Impaired angiotensin II regulation of renal C-type natriuretic peptide mRNA expression in experimental diabetes mellitus

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Abstract

Objective: Abnormalities in the regulation of natriuretic peptides (NP) associated with major diseases such as hypertension, heart failure, and diabetes mellitus (DM) have been reported. We investigated levels of mRNA for the vasodilator C-type natriuretic peptide (CNP) in the renal cortex of streptozotocin (STZ)-diabetic rats and the influence of an angiotensin II inhibition.

Methods: DM was induced in Wistar rats by a single STZ injection. Rats were kept for 12 weeks. Additionally, the influence of the ACE inhibitor ramipril (Ram: 3 mg/kg/day) and the AT1 receptor antagonist losartan (Los: 20 mg/kg/day) on CNP expression in the STZ-diabetic and control groups was studied (each group n=6). Animals were characterized by their mean arterial blood pressure, plasma glucose levels, and renal function (each group n=9). After extraction of total renal cortical RNA, CNP expression was analyzed by Northern blots.

Results: Renal function was impaired in STZ-diabetic rats which has been improved by Ram and Los treatment. Untreated STZ-diabetic rats showed no difference in renal CNP expression compared to untreated controls. Ram and Los treatments led to an increase in renal cortical CNP mRNA in both diabetic and non-diabetic rats. This effect was weaker in STZ-diabetic rats (Ram: control 5.4-fold, STZ 3.5-fold; Los: control 4.2-fold, STZ 1.9-fold).

Conclusion: These results clearly demonstrate a direct regulatory effect of the renin–angiotensin system (RAS) on renal mRNA levels of CNP. We suggest that RAS inhibition not only prevents the generation of angiotensin II (AngII) but also leads to a stimulation of CNP expression. We conclude that AngII suppresses CNP expression via the AT1 receptor and this mechanism is impaired in STZ-diabetic rats.

Keywords: Angiotensin; Diabetes; Natriuretic peptide; Renin–angiotensin system; Gene expression

1. Introduction

C-type natriuretic peptide (CNP) is a 22-amino acid peptide that shows a high structural homology to the two other members of the natriuretic peptide family, atrial (ANP) and brain (BNP) natriuretic peptide. Whereas ANP and BNP are secreted in an endocrine fashion mainly from the heart and are ligands for the natriuretic peptide receptor A (GC-A), a guanylyl-cyclase-coupled receptor on the cell membrane, C-type natriuretic peptide acts predominantly in an autocrine/paracrine fashion and binds specifically to the natriuretic peptide receptor B (GC-B) [1].

It was shown that CNP-precursor release is triggered by shear stress [2] and septic shock [3]. CNP itself is discussed as an endothelium-derived vasodilator [3], a selective cardiovascular peptide [4], a growth inhibitor of vascular smooth muscle cells [5], and a modulator of ACTH-induced aldosterone secretion [6]. First identified in the porcine brain [7], CNP expression could be also detected in the human endothelium [8] and kidney, including glomeruli and renal vasculature as well as walls of large extrarenal vessels [6]. The biological role of CNP in the kidney is not fully understood, but the existence of a glomerular CNP system that may regulate tissue homeostasis and control mesangial cell proliferation has been proposed [9].

Little is known about the renal pathophysiological function of CNP. In experimental renal mesangioprolifer-
tive syndromes, CNP treatment led to renal protective effects including inhibition of mesangial cell proliferation and matrix accumulation [9]. Since nothing was known about CNP regulation in diabetic nephropathy, which is also accompanied by mesangial cell proliferation, we investigated the renal cortical expression of CNP in streptozotocin (STZ)-diabetic rats for 12 weeks and in a control group of non-diabetic rats. At present, STZ treatment currently provides the best characterized model for type I diabetes mellitus (DM) in rats. Additionally, CNP expression under either an angiotensin-converting enzyme inhibitor (ACEI) or an AT1 receptor antagonist was investigated in both groups to clarify the renal interaction between the renin-angiotensin system (RAS) and CNP.

2. Methods

2.1. Animal experiments

Experiments were performed in conscious male Wistar rats weighing 300–330 g (Dr Karl Thomae, Germany). This research was in compliance with the Guide for the Care and Use of Laboratory Animals published by the OPRR (Office for Protection against Research Risks) of the US National Institutes of Health, Washington, DC (NIH Publication No. 85-23, revised 1996) and the institutional Animal Ethics Committee. To prevent any influence of a high protein intake on renal CNP expression and kidney function, the diabetic rats were pair-fed with control animals in the present study.

DM was induced by a single intraperitoneal injection of STZ (70 mg/kg, diluted with 0.4 ml of sodium citrate buffer [0.1 M, pH 4.5]; Sigma Chemical Co., Germany), the single-shot STZ injection providing the best characterized model for type 1 DM model in rats.

Hyperglycemia was confirmed 48 h later by a reflectance meter (Acutrend, Boehringer Mannheim, Germany). Non-diabetic rats were used as controls (A), treated with Ram (ACEI; 3 mg/kg/day per gavage) (B), or treated with Los (AT1 receptor antagonist; 20 mg/kg/day per gavage) (C). In a pilot experiment, these doses have been demonstrated to inhibit 90% of the vasopressor effect of exogenous AngI (Ram) or AngII (Los).

The STZ-diabetic rats were randomized to receive one of the following three regimens for a period of 12 weeks: no treatment (D), Ram (E), or Los (F). This time span was chosen because rats are known to develop severe diabetic nephropathy accompanied by increased glomerular basal membrane thickness, mesangial cell proliferation and glomerular matrix accumulation 12 weeks after STZ injection [12]. Throughout the 12-week study period, STZ-treated rats (n=15 per group) displayed severe hyperglycaemia (>600 mg/dl).

After the 11th week, glomerular filtration rate (GFR) was measured by a single inulin injection method (800 mg/kg) after implanting femoral arterial and venous catheters [10]). Finally, mean arterial pressure (MAP) was measured in the conscious state via the arterial catheter (each group n=9), as previously described [11]. At the end of the study (12th week), these rats were placed in metabolic cages and urine was collected for 24 h to measure total protein excretion using the pyrogallol red method (Analyticon, Germany).

2.2. CNP detection

To determine renal cortical RNA expression, six anaesthetized rats of groups A–F were exsanguinated and the kidneys excised. Renal cortices were macroscopically separated from renal medullae and rapidly frozen in liquid nitrogen.

Northern blot analysis of renal cortices was performed after homogenisation and total RNA extraction using the Trizol reagent (Gibco, Germany) according to the manufacturer’s directions. For each blot, 20 μg of total RNA was loaded per lane. A CNP cDNA fragment with the length of 369 bp was amplified via the PCR method and sub cloned in a T-vector (Promega). This probe or a 250 cDNA specific for β-actin mRNA as a housekeeping gene were radiolabeled and hybridised with the blot. After a washing procedure, the signals were analysed by a FUJIX BAS 2000 Phosphor-Imager system (Raytest, Straubenhardt, Germany). Quantitative analysis was performed by measuring the intensity of the CNP bands normalised by the intensity of the β-actin bands.

2.3. Statistical analysis of data

All data were expressed as means±S.E.M. Data were analysed using a two-way analysis of variance (ANOVA) in conjunction with Student’s t-test. P values <0.05 were accepted as significant.

3. Results

3.1. Characteristics of diabetic rats

As summarised in Table 1, all groups of STZ-diabetic rats developed severe hyperglycaemia after 12 weeks. The increase was significant compared to controls (P<0.01).

MAP did not differ significantly between treated and untreated non-diabetic rats, and STZ-diabetic rats. Diabetic rats treated with Ram or Los showed no significant changes in MAP compared to controls (Table 1).

All treatment groups of STZ-diabetic rats showed similar blood glucose and MAP values. However, GFR and renal protein excretion levels differed. Although all groups of STZ diabetic rats showed a marked GFR reduction and an increase in urinary protein excretion compared to non-diabetic animals, Ram- or Los-treated
4. Discussion

The aim of the study was to investigate renal cortical CNP mRNA in STZ-diabetic rats compared to controls and the influence of an ACEI or an AT1 receptor antagonist on CNP expression under diabetic and control conditions. Since plasma, renal and urinary CNP peptide concentrations are influenced by glomerular hypofiltration and proteinuria in our DM type I rat model, the detection of renal cortical CNP mRNA is a more specific marker of CNP regulation than measurement of peptide concentrations.

Our results are the first to demonstrate that renal CNP mRNA levels in STZ-diabetic rats with reduced GFR and glomerular damage indicated by increased urinary protein levels, do not differ from those in controls with normal renal function 12 weeks after STZ injection. Since Shin et al. [12] found renal upregulation of CNP mRNA 8 weeks after STZ injection, more severe nephropathy [12] 12 weeks after the induction of experimental diabetes enables the kidney to upregulate CNP gene expression. Thus, in contrast to renal ANP [14], the development of diabetic nephropathy, which is characterised by increased glomerular pressure and mesangial cell proliferation does not influence CNP gene expression after 12 weeks, although the peptide is a vasodilator and antiproliferative agent [9].

Additionally, under diabetic conditions, renal CNP mRNA is regulated differently from cardiac CNP gene expression, which we recently found to be downregulated in diabetic cardiopathy [15]. Moreover, an inhibition of the RAS in control and diabetic rats led to a significant increase of CNP expression. The weaker increase in CNP mRNA in STZ-diabetic rats compared to controls after RAS inhibition may indicate diabetic-induced interstitial changes including cell atrophy and fibrosis. However, since an up regulation of CNP mRNA by AT1 blockade is a direct proof for CNP mRNA suppression by AngII, we are the first to demonstrate that AngII down regulates renal CNP expression under physiological and diabetic conditions.

Since CNP expression was more pronounced under ACEI treatment than under AT1 receptor blockade, either ACE itself triggers CNP mRNA to a small degree via angiotensin-independent pathways, or the angiotensin-receptor AT2 is also involved in the regulation of CNP expression. In addition, since plasma concentrations of CNP were not significantly affected by AngII infusion [16], whereas a renal RAS blockade in our study led to augmented CNP-mRNA concentrations in kidney, our findings give a new indication of an autocrine function of CNP.

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Moreover, an inhibition of the RAS in control and diabetic rats led to a significant increase of CNP expression. The weaker increase in CNP mRNA in STZ-diabetic rats compared to controls after RAS inhibition may indicate diabetic-induced interstitial changes including cell atrophy and fibrosis. However, since an up regulation of CNP mRNA by AT1 blockade is a direct proof for CNP mRNA suppression by AngII, we are the first to demonstrate that AngII down regulates renal CNP expression under physiological and diabetic conditions.

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Fig. 1. Renal cortical CNP expression in control rats without, after Ram, or Los treatment and in diabetic rats without, after Ram, or Los treatment. (A) Representative Northern blot assay showing the CNP signals for three samples per group. (B) Quantitation of CNP-mRNA expression after autoradiographic signal analysis (n=6). Data are shown as multiples after normalization to β-actin mRNA levels. Values are means±S.E.M. * P<0.01 vs. control, † P<0.01 vs. STZ controls.

mesangial proliferation under diabetic conditions. CNP seems not to be an inhibitor of the physiological actions of AngII, such as pulmonary pressor effects and stimulation of aldosterone secretion [19], but an inhibitor of the AngII peptide level, since Davidson et al. [20] could show that CNP is an endogenous inhibitor of vascular ACE activity. Therefore, CNP stimulation may lead to an additional down regulation of the RAS after an initial blockade of the
AngII activity. Thus, one of the physiological functions of CNP up regulation under pathophysiological conditions could be the prevention of negative actions of AngII. Therefore, an impaired increase of CNP after RAS blockade under diabetic conditions could be a reason for the impaired renal function in DM under ACEI compared to non-diabetic rats. Since Igaki et al. [21] demonstrated an increased GFR after CNP infusion in healthy humans and patients with chronic renal failure, an elevated CNP level by itself could be beneficial for the diabetic renal function. Our results are the first to demonstrate a direct regulatory effect of RAS on renal CNP expression. We also suggest that AngII suppresses CNP expression via the AT1 receptor. This effect is reduced in STZ-diabetic rats. However, RAS inhibition not only prevents the generation of AngII but also leads to a stimulation of CNP expression. Further experiments have to show whether this stimulation could provide renal protection in diabetic nephropathy.

References