of the drawbacks of systemic PACAP treatment is the rapid degradation of the peptide in the blood by dipeptidyl peptidase-IV [DPP-IV, 42]. Inhibitors of this enzyme are being used in the treatment of diabetic patients for the increased level of glucagon-like peptide-1 [8]. Protective effects have been described even in diabetic nephropathy [21]. A recent paper has suggested that the therapeutic effects of DPP-IV inhibitors could involve so far unknown mechanisms, such as increased PACAP levels [8]. Whether the prolonged presence of PACAP in the circulation is protective in diabetes-related complications, is to be determined by future investigation.

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References

[1] Abbate M, Zoja C, Remuzzi G. How does proteinuria cause progressive renal damage? J Am Soc Nephrol 2006;17:2974–84.
[2] Arimura A, Li M, Batuman V. Potential protective action of pituitary adenylate cyclase-activating polypeptide (PACAP38) on in vitro and in vivo models of myeloma kidney injury. Blood 2006;107:661–8.
[3] Brenneman DE, Hauser JM, Spong C, Phillips TM. Chemokine release is associated with the protective action of PACAP-38 against HIV envelope protein neurotoxicity. Neuropeptides 2002;36:271–80.
[4] Bruno CM, Valenti M, Bertino G, Ardiri A, Bruno F, Cunsolo M, et al. Plasma ICAM-1 and VCAM-1 levels in type 2 diabetic patients with and without microalbuminuria. Minerva Med 2008;99:1–5.
[5] Catania JM, Chen G, Parrish AR. Role of metalloproteinases in renal pathophysiologies. Am J Physiol Renal Physiol 2007;292:F905–11.
[6] Chen S, Hong SW, Iglesias-de la Cruz MC, Isono M, Casaretto A, Ziyadeh FN. The key role of the transforming growth factor-beta system in the pathogenesis of diabetic nephropathy. Ren Fail 2001;23:471–81.
[7] Cugini D, Azzollini N, Gagliardini E, Cassis P, Bertini R, Colotta F, et al. Inhibition of the chemokine receptor CXCR2 prevents kidney graft function deterioration due to ischemia/reperfusion. Kidney Int 2005;67:1753–61.
[8] Darsalia V, Ortsater H, Olverling A, Darlof E, Wolbert P, Nystrom T, et al. The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain. Diabetes, PMID 23209191, in press.
[9] Delgado M, Jonakait GM, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit chemokine production in activated microglia. Glia 2002;39:148–61.
[10] Dragun D, Hoff U, Park JK, Qun Y, Schneider W, Luft FC, et al. Ischemia-reperfusion injury in renal transplantation is independent of the immunologic background. Kidney Int 2000;58:2166–77.
[11] Elekes K, Sandor K, Moricz A, Kereskai L, Kemeny A, Szoke E, et al. Pituitary adenylate cyclase activating polypeptide plays an anti-inflammatory role in endotoxin-induced airway inflammation: in vivo study with gene-deleted mice. Peptides 2011;32:1439–46.
[12] Falk RJ, Scheinman JI, Mauer SM, Michael AF. Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. Diabetes 1983;32(Suppl. 2):34–9.
[13] Girard BM, Malley SE, Braas KM, May V, Vizzard MA. PACAP/VIP and receptor characterization in micturition pathways in mice with overexpression of NGF in urothelium. J Mol Neurosci 2010;42:378–89.
[14] Higurashi M, Ohya Y, Joh K, Muraguchi M, Nishimura M, Terawaki H, et al. Increased urinary levels of CXCL5, CXCL8 and CXCL9 in patients with Type 2 diabetic nephropathy. J Diabetes Complications 2009;23:178–84.
[15] Horvath G, Brubel R, Kovacs K, Reglodi D, Opper B, Ferencz A, et al. Effects of PACAP on oxidative stress-induced cell death in rat kidney and human hepatocyte cells. J Mol Neurosci 2011;43:67–75.
[16] Kanauchi M, Nishioka H, Nakashima Y, Hashimoto T, Dohi K. Role of tissue inhibitors of metalloproteinase in diabetic nephropathy. Nippon Jinzo Gakkai Shi 1996;38:124–8.
[17] Khan AM, Li M, Abdulnour-Nakhoul S, Maderdrut JL, Simon EE, Batuman V. Delayed administration of pituitary adenylate cyclase-activating polypeptide 38 ameliorates renal ischemia/reperfusion injury in mice by modulating Toll-like receptors. Peptides 2012;38:395–403.
[18] Khan AM, Li M, Brant E, Maderdrut JL, Majid DS, Simon EE, et al. Renoprotection with pituitary adenylate cyclase-activating polypeptide in cyclosporine A-induced nephrotoxicity. J Investig Med 2011;59:793–802.
[19] Kock KF, Vestergaard V. Armanni-Ebstein lesions of the kidney: diagnostic of death in diabetic coma. Forensic Sci Int 1994;67:169–74.
[20] Li M, Maderdrut JL, Lertora JJ, Arimura A, Batuman V. Renoprotection by pituitary adenylate cyclase activating polypeptide in multiple myeloma and other kidney diseases. Regul Pept 2008;145:24–32.
[21] Liu WJ, Xie SH, Liu YN, Kim W, Jin HY, Park SK, et al. Dipeptidyl peptidase IV inhibitor attenuates kidney in streptozotocin-induced diabetic rats. J Pharmacol Exp Ther 2012;340:248–55.

[22] Matavelli LC, Siragy HM. Reduction of aldosterone production improves renal oxidative stress and fibrosis in diabetic rats. J Cardiovasc Pharmacol 2013;61:17–22.
[23] Mizutani A, Okajima K, Uchiba M, Noguchi T. Activated protein C reduces ischaemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation. Blood 2000;95:3781–7.
[24] Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol 2011;7:327–40.
[25] Nedvig K, Weber Gy Nemeth J, Kovacs K, Reglodi D, Kemeny A, et al. Intestinal autotransplantation using PACAP-38-containing preservation solution. J Mol Neurosci 2012;48:788–94.
[26] Qi W, Chen X, Zhang Y, Holian J, Mreich E, Gilbert RE, et al. High glucose induces macrophage inflammatory protein-3 alpha in renal proximal tubule cells via a transforming growth factor-beta 1 dependent mechanism. Nephrol Dial Transplant 2007;22:3147–53.
[27] Reglodi D, Kiss P, Horvath G, Lubics A, Laszlo E, Tamas A, et al. Effects of pituitary adenylate cyclase activating polypeptide in the urinary system, with special emphasis on its protective effects in the kidney. Neuropeptides 2012;46:61–70.
[28] Reglodi D, Kiss P, Szabadfi K, Atlasz T, Gabriel R, Horvath G, et al. PACAP is an endogenous protective factor – insights from PACAP deficient mice. J Mol Neurosci 2012;48:482–92.
[29] Rezende LF, Santos GJ, Carneiro EM, Bodschero AC. Ciliary neurotrophic factor protects mice against streptozotocin-induced type I diabetes through SOCS3: the role of STAT1/STAT3 ratio in β-cell death. J Biol Chem 2012;287:41628–39.
[30] Sakuma Y, Ricordi C, Miki A, Yamamoto T, Mita A, Barker S, et al. Effect of pituitary adenylate cyclase-activating polypeptide in islet transplantation. Transplant Proc 2009;41:343–5.
[31] Sanchez A, Tripathy D, Grammas P. RANTES release contributes to the protective action of PACAP38 against sodium nitroprusside in cortical neurons. Neuropeptides 2009;43:315–20.
[32] Shikata K, Suzuki Y, Wada J, Hirata K, Matsuda M, Kawashima H, et al. L-selectin and its ligands mediate infiltration of mononuclear cells into kidney interstitium after ureteric obstruction. J Pathol 1999;188:93–9.
[33] Shintani N, Tomimoto S, Hashimoto H, Kawaguchi C, Baba A. Functional roles of the neuropeptide PACAP in brain and pancreas. Life Sci 2003;74: 337–43.
[34] Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994;76:301–14.
[35] Stout LC, Kumar S, Whorton EB. Insudative lesions-their pathogenesis and association with glomerular obsolescence in diabetes: a dynamic hypohesis based on single views of advancing human diabetic nephropathy. Hum Pathol 1994;25:1213–27.
[36] Szabadfi K, Atlasz T, Kiss P, Reglodi D, Szabo A, Kovacs K, et al. Protective effects of the neuropeptide PACAP in diabetic retinopathy. Cell Tissue Res 2012;348:37–46.
[37] Szabo A, Danyadi B, Bognar E, Szabadfi K, Fabian E, Kiss P, et al. Effect of PACAP on MAP kinases, Akt and cytokine expressions in rat retinal hypoperfusion. Neurosci Lett 2012;523:93–8.
[38] Szakaly P, Kiss P, Lubics A, Magyarlaki T, Tamas A, Racz B, et al. Effects of PACAP on survival and renal morphology in rats subjected to renal ischemia/reperfusion. J Mol Neurosci 2008;36:89–96.
[39] Taft JL, Nolan CJ, Yeung SP, Hewitson TD, Martin FI. Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. Diabetes 1994;43:1046–51.
[40] Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 2010;21:556–63.
[41] Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology (Carlton) 2007;12:261–6.
[42] Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 2009;61:283–357.
[43] Woltman AM, de Fijter JW, van der Kooij SW, Jie KE, Massacrier C, Caux C, et al. MIP-3alpha/CCL20 in renal transplantation and its possible involvement as dendritic cell chemoattractant in allograft rejection. Am J Transplant 2005;5:2114–25.
[44] Yamamoto K, Hashimoto H, Tomimoto S, Shintani N, Miyazaki J, Tashiro F, et al. Overexpression of PACAP in transgenic mouse pancreatic beta-cells enhances insulin secretion and ameliorates streptozotocin-induced diabetes. Diabetes 2003;52:1155–62.
[45] Yu R, Guo X, Huang L, Zeng Z, Zhang H. The novel peptide PACAP-TAT with enhanced traversing ability attenuates the severe lung injury induced by repeated smoke inhalation. Peptides 2012;38:142–9.
[46] Zandi-Nejad K, Eddy AA, Glassock RJ, Brenner BM. Why is proteinuria an ominous biomarker of progressive kidney disease. Kidney Int Suppl 2004;92:S76–89.
[47] Zhang H, Yu R, Liu X, Guo X, Zeng Z. The expression of PAC1 increases in the degenerative thymus and low dose PACAP protects female mice from cyclophosphamide induced thymus atrophy. Peptides 2012;38:337–43.
[48] Zhou C, Yool AJ, Nolan J, Byard RW. Armanni-Ebstein lesions: a need for clarification. J Forensic Sci 2013;58(Suppl 1):S94–8.