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Visinin-like peptide 1 in adrenal gland of the rat. Gene expression and its hormonal control

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ABSTRACT

VSNL1 encodes the calcium-sensor protein visinin-like 1 and was identified previously as an upregulated gene in a sample set of aldosterone-producing adenomas. Recently, by means of microarray studies we demonstrated high expression of Vsnl1 gene in rat adrenal zona glomerulosa (ZG). Only scanty data are available on the role of this gene in adrenal function as well as on regulation of its expression by factors affecting adrenal cortex structure and function. Therefore we performed relevant studies aimed at clarifying some of the above issues. By Affymetrix® Rat Gene 1.1 ST Array Strip, QPCR and immunohistochemistry we demonstrated that expression levels of Vsnl1 in the rat adrenal ZG are notably higher than in the fasciculata/reticularis zone. In QPCR assay this difference was approximately 10 times higher. Expression of this gene in the rat adrenal gland or adrenocortical cells was acutely down regulated by ACTH, while chronic administration of corticotrophin or dexamethasone did not change Vsnl1 mRNA levels. In enucleation-induced adrenocortical regeneration expression levels of both Vsnl1 and Cyp11b2 were notably lowered and positively correlated. Despite these findings, the physiological significance of adrenal Vsnl1 remains unclear, and requires further investigation

Introduction

Calcium, an intracellular signal pathways second messenger is involved in numerous processes in the cell, for example cellular transport and gene expression [19,22]. Changes of intracellular concentration of Ca^{2+} are primary regulated by mitochondria and have many consequences, for example physiological – increased of NADH and NADPH formation in neurons and zona glomerulosa cells (ZG) of adrenal gland, or pathological – apoptosis induced by the loss of mitochondrial membrane potential and a sudden drop in NADPH oxidation [4,9,34,42,43]. Vsnl1 is involved in modulation of intracellular calcium-dependent signaling pathways, including cAMP-, cGMP- and MAPK signaling [6,30,31]. In murine pancreatic β cells overexpression of Vsnl1 led to increase of cAMP levels and transcription of insulin precursor mRNA. Furthermore, Vsnl1 affects the expression of genes involved in cellular proliferation (cyclin D2, Ccnd2), as well as glycogen synthase kinase 3 beta (Gsk3b) and transcription factors (cAMP responsive element binding protein 1 – Creb1) [8].

In 1992 group of Kuno et al. [25] identified calcium binding protein named visinin-like protein 1 (Vsnl1). Together with *Vsnl2*, *Vsnl3*, hippocalcin (*Hpca*) and neurocalcin δ (*Ncald*) genes, *Vsnl1* gene is a member of the visinin-like proteins subfamily that belongs to the bigger visinin/recoverin subfamily of neuronal calcium sensor (NCS) proteins [20,24]. Common feature of all NCS proteins are calcium-binding regions, called EF hands. Visinin-like proteins have 4 EF hand motifs, however only three potential Ca^{2+}-binding sites (EF hands 2, 3 and 4) are active. EF hand 1 is inactive in all visinin-like peptides [30,31]. These peptides undergo myristoylation and in a calcium-dependent manner they may associate with biological membranes (so called calcium-myristoyl switch mechanism to translocate to cellular membranes) [6,29,62]. Visinin-like peptides amino acid sequence show high homology –89% between Vsnl1 and Vsnl2, 91% between Vsnl1 and Ncald, and also 94% Vsnl1 with Vsnl3 and hippocalcin [7,50]. Vsnl1, as well as other Vsnls, is highly expressed in nerve cells of the central nervous system (CNS) of different species, for example in rat cerebellum and hippocampus [6,20,27,28,49]. Vsnls are also found in peripheral organs. By means of RT-PCR, Northern blot and Western blot Vsnls were demonstrated among others in human and rat heart, liver, lung, testis, and in rat skin and stomach [14].

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The production of aldosterone is modulated by many factors, such as angiotensin II (AII), potassium and adrenocorticotropic hormone (ACTH). Many signal-transducing elements (protein kinase C, cAMP, cGMP) affect aldosterone secretion [for review see 50]. Among those modulators calcium play significant role in the production of aldosterone, since it induced acute and long-term responses. Elevated mitochondrial Ca^{2+} concentration and increased in NADPH level in adrenocortical cells correlated with the rate of aldosterone synthesis [41,42,51,52,57].

In relation to the role of calcium in adrenal cortex physiology and pathology, of interest is up-regulation of *VSNL1* (visinin-like 1 gene) in aldosterone-producing adenomas (APA) [58,59]. Recently we performed microarray studies on zone- and sex-specific transcripts in adult male and female rat. One of genes, identified as Vsnl1 was highly expressed in the adrenal ZG. However, only scanty data are available on the role of this gene in adrenal function as well as on regulation of its expression by factors affecting adrenal cortex structure and function. Therefore performed studies aimed to investigate the expression of Vsnl1 gene and protein in rat adrenal cortex. Since this gene is exclusively expressed in the ZG, we also investigated effects of known aldosterone secretagogues on expression levels of this gene.

Materials and methods

Animals, reagents

Wistar rats from the Laboratory Animal Breeding Center, Department of Toxicology, Poznan University of Medical Sciences were used. The animals were maintained under standardized conditions of light (14:10 h light-dark cycle, illumination onset at 06.00 a.m.), at 23 °C, with free access to standard pellets and tap water. The study protocol was approved by the Local Ethics Committee for Animal Studies. If not otherwise stated, all reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA) or from Avantor Performance Materials Poland S.A. (Gliwice, Poland).

Freshly isolated rat adrenal zona glomerulosa cells

Immediately after decapitation adrenal glands of adult female rats were removed and freed of adherent fat. Next, under a stereomicroscope glands were decapsulated to separate ZG from the zona fasciculata/reticularis (ZF/R). Pieces of connective tissue capsule with adjacent ZG cells were mechanically chopped using surgical instruments and then they were digested with collagenase in Krebs-Ringer solution (1 mg/ml). Digestion was performed in 37 °C for 60 min in water bath with shaking. Obtained suspension was filtered and again subjected to mechanical grinding by pipetting (10–15 times). Cells were centrifuged and again suspended in Krebs-Ringer solution (with addition of 0.3% glucose and 0.2% bovine serum albumin (KRGBA)). Incubation of cells (5000 ml^{-1}) with angiotensin II (AII, 100 nM) or potassium ions (K^+, 16 mM) was performed in water bath in 37 °C for 120 min. This technique was described in greater detail by Hinson et al. [17] and Malendowicz et al. [32,33,48]. Incubation medium was centrifuged and collected cells frozen in −36 °C. Obtained material was used for microarray experiments.

Rat primary adrenocortical cell culture

Method of culturing rat adrenocortical cells was described earlier [61]. Briefly, adrenals were obtained from 20 to 22 days old rats. Glands were immediately transferred into vessel with culture medium (DMEM – Dulbecco's modified Eagle's medium, Sigma–Aldrich), mechanically chopped and digested with collagenase (in water bath at 37 °C for 30 min). The suspension was further mechanically disintegrated using glass pipette and then poured through a nylon filter into a test tube and centrifuged for 10 min at 1000 × *g*. The collected cells were then suspended in DMEM, and plated into culture plates (Nunc International) (1×10^4/hole). Culture medium was changed every 24 h. At day 4 of culture ACTH at 10^{-7} M was added and cell harvested after 24 h. The last incubation was carried out in the presence or absence of bovine serum albumin (BSA, 0.3%). Collected cells were used for microarray experiments.

Enucleation-induced rat adrenal gland regeneration

In the rats (Female Wistar rats, final body weight 100–150 g), under standard ketamine and xylazine anesthesia, via dorsal approach, both adrenal glands were enucleated according to the classic method [18]. The operated rats were given 0.9% NaCl to drink for 3 days. One, 2, 3, 5, 8 and 15 days after surgery the rats were sacrificed, and their regenerating adrenals immediately removed, freed of adherent fat and sank in RNA-later. Other glands were frozen at −20 °C for gene expression studies (microarray, QPCR) or fixed in Bouin's solution and embedded in paraffin for immunohistochemistry (ICC). Adrenals from sham operated rats (day 1 after sham surgery) served as a control adrenal glands.

Effects of ACTH or dexamethasone (DEX) a on Vsnl1 gene expression

Adult male rats were subjected to prolonged (ACTH12, 5 μg ACTH/rat administered at hours 0, 12, 24 and decapitated 12 h after the last injection) or acute (ACTHA, 5 μg/rat injected 1 h before decapitation) ACTH action, or prolonged dexamethasone (DEX, 5 μg rat administered at hours 0, 12, 24 and decapitated 12 h after the last injection) suppression. Detailed description of experiments is given elsewhere [39].

Sampling of adrenals of intact rats

Under a stereomicroscope, male and female (estrus cycle phase determined by vaginal smears) adrenal glands were decapsulated to separate ZG. From the remaining part of the gland samples of zona fasciculata/reticularis (ZF/R) and adrenal medulla (M) were taken. Samples were used for microarray experiments.

RNA isolation

The applied methods were described earlier [1,2,45–47,53–56]. From collected cells, samples of adrenal zones and from samples of entire adrenal glands, total RNA was extracted using TRI Reagent (Sigma) and, then, purified on columns (Rnasy Mini Kit, Qiagen). The amount of total mRNA was determined by optical density at 260 nm and its purity was estimated by 260/280 nm absorption ratio (higher than 1.8) (NanoDrop spectrophotometer, Thermo Scientific).

Reverse transcription

Reverse transcription was performed using AMV reverse transcriptase (Promega) with Oligo dT (PE Biosystems, Warrington, UK) as primers in the temperature of 42 °C for 60 min (thermocycler UNO II, Biometra). The primers used were designed by Primer 3 software (Whitehead Institute for Biomedical Research, Cambridge, MA) (Table 1). The primers were purchased from the Laboratory of DNA Sequencing and Oligonucleotide Synthesis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw.