RENAL ORIGIN OF A NATRIURETIC MATERIAL

J P Godon, P Cambier, A Nizet

Université de Liège, Institut de Médecine, Liège, Belgium

Introduction

The purpose of our work was to study the pathogenesis of oedema formation during experimental and human glomerulonephritis.

We have demonstrated that rats with experimental nephrotoxic serum nephritis as well as men with chronic glomerulonephritis or nephrotic syndrome were unable to increase fractional or absolute sodium excretion after intravenous sodium loading [1].

The so-called ‘physical factors’ are not involved in this sodium retention because their variations are quite similar in normal and in glomerulonephritic rats. Therefore, we have looked for the presence or the absence of a natriuretic substance in experimental or human glomerulonephritis.

Methods and Results

Urines and plasma extracts were prepared under three experimental conditions:

1. Normal rats of Wistar strain were submitted to a high sodium diet containing 9 to 10 mEq of Na per day.

2. Normal rats were submitted to a low sodium diet containing 0.1 to 0.2 mEq of sodium per day. They were in negative sodium balance.

3. Rats with experimental glomerulonephritis induced by a single intravenous injection of anti-glomerular basement membrane IgG and receiving the same high sodium diet as normal salt loaded rats. They were studied after 4 weeks of evolution of the disease.

The preparation of plasma and urinary extracts has been described previously [3,4].

The bio-assay was performed as soon as possible after preparation of the extracts, in normal rats, on a diet of 2–3 mEq of Na/day and fasting for 12 hr, by injection of extracts into the renal arteries. The results were sometimes
expressed as the variations in fractional sodium excretion between the periods before and after the injection of extracts, or more often by the mean differences in sodium excretion between both periods. The comparison of the results is expressed, for non-paired samples, according to Student’s ‘t’ test.

The extract for one bio-assay was produced from 50 ml of urine and the plasma extract, from 0.5 cc of plasma.

In Figure 1, we see on the ordinate, the effect on fractional sodium excretion of the injection, after a control period, of material prepared from the urine of salt loaded normal rats in A, salt depleted rats in B, salt loaded glomerulonephritic rats in C and the effect of the solvent. An important statistical difference is observed between the effect of the material prepared from normal salt loaded rats as compared with the other series: normal salt depleted rats, salt loaded GN rats and the solvent of the extracts.

The same results are found with plasma extracts. Figure 2 demonstrates that significant natriuretic activity is observed with plasma extracts prepared

![Figure 1](image1.png)

**Figure 1.** Fractional sodium excretion (% of filtered load) of normal rats before (1) and after (2) the injection of the urinary extracts prepared from normal salt loaded rats (A), glomerulonephritic (GN) salt loaded rats (B), normal salt depleted rats (C). D represents the effect on fractional sodium excretion of the solvent (0.5 ml of saline). Mean ± MSD. Comparison of mean differences between A and B: 2 P<0.001, between B and C, C and D, B and D: 2 P>0.1
Plasma extract from salt loaded

Normal rats

G.F.R. \( \mu l/min/100 \text{ g} \) B.W.

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R.P.F. \( \mu l/min/100 \text{ g} \) B.W.

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T.R.F. Na %

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G.N. rats (autologous stage)

Figure 2. GFR (\( \mu l/min/100 \text{ g B.W.} \)), RPF (\( \mu l/min/100 \text{ g B.W.} \)), fractional sodium excretion (% of filtered load), before (1) and after (2) the injection into normal rats of the plasma extracts prepared from normal salt loaded and GN salt loaded rats. No significant variations of GFR and RPF (2P > 0.1).

The increase of fractional sodium excretion after the injection of the normal extracts is significantly higher (2P < 0.001) than after GN extracts which induce an effect comparable with the injection of the solvent.

from normal salt loaded rats as compared with the extracts from GN or salt depleted rats. No modifications of GFR or RPF are observed.

In men, we have observed the same results [2]. Indeed, if we inject into normal rats a plasma extract prepared from normal salt loaded men, we induce a very significant natriuresis as compared with the injections of the solvent, or of extracts prepared from men in negative sodium balance. If the extracts are prepared from the plasma of men with nephrotic syndrome, acute or mem-

brano-proliferative glomerulonephritis, we do not observe any significant natriuretic activity as compared with controls. This natriuretic activity reappears after recovery from nephrotic syndrome as well as after recovery from acute glomerulonephritis. Therefore, if the only detectable lesions are renal in human and experimental glomerulonephritis, a renal origin of our material can be

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strongly inferred.

We have tried to confirm the renal origin of our natriuretic material, looking for its production by a totally isolated dog kidney. Both kidneys from a salt depleted dog were perfused on the pump-oxygenators of Prof Nizet with the blood of another salt depleted dog. On one side, we added one hundred ml of saline. The extracts were prepared as described previously and tested in normal rats. The results are expressed as the mean differences from initial values. The extracts prepared from the blood of the salt depleted dog induced a slight increase of fractional natriuresis just comparable with the injection of the solvent (2 P>0.1). The extracts prepared from Na loaded and diluted perfusing blood induced a very significant natriuresis as compared with the plasma extracts from the control kidney without salt load (2 P<0.001) (Figure 3).

Figure 3. Effect on fractional sodium excretion of the extracts prepared from blood donor (C), of the extracts prepared from the perfusing blood without salt load (control) and with salt load (Na load). On abscissa, the perfusion time when the samples are withdrawn

In these experiments, the renal origin of the natriuretic material is evident.

We have tried to produce this material in vitro using incubation of cortical kidney slices and glomerular and tubular epithelial cell cultures. Cortical kidney slices were obtained from normal and glomerulonephritic salt loaded kidneys and from normal salt depleted kidneys. They were incubated in a Krebs-Ringer solution. Their weight was 650 mg per 15 cc of solution. This solution served for one bio-assay in duplicate after preparation as described for plasma and urine extract. The final volume of solubilised extract was 0.5 cc.
The extracts prepared from the incubation medium of salt depleted kidney and glomerulonephritic kidney did not induce any significant natriuresis as compared with the injection of the solvent (Figure 4). Conversely, the extracts prepared from the incubation solution of a salt loaded kidney induced a very significant natriuresis as compared with the other preparations (Figure 4).

![Diagram](image)

Figure 4. Effect of the extracts prepared from the incubation medium of kidney slices taken from normal salt depleted rats (N.Na (-), salt loaded normal rats (N.Na (+)) and glomerulonephritic rats (G.N.Na (+)). C: injection of the solvent

These experiments indicate that, in vitro, kidney slices release a natriuretic material according to the previous state of the animals, i.e. only if they are previously salt loaded and not if they are glomerulonephritic or salt depleted.

Cultures of glomerular and cortical tubular epithelial renal cells were performed from the same three series of animals under the following conditions: we used 10 ml of 199 Medium (Flow Laboratories) in Falcon ® plastic flasks; the medium was supplemented with foetal calf serum at a final concentration of 20%, Penicillin (200 u/ml) and Gentamycin (50 μg/ml). The pH of the medium was 7.3 and the culture was performed at 37°C. Figure 5 shows the epithelial aspects of cortical tubular cells in culture, from a normal salt loaded rat. They exhibit large interconnections with clearly visible junctional complexes. We see many oval mitochondria and the rough endoplasmic reticulum is well developed. Conversely, the tubular cells taken from salt loaded glomerulonephritic animals exhibit more enlarged intercellular spaces with many interconnected villi. The rough endoplasmic reticulum is more developed and surrounds the mitochondria which are more numerous than in normal cells.
Figure 6. Appearance of the cultured tubular epithelial cells from glomerulonephritic kidneys (see text). Lead citrate staining. x 6000 × 2.4 provided by C. Dethenne
There are more autosomes and lysozomal vacuoles (Figure 6). These appearances demonstrate the epithelial origin and the viability of our cultured cells.

The extracts were prepared in the same way as described before, and the medium from one 10 ml culture box was used for one bio-assay. The extracts prepared from the medium of the glomerular epithelial cell culture taken from normal salt loaded rats induced a slight natriuresis when the medium was used after 3 days of culture (Figure 7). This natriuretic effect disappeared during the next few days. The extracts prepared from the culture medium of tubular epithelial cells from salt loaded kidneys were very natriuretic and this effect was detectable for more than 15 days. Conversely, the extracts prepared from tubular or glomerular cell cultures removed from either salt depleted kidneys or glomerulonephritic kidneys did not exhibit any natriuretic activity (Figure 7). Moreover, the addition of cycloheximide to the culture medium inhibited the production of our material by normal salt loaded cells.

Since the natriuretic activity disappeared when the extracts were stored for 4 hours at 37°C, and since the natriuretic effect was consistently present in the supernatant of the culture for more than 15 days, when the culture was performed at 37°C, and since the cycloheximide inhibited its production, we can assume that it really was synthesised in vitro.

The absence of synthesis by the cells removed from salt depleted rats or from glomerulonephritic rats can be used as an argument to say that the kidney is affected by the previous sodium diet as well as by the immunological injury even when any extra-renal influence has been excluded by culture.
Conclusions

Johnston [6] was the first one to suggest by means of an indirect technique (cross-circulation) the renal origin of a natriuretic factor, whereas Anderson [7] suggests its cerebral origin, Lichardus and Ponec [8] underline the role of hypophysis in its production and Daly [9] the role of liver. Recently, Gonick and Saldanha [10] have isolated, from a kidney previously salt loaded, a natriuretic extract but do not suggest a renal origin and think that their material is coming from another part of the organism and is stored within the kidney. From 1968, Nizet and ourselves [11] have evidence that a totally isolated dog kidney is able to excrete quantitatively a salt load and, therefore, that intrarenal mechanisms are sufficient to regulate sodium excretion.

The present work gives additional proof for this hypothesis:

1. The glomerulonephritic kidney is unable to respond adequately to an acute saline load by adjusting fractional reabsorption of sodium. This absence of response depends on the kidney itself. The ‘so-called’ physical factors cannot be involved [1].

2. A natriuretic material, present in the blood of normal salt-loaded animals is absent in glomerulonephritis. This material restores the renal response to the acute saline load. Its absence explains the oedema of glomerulonephritis.

Since the only detectable lesions are in the kidney the renal origin of this material is suggested.

3. The renal origin is confirmed by the production of a natriuretic material by an isolated kidney, cortical kidney slices in vitro and tubular cells in culture and by its absence, in these three conditions, in salt depletion as well as in glomerulonephritis. It seems that its origin is in the cortical tubules, which are able to synthesise it in culture if they are removed from normal salt loaded rats.

4. The similarity of the results obtained in glomerulonephritis and in sodium depletion suggests strongly that the renal functional changes involved in both conditions are the same [5], i.e. the absence of a renal natriuretic factor.

References

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Open Discussion

DZURIK (Bratislava) Have you found this natriuretic factor in urine or in the serum of uraemic subjects, because Bricker has found his natriuretic factor in these patients?

GODON No, we have not observed our factor in uraemic subjects, but this factor disappears during glomerulonephritis with normal function. We did not try to find the low molecular weight factor during glomerulonephritis, but I think that Bricker's factor is quite a different factor. The molecular weight of Bricker's factor is low, and our factor is a big one.