

PACAP 38 inhibits adiponectin release

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Abstract

BACKGROUND: Pituitary adenylate cyclase activating peptide (PACAP 38) is a neuropeptide with anti-inflammatory activity. Vasoactive intestinal peptide (VIP)/PACAP receptors are found in immune cells, endocrine glands and also in adipose tissue. Adiponectin is an adipocyte-derived protein hormone which possesses anti-inflammatory, antidiabetic and antiatherogenic properties.

The aim of this study was to examine the influence of PACAP 38 on adiponectin release in basal conditions and during lipopolysaccharide (LPS)-induced acute inflammation.

METHODS: Male Wistar-Kyoto rats were divided into four groups which received intraperitoneal injections of 0.9% NaCl, LPS, PACAP 38 or LPS+PACAP 38, respectively. Serum adiponectin concentrations were measured using an ELISA test.

RESULTS: LPS administration did not change adiponectin concentration; however, PACAP 38 administered alone decreased serum adiponectin concentration after 2 h ($p<0.05$) and 4 h ($p<0.01$).

In the group that received LPS+PACAP38, compared with LPS alone, no difference in adiponectin concentration was observed.

CONCLUSIONS: We conclude that PACAP 38 may directly modulate adiponectin secretion by adipocytes in basal conditions.

INTRODUCTION

Pituitary adenylate cyclase activating peptide (PACAP) is the most highly conserved member of the vasoactive intestinal peptide (VIP)/PACAP/glucagon superfamily. PACAP exists in two forms, PACAP 27 and PACAP 38, the latter being the more biologically active [1,2].

The biological activities of PACAP 38 and its structurally-related molecule VIP are mediated through specific G-protein-coupled receptors. The receptors VPAC1 and VPAC2 bind both peptides

and a third type of receptor, PAC1, selectively binds only PACAP. These receptors have been demonstrated in immune cells and endocrine glands such as the adrenals, pituitary, pancreas and gonads [3,4,5]. The presence of the receptors in adipocytes has been claimed but the published data are equivocal [6,7,8]. Adipose tissue participates not only in the regulation of energy storage and homeostasis but is now also recognized as an important endocrine organ. This tissue secretes

many biologically active adipokines like leptin, resistin, TNF α , IL-6, adiponectin [9,10]. Adiponectin is one of the most important peptide hormones linking obesity with insulin resistance, cardiovascular disease and metabolic syndrome [11,12]. Moreover, adiponectin possesses antidiabetic, antiatherogenic and anti-inflammatory properties, although the latter attribute remains controversial [13,14,15,16].

PACAP has important anti-inflammatory properties that are expressed through the inhibition of proinflammatory cytokine production and stimulation of anti-inflammatory cytokine secretion during acute inflammation. This polypeptide has been shown to protect mice against LPS-induced lethal endotoxemia [17,18].

In a previous study we examined the influence of PACAP 38 on the immune and endocrine systems during LPS-induced acute inflammation and confirmed its anti-inflammatory properties. In this septic shock model, PACAP 38 decreased serum TNF α concentration and modulated the secretion of corticosterone, T4 and GH [19].

As both PACAP 38 and adiponectin are recognized as anti-inflammatory factors, and considering the presence of VIP/PACAP receptor in adipose cells, we examined the influence of PACAP 38 on adiponectin secretion in basal conditions and during LPS-induced acute inflammation.

MATERIALS AND METHODS

Animals

Adult male Wistar-Kyoto rats were kept under conditions of controlled light (14 h light/10 h dark cycle) and temperature (22–23 °C) with free access to food and water. The animals were randomly assigned to one of four experimental groups (7–10 rats each) and submitted to two intraperitoneal (i.p.) injections separated by less than 1 min according to the protocol shown in Table 1.

Half of the animals in each group were then sacrificed by decapitation after 2 h and the remaining half after 4 h. Trunk blood was collected, the serum separated and kept at –60 °C before further analysis.

All animal procedures were in accordance with the Guiding Principles for the Care and Use of Research Animals and were approved by the First Warsaw Ethics Committee for Experiments on Animals.

Reagents

Lipopolysaccharide (LPS) from *Escherichia coli* serotype O55:B5 and pituitary adenylate cyclase activating peptide 38 (PACAP 38) were obtained from Sigma-Aldrich Co., St. Louis, MO (USA).

Assays

The concentration of adiponectin in rat serum was assayed using an ELISA kit (AdipoGen Inc., Seoul, South Korea). The sensitivity of the assay was 50 pg/ml. Serum adiponectin concentration was measured 2 h and 4 h after i.p. injections, respectively.

Statistical analysis

The results were analyzed using the Kruskal-Wallis rank test followed by the Mann-Whitney U test to identify significant differences between groups. All results were expressed as means ± SD. To calculate coefficients of correlation between cytokine and adiponectin levels, the Spearman test was applied. The data about changing of serum Tumor Necrosis Factor α (TNF α) interleukin 6 (IL-6) and interleukin 10 (IL-10) were shown in our previous study [19].

Results

Treatment with LPS as compared with 0.9% NaCl (control group) did not change serum adiponectin concentrations measured 2 h and 4 h after the injections (Figures 1 and 2). Administration of PACAP 38 alone, compared with the control group, decreased serum adiponectin concentrations measured after 2 h and 4 h ($p<0.05$; $p<0.01$, respectively) (Figures 1 and 2).

No differences in serum adiponectin concentration were observed between the group receiving LPS+ PACAP 38 and that given LPS alone. There were no significant correlation between all investigated cytokines and adiponectin level.

DISCUSSION

Adipose tissue is not only a reservoir of energy, it also has many important endocrine and immune functions. Adiponectin is thought to regulate metabolic functions in the adipose tissues, liver and muscles, and it also plays an important role in the modulation of inflammatory reactions [9,10,13]. This peptide attenuates LPS-dependent

Table 1. Experimental protocol

| Experimental group of rats | Number of rats | Intraperitoneal injection (i.p.) | |
|----------------------------|----------------|---|---------------------------------------|
| | | 1 st | 2 nd |
| LPS | 19 | 0.15 ml of 0.9% NaCl | 600 µg of LPS in 0.15 ml of 0.9% NaCl |
| PACAP | 14 | 30 nmol of PACAP 38 in 0.15 ml of 0.9% NaCl | 0.15 ml of 0.9% NaCl |
| PACAP + LPS | 14 | 30 nmol of PACAP 38 in 0.15 ml of 0.9% NaCl | 600 µg of LPS in 0.15 ml of 0.9% NaCl |
| Vehicle (control) | 20 | 0.15 ml of 0.9% NaCl | 0.15 ml of 0.9% NaCl |

Figure 1. Serum adiponectin concentration 2 h after injection in all investigated groups. Results are expressed as mean \pm SD for 7 to 10 animals per group. ns-not significant

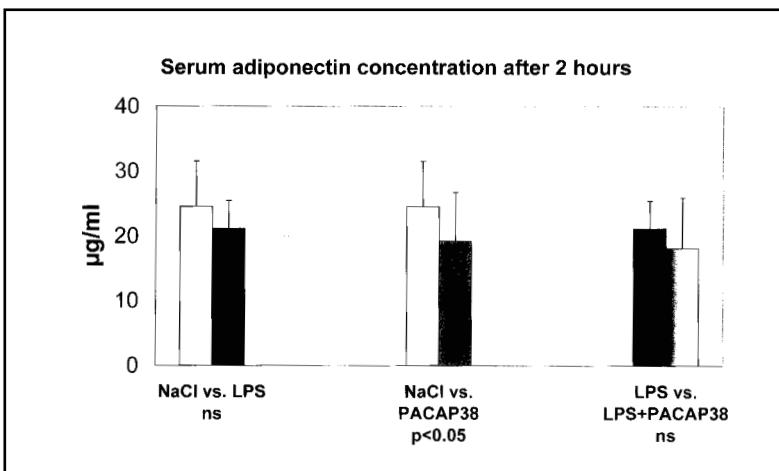
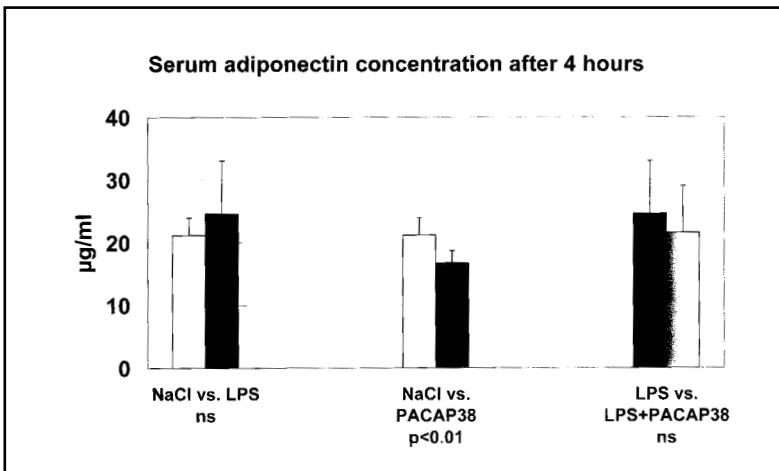


Figure 2. Serum adiponectin concentration 4 h after injection in all investigated groups. Results are expressed as mean \pm SD for 7 to 10 animals per group. ns-not significant



increases in TNF α and IL-6 production in LPS-activated porcine macrophages. The observed decrease in TNF α and IL-6 synthesis is dependent on suppression of the activity of nuclear factor κ B, a key transcription factor responsible for the expression of proinflammatory cytokines [14,20]. Furthermore, adiponectin stimulates LPS-dependent production of IL-10 and IL-1 receptor antagonist in human monocytes and dendritic cells, and reduces both interferon γ secretion and macrophage phagocytosis [14,21]. The anti-inflammatory properties of this peptide are similar to PACAP 38. It has also been reported that adiponectin may show pro-proliferative and pro-inflammatory actions in colonic epithelial cancer cells [22], which is contrary to previous findings. In the current study, we showed that the serum concentration of adiponectin did not change 2 h and 4 h after LPS injection. PACAP 38 administered simultaneously with LPS had no influence on serum adiponectin concentration compared with the group that received LPS only. There were no significant correlations between adiponectin and any of the investigated cytokines, which were estimated previously in the same serum samples [19].

The lack of correlation between serum cytokine levels (proinflammatory and anti-inflammatory) and

adiponectin concentration suggested that adiponectin secretion is not dependent on cytokine concentration during acute inflammation.

PACAP 38 is a well recognized anti-inflammatory polypeptide that has also been named "macrophage deactivating factor". VIP/PACAP receptors are expressed in human and rat adipocytes [6,8]. However, a study conducted in mice failed to confirm the presence of the VPAC2 receptor in mouse adipose tissue [7]. These findings indicate that adipose tissue could be a target for VIP and PACAP action. It has also been demonstrated that both of these peptides play a role in carbohydrate and lipid metabolism [23,24]. PACAP, like VIP, is a strong activator of cAMP synthesis in different cell types. Increased production of cAMP in adipocytes stimulates the activity of protein kinase A (PKA). PACAP 38 can mediate two opposite effects in adipose cells: in the absence of insulin it stimulates lipolysis by raising cAMP levels and activating PKA, but with insulin present it potentiates lipogenesis [25]. Beta adrenergic drugs and catecholamines also increase cAMP levels, stimulate the activity of PKA, activate the hormone sensitive lipase and lipolysis as well as decrease the level of adiponectin, probably via this pathway [26,27].

In this study we found that, compared with the control group (0.9% NaCl), PACAP 38 administration caused a decrease in serum adiponectin concentration after 2 h and 4 h. This response may be dependent on increases in cAMP and PKA activity in adipose cells caused by PACAP 38. The existence of VIP/PACAP receptors on adipocyte cells suggests that PACAP 38 can modulate adiponectin secretion directly. To our knowledge this is the first evidence indicating such an influence of PACAP on adiponectin secretion.

We conclude that the anti-inflammatory properties of PACAP 38 had no influence on adiponectin secretion during LPS-induced acute inflammation but that this neuropeptide could directly modulate adiponectin secretion in basal conditions.

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