Discussion

It has been shown [10] that caloric intake is one of the most influential modulators on hepatic 5'-deiodinase activity. The calories can be composed of a single carbohydrate such as glucose without protein or lipid [3]. The mechanism by which glucose enhances the recovery of 5'-deiodinase activity in fasted animals remains unclear. Since glucose ingestion elevates blood glucose and insulin levels in addition to the enzyme activity, one can expect that the glucose-evoked deiodinase activity might be mediated through a new protein synthesis via insulin action [4]. The recovery of 5'-deiodinase activity seems to be in parallel to the raised insulin levels. Recently Nishida et al. [11] observed a similarity in responses of thyroxine 5'-deiodinase and of a hepatic stearoyl CoA desaturase to starvation and refeeding. The desaturase enzyme activity was obligatorily mediated by insulin and only secondarily by thyroid hormone [12]. Grau et al. [4] had reported that the T_3 production in hepatic microsomes was elevated when insulin was infused in vivo and the addition of CH to the infusion did not affect T_3 production. Gavin and colleagues [13] studied the effect of glucose on type II 5'-deiodinase in cultured mouse neuroblastoma cells. They found both the type II 5'-deiodinase activity and the uptake of ^3H labeled amino acid reduced after incubating the cells with puromycin for 8 h. Their study did not include the effect of actinomycin on the uptake of ^3H labeled amino acid for comparison. Therefore the differences in our observations and conclusions may be accounted for by differences in our methods and the different enzyme sources used, as the type I and type II 5'-deiodinase have very different characteristics.

In our study, CH was used as an inhibitor of protein synthesis at the translational stage. Puromycin, being another inhibitor at the translational stage of nuclear protein, and actinomycin, being an inhibitor at the transcriptional stage, were also tested. We had hoped that comparing the CH effect with those of PM and AD may help to indicate the specific stage where these inhibitors block the 5'-deiodinase enzyme recovery. Both CH and PM inhibited the hepatic [^3H]-Leu uptake in the refed group but not the type I 5'-deiodinase activity. AD did not suppress the leucine uptake nor the hepatic 5'-deiodinase activity in the refed group. From these results the specific stage where glucose exerts its influence on the enzyme activity is not identified, but these findings have provided strong evidence that the glucose-dependent recovery of type I 5'-deiodinase activity does not require new protein synthesis in vivo.

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Influence of sodium balance on atrial natriuretic factor in rats with one-kidney, one-clip renal hypertension

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Summary. The influence of sodium intake on the gene expression and circulating levels of atrial natriuretic factor (ANF) was investigated in unanesthetized rats with one-kidney, one-clip renal hypertension. After clipping, the rats were maintained for 3 weeks either on a salt-deficient (n = 11) or a regular-sodium diet (n = 10). Animals which had received the regular-sodium diet exhibited significantly higher ANF mRNA levels in their right and left atria than salt-restricted animals, whereas there was no significant difference in plasma ANF levels.
Key words. Renal hypertension; sodium; atrial natriuretic factor; messenger RNA.

Atrial natriuretic factor (ANF) represents a family of peptides that have powerful diuretic, natriuretic and vasorelaxant activities and that are thought to play an important role in the regulation of body fluid and sodium homeostasis[1-4]. Synthesized in mammalian cardiocytes, they are stored in atrial granules. The hormone released into the circulation consists of a 28 amino-acid peptide. In recent years, the regulation of ANF secretion has been explored mainly by measuring either the atrial content or the plasma concentration of the peptides[1-4]. More recently, molecular cloning and sequencing of the cDNA coding for the common precursor of these peptides (pre-proANF) allowed the investigation of ANF gene transcription activity in the rat[5-7].

The present study was undertaken to assess the influence of sodium balance on gene expression and plasma levels of ANF in rats with established one-kidney, one-clip renal hypertension (1-K, 1-C). For this purpose, rats were first subjected to uninephrectomy and partial occlusion of the contralateral kidney. They were then kept for three consecutive weeks either on a regular or a salt-deficient diet. In this experimental model of high blood pressure, manipulation of dietary sodium intake is known to have no effect on the development of hypertension. Characteristically, however, animals given a regular level of sodium intake become progressively unresponsive to blockade of the renin-angiotensin system whereas salt-restricted animals maintain an angiotensin II-dependent form of hypertension[8,9].

Methods

Animals. Male Wistar rats (Madörin, Füllinsdorf, Switzerland) weighing between 150 and 180 g had a solid silver clip (internal diameter 0.2 mm) placed on the left renal artery under ether anesthesia. At the same time, a right nephrectomy was performed. The rats were then returned to their cages, left in a conditioned environment (constant temperature and humidity, and regular light/dark cycles) and given a normal chow diet (Indulab, Buchs, Switzerland) containing 0.27% Na. After 2 days, half of the animals were put on a salt-deficient diet (Indulab) containing only 0.006% Na. Food and tap water were provided at libitum throughout the study.

The rats were studied 3 weeks after the initial surgery. On the day of the experiment, mean intra-arterial pressure and heart rate were measured as previously described[9]. Under ether anesthesia the animals had a PE-50 catheter inserted in the right femoral artery. They were placed thereafter in plexiglas tubes to restrict their movements and left to recover for 90 min. A 2-ml blood sample was drawn through the arterial line at a time when blood pressure and heart rate were stable. The rats were then immediately sacrified by an overdose of barbiturates and their hearts rapidly excised. Atria were then separated, weighed, immediately frozen and stored at − 70 °C.

Determination of plasma ANF. Immunoreactive ANF was measured by radioimmunoassay after solid-phase extraction of plasma on Bond-Elut cartridges (Analytichem International, Harbor City, CA, USA) using a commercially available antiserum (Peninsula Laboratories, RAS 8798, St Helens, England), standard (1-28) ANF (Peninsula Lab.) and iodinated (1-28) ANF (Amersham, Zürich, Switzerland). The characteristics of this assay have been described elsewhere[10].

ANF mRNA determination. Total RNA was extracted by the guanidine-cesium chloride method[11]. Dot-blot hybridization and ANF mRNA quantification were performed as described previously, using a synthetic 40-base oligonucleotide complementary to nucleotides 393-432 numbered from the first ATG triplet of the ANF cDNA (synthesized and kindly provided by Ciba-Geigy AG, Basel, Switzerland)[12,13]. Previous experiments provided evidence that the synthetic probe hybridizes specifically with ANF mRNA. Samples obtained from the animals fed with a regular-(RNa) and low-sodium (LNa) diet were applied on the same dot-blot. The same procedure was repeated 3 times. The quantitation of ANF mRNA was adapted from the method of Heinrich et al.[14]. The absorbances of the hybrid images were plotted against increasing amounts of total dotted RNA. Values are expressed as the slope of the resulting regression line.

As a negative control, liver total RNA was dotted on each blot. As an internal control of homogeneity, blots were hybridized again to an oncogene RAS cDNA probe which is expressed at constant levels in most tissues. In order to estimate poly(A)-containing mRNA, blots were then hybridized to a poly(dT)probe.

Statistics. The data described in the table were analyzed using Student's t-test for unpaired data. Linear regression curves and correlation coefficients between optical density and concentrations of total RNA were obtained by the method of least squares. The probability that the slope of the linear regression was different from zero was tested using a one-way analysis of variance. Analysis of covariance was performed to test the influence of sodium

Characteristics of rats with 1-K, 1-C renal hypertension maintained for 3 weeks on either a regular or a low-sodium intake (Means ± SEM)

	Regular Na (n = 10)	Low Na (n = 11)
Mean blood pressure (mmHg)	170 ± 7	174 ± 2
Heart rate (beats/min)	351 ± 8	382 ± 3*
Body weight (BW) (g)	266 ± 8	175 ± 3***
Weight of right atrium (RA) (mg)	28 ± 2	17 ± 1***
Weight of left atrium (LA) (mg)	26 ± 2	18 ± 1***
RA/BW	0.104 ± 0.008	0.103 ± 0.005
LA/BW	0.106 ± 0.008	0.097 ± 0.007
Plasma ANF (fmol/ml)	71.6 ± 19.1	41.6 ± 9

*$p < 0.05$; ***$p < 0.001$, regular Na vs low Na

intake on the relationship between optical density of spots and concentration of total RNA. Results are given as means ± SEM.

Results

The characteristics of the two study groups are given in the table. Mean blood pressure was similar in RNa and LNa rats. Heart rate was higher ($p < 0.05$) and body weight lower ($p < 0.001$) in LNa than in RNa animals. Both the right and the left atria weighed less ($p < 0.001$) in LNa rats. With regard to the ratio between the weight of the atria and the body weight, no significant difference was observed between RNa and LNa animals. This was true both for the right and for the left atrium. Plasma ANF levels tended to be higher (71.6 ± 19.1 fmol/ml) in the RNa than in the LNa group (41.6 ± 9.0 fmol/ml). The difference did not reach a significant level due to a large scatter of the values.

The figure depicts the results of atrial ANF gene expression obtained in the 2 study groups. LNa rats exhibited a significantly lower concentration of ANF mRNA ($p < 0.05$) in both the right and the left atria. In LNa rats the values for relative ANF mRNA content (expressed as the slope of the regression line between total RNA concentrations and the respective optical density of their spots) were 156 ± 33 and 181 ± 11 for the left and for the right atrium, respectively. The corresponding values in RNa rats were 521 ± 85 and 430 ± 47.

Discussion

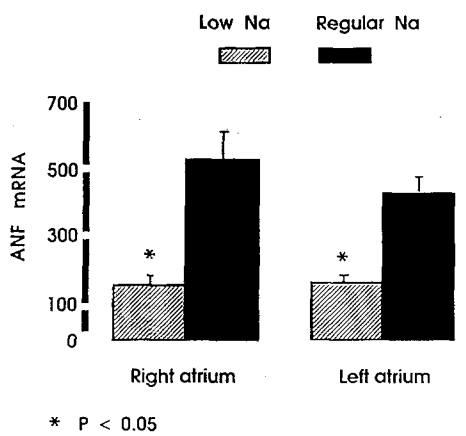
In rats fed with a regular-sodium diet and subjected to renal artery clipping and removal of the contralateral kidney, the renin-angiotensin system is stimulated only during the early developmental phase of hypertension[8,9,15,16]. In these animals, sodium retention progressively develops, which leads to a suppression of renin secretion[17]. Most probably, this is the reason why circulating angiotensin II is not involved in the maintenance of high blood pressure in rats with the established form of 1-K, 1-C renal hypertension[8,9]. Rats kept on a salt-deficient diet during the developmental phase of hypertension increase their blood pressure to a similar extent to animals which have received a regular-sodium diet[9,18,19]. In the salt-restricted rats, however, the renin-angiotensin system remains stimulated and plasma angiotensin II is responsible for the elevation of blood pressure even during the phase of established hypertension[9]. In the present study, the experiments were performed 3 weeks after clipping. As anticipated, there was no difference in blood pressure levels between animals given low-sodium and regular-sodium diets.

As previously observed[9], rats maintained on a salt-deficient diet did not gain weight as much as rats given a regular-sodium diet. Food intake was not checked in the present study. Most probably, rats kept on the salt-deficient diet had a lower caloric intake than those which had received the regular-sodium diet. Rats are indeed prone to eat more when given food containing some salt.

The difference in body weight between our two experimental groups of animals has to be taken into account when comparing the mass of the right and the left atrium. Looking only at the atrial weight, there appeared to be significant differences between animals on low- and regular-sodium diets. There was, however, no difference when the ratio between the weight of the atria and the body weight was used for comparisons.

Sodium loading is expected to increase the release of ANF into the circulation[1-4]. The finding of a quantitatively enhanced ANF gene transcription in both the right and the left atrium of our regular-sodium rats is well compatible with such a view. Plasma ANF levels also tended to be higher in regular- than in low-sodium animals. Due to a wide distribution of the values, however, the difference did not reach statistical significance. In fact, the range of plasma ANF concentrations was particularly wide in those rats which had a regular level of sodium intake. In this context, it is of interest that 1-K, 1-C renal hypertensive rats maintained on a diet containing a normal amount of sodium have been reported to have a low atrial ANF content[20,21]. It is therefore possible that, due to a depletion of tissue storage sites, circulating levels of ANF were not elevated in all of our regular-sodium rats. Other investigators have reported an increase in plasma levels of ANF in regular-sodium rats with 1-K, 1-C renal hypertension[20]. The increase was shown to occur already 1 week after clipping and persisted throughout the 8-week observation period.

Even though regular-sodium animals could be expected to exhibit higher concentrations of ANF mRNA and of circulating ANF, this is not necessarily the case. Indeed, in normal animals fed three different sodium diets, salt loading raised ANF mRNA and plasma ANP levels within one week[13]. However, at the end of three weeks,



ANF mRNA concentration in atria of regular- and low-sodium rats with 1-K, 1-C renal hypertension. Regression lines were constructed between total RNA dilutions and the respective standardized optical density of their spots. ANF mRNA levels are represented here as the slope of the regression line (mean ± SEM of the slopes).

no difference in these variables could be seen between the groups of rats maintained on different sodium diets. Thus, sodium diet maintains an influence on gene expression of ANF after three weeks in rats with 1-K, 1-C renal hypertension but not in normotensive animals. At the present time, it is impossible to provide any clear reason for this different behavior of sodium-dependent ANF synthesis. It is nevertheless attractive to speculate that the concomitant hypertension may somehow contribute to the different secretory profile of the 1-K, 1-C renal hypertensive animals.

In summary, ANF gene transcription activity in the right and in the left atrium was, for a similar level of blood pressure, higher on a regular- than on a low-sodium diet in rats with established 1-K, 1-C renal hypertension, i.e. in animals known to have a suppressed and an activated renin-angiotensin system, respectively. The enhanced gene expression was associated with increased circulating levels of ANP only in some of the animals.

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Peptidylarginine deiminase in rat and mouse hemopoietic cells

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Summary. Peptidylarginine (protein-L-arginine) deiminase activities have been demonstrated in extracts of rat and mouse peritoneal macrophages, bone marrow cells, splenic adherent cells, neutrophils, and mouse monocyte/macrophage cell lines. The enzyme in these cells is indistinguishable from the skeletal muscle enzyme with respect to immunochemical properties.
Key words. Peptidylarginine deiminase; L-citrulline; hemopoietic cells.

Peptidylarginine (protein-L-arginine) deiminase (EC 3.5.3.15; PAD), which catalyzes the conversion of L-arginine residues in proteins to citrulline residues, has been described in various vertebrate tissues[1-5]. Although the distribution of PAD in functionally distinct mammalian tissues, and its unequivocal enzyme reaction, suggest the importance of this enzyme in the functional and metabolic modification of proteins, its role in cellular physiology is still largely unknown. Recently, it has been reported that murine bone marrow cells[6,7] and cytotoxic activated macrophages[8,9] can produce L-citrulline without the involvement of the urea cycle. The authors of these papers rule out a possible involvement of PAD in this citrulline-producing pathway, since they failed to demonstrate PAD activity in the lysates of these cells. But, in the study described here, we were able to demonstrate that rat and mouse hemopoietic cells, including macrophages and bone marrow cells, and some mouse leukemia cell lines do have PAD. Evidence was also obtained that the PAD in these cells is indistinguishable from the rat skeletal muscle enzyme.