The Site of Sodium Retention in Glomerulonephritis. Role of a Natriuretic Factor of Renal Origin

J P GODON
University of Liege, Belgium

Summary

In previous work, we demonstrated that rats with experimental glomerulonephritis (G.N.) were unable to increase fractional and absolute sodium excretion after saline loading. This inability is not due to changes in blood dilution factors such as plasma oncotic pressure but rather to the disappearance of a natriuretic material that we have shown to be of renal origin. Analogous results were obtained in human G.N. In order to identify the nephronic site of the enhanced tubular sodium reabsorption two techniques were used: a. Study of TmG in normal and glomerulonephritic rats during progressive saline loading; 2. Micropuncture studies of sodium and water reabsorption.

The results clearly demonstrate that: 1. In G.N. rats, TmG is increased in proportion to Na reabsorption; 2. TmG decreases, by the same order of magnitude, in G.N. and normal rats during progressive saline loading; 3. One site of the increased sodium and water reabsorption is proximal as evidenced by glucose titration curve and micropuncture studies; 4. There is a disruption of glomerulotubular balance in G.N. rats.

Moreover, the parallel behaviour of TmG and proximal sodium reabsorption strongly suggests that the part of sodium transport presumably linked to glucose transport is selectively involved. These results also suggest that the observed disorders are due to the disappearance of a renal natriuretic factor, the site of action of which is presumably proximal.

Introduction

Our previous work has demonstrated that rats with experimental glomerulonephritis (G.N.) induced by antiglomerular basement membrane IgG, were unable
to decrease fractional sodium reabsorption after an acute intravenous saline load (Godon, 1972). We have given the proof that this phenomenon is not related to a modification of the so-called 'physical factors', i.e. blood dilution, but to the disappearance of a natriuretic material (Godon, 1974, 1975, a.b.) which was found to be of renal origin (Godon and Nizet, 1974, Godon 1975, a, b, c). There was a resemblance between the behaviour of rats with experimental G.N. and of patients with glomerular diseases such as membrano-proliferative glomerulonephritis at an early stage (Godon, 1975,a.). Therefore, the conclusions that were reached concerning the disturbances of sodium excretion by the glomerulonephritic kidney can be applied to men as well as animals.

In the present work, we attempt to investigate the site of the nephron which is responsible for the observed increase of sodium reabsorption in glomerulonephritis. There is good evidence that this increased sodium reabsorption is due to the disappearance of a renal natriuretic factor and the site in the nephron of the enhanced sodium reabsorption could well be the site of action of such a factor.

We have studied the problem by means of an indirect technique, the measurement of glucose maximum transport (TmG), and a direct method, tubular micropuncture. Indeed, it was proved (Vogel et al, 1965, Robson et al, 1968, Keyes and Swanson, 1971, Von Bayer et al, 1972) that there is a relationship between the decrease of fractional sodium reabsorption and TmG during saline infusion. Thus we have studied the TmG modifications during progressive saline load.

Each experiment on diseased animals is performed at least four weeks after the induction of the glomerulonephritis. Some authors (Lubowitz et al, 1974, Rocha et al, 1973, Allison et al, 1974, Mazumdar et al, 1975) have claimed that there is no sodium retention during Masugi nephritis but their studies were always performed, either during the heterologous stage (Hammer and Dixon, 1963), or before the third week of evolution of the disease; we have demonstrated that glomerulonephritic rats do not exhibit any sodium retention during these periods (Godon, 1972, Godon, submitted for publication).

MATERIAL AND METHODS

1. Experimental animals

Wistar rats are used, weighing 150 to 250 g. Their food contains 2.5 to 3 mEq of Na per day; they have free access to tap water. Glomerulonephritis is induced by a single I.V. injection of rabbit anti-rat glomerular basement membrane IgG. The amounts of IgG are adjusted to induce, during the autologous phase, a proteinuria of 50 to 100 mg per 24 hours; the amount required being between 5 and 10 mg of IgG (Godon, 1972). Each nephritic rat is studied after 4 to 8 weeks of the evolution of the disease. Each rat is fasting (except for water) for 12 hours before an experiment.
2. TmG measurements

Twelve glomerulonephritic rats and 8 normal rats are anaesthetised by Inactin (I.P., 10 mg/100 g B.W.). They are prepared for inulin clearance as previously described (Godon, 1972). They received a 0.5 cc priming injection of a 30% glucose solution, followed by a continuous infusion of the same solution at a rate of 62.5 µl/min.

After an equilibration period of one hour, we started an infusion of NaCl (145 mEq/l) at a rate of 100 µl/min. Two '30 minutes' urine collection periods are performed at this flow rate with blood sampling at their mid-point. The infusion rate is increased successively to 375, 750 and 1000 µl/min, each time for 30 minutes (periods 3, 4, 5).

Inulin values (Von Führ et al, 1965) are corrected by subtraction from observed values, of the product of glucose concentration (in blood and urine) and a coefficient (0.0649) corresponding to the interference of glucose in the anthrone reaction. Glomerular filtration rate, absolute and fractional sodium excretion, TmG are calculated according to the usual formulae; since it is well known that TmG is proportional to GFR, we have expressed the values of TmG per µl of GFR in order to reduce variation.

Moreover, plasma samples are withdrawn from 5 normal and 5 glomerulonephritic rats at the end of the last period of NaCl infusion to look for the presence of natriuretic activity. These samples are prepared and tested according to a previously described technique (Godon, 1974, 1975a).

The results are expressed as the mean differences between the values of the fractional sodium rejection before and after the injection of the extracts.

3. Micropunctures

Five normal and 6 glomerulonephritic rats are anaesthetised by Inactin (10 mg/100 g I.P.). The left kidney is exposed using a flank incision. From the beginning of surgical procedures, Ringer solution is perfused at a flow rate of 37.5 µl/min. The temperature of the animals is monitored at 37°C. After the surgical manipulations a priming dose of Inulin (2 ml of a 2% inulin solution in Tyrode, pH 7.4) is given, followed by a continuous infusion of the same solution at a flow rate of 37.5 µl/min.

The micropipettes have an outer diameter of 12 µ. Lissamin green (10% solution, 25 µl/100 g B.W.) is used to measure the proximal 'transit time' and to identify the last apparent proximal convolution. Five to 8 proximal tubular fluid samples are taken at this site from each rat. The diameters of punctured tubules are measured. Inulin microanalysis is performed according to the technique of Von Führ et al (1965).

The isosmotic water and Na⁺ reabsorption is calculated by the TF(In/P)in where TF(In is the inulin concentration in the punctured tubular fluid and P)in, the
plasma inulin concentration or, in percentage of the filtered amount, by
1 - (P_in/TF_in) x 100. The single nephron GFR (SNGFR) is calculated accord-
ing to the usual formula and expressed in nanolitres per minute (nl/min).

Results are presented individually or by the mean ± the standard error of the
mean. Limits of confidence for the comparison of average values and mean
differences of both series are given, according to Student’s ‘t’ test, for nonpaired
samples.

RESULTS

1. TmG measurements

The diuresis, GFR, absolute and fractional Na excretion are significantly lower
(2 P always < 0.05) in G.N. rats than in control animals during the first collection
period. During progressive saline loading, the diuresis and sodium excretion increase
comparatively in both series. GFR becomes comparable (2 P > 0.1) in both
series when the saline perfusion flow rate reaches 750 µl/min. TmG is always
higher in glomerulonephritic rats than in control group (2 P < 0.05). During
progressive saline loading, it decreases similarly in both series. When we compare
(Figure 1), the maximal glucose transport, expressed by microlitres of glomerular
filtrate, and the fractional sodium excretion, we observe an inverse relationship
between the lowering of TmG and the increase of the fractional sodium excretion;
the two slopes are parallel but the slope giving the parameters of G.N. rats is
shifted to the abscissa origin and to the upper part of the graph: sodium ex-
cretion is always lower and the TmG higher, in G.N. rats than in normal
animals.

Plasma extracts prepared for investigation of their natriuretic activity induce
a significant natriuresis when they are taken from control rats and do not exhibit

Figure 1. Modifications of TmG, expressed by µl of glomerular filtrate and by 100 g body
weight (ordinate) as compared to fractional Na excretion (TRF Na%) (abscissa). The
difference between both series is significant, at least 2P < 0.05.
N: normal rats, G.N: glomerulonephritic rats

523
any natriuretic effect (comparable to the solvent of extracts : 2 P > 0.1) when they are prepared from glomerulonephritis rats (Figure 2) (comparison between both series : 2 P < 0.001).

2. Micropuncture

In normal rats, the mean fractional sodium reabsorption, at the last apparent proximal tubular convolution is 57 ± 3% of filtered load whereas it is 79 ± 4.7% in G.N. rats. The proximal transit time is 11.5 ± 0.9 sec. in normal rats and 19.2 ± 1.14 sec. in nephritic animals. There is quite a good relationship between proximal transit time and the measured proximal fractional sodium reabsorption in normal rats. While this relationship is not so clear in G.N. animals, the proximal transit time seems to be longer in these rats than its theoretical calculation from TF_in/P_in ratio (Figure 3).

The inner diameter of proximal tubules of both series is quite comparable; (2 P > 0.1) 19 ± 2μ in normal rats and 21 ± 2μ in G.N. animals. Glomerular filtration rate of single superficial nephrons is 40.3 ± 5.05 nl/min in control rats and 29.7 ± 2.4 nl/min in G.N. rats; this last value corresponds to 73% of normal values; overall glomerular filtration rate is reduced by the same magnitude (2 P > 0.1) in diseased animals (673 ± 37 μl/min/100 g B.W.) compared with control rats (843 ± 32 μl/min/100 g B.W.).

In normal rats, proximal fractional sodium and water reabsorption is constant at different levels of SNGFR while, in G.N. rats, they are very scattered but always higher than in the normal rats at identical SNGFR (Figure 4).
Figure 3. Relationship between proximal fractional sodium and water reabsorption \((1 - [P/TF]_{in})\) and proximal transit time of Lissamine Green in normal (N.) and glomerulonephritic rats (G.N.).

Figure 4. Relationship between \(TF_{in}/P_{in}\) ratio and single nephron glomerular filtration rate (nl/min), (S.N.G.F.R.) in normal (N.) and glomerulonephritic (G.N.) rats.
CONCLUSIONS

Several authors (Rocha et al, 1973; Lubowitz et al, 1974; Allison et al, 1974; Mazumdar et al, 1975) have demonstrated that there were no disturbances in sodium excretion in experimental glomerulonephritis but these authors have studied this disease during its early stage whereas we have demonstrated that an increased sodium reabsorption became constant after 4 weeks evolution of nephrotoxic serum nephritis (Godon, 1972). For this reason, all our studies of the pathophysiology of oedema formation during glomerular diseases were performed 4 weeks after the induction of the glomerulonephritis, when the ultrastructural alterations of glomeruli and tubules seems to be constant. At this time, all the G.N. rats exhibit sodium retention as compared with the control group.

We have shown that TmG increases in G.N. rats simultaneously with a decreased absolute or fractional sodium excretion. Therefore, it can be suggested that at least one part of sodium reabsorption is linked to glucose transport; however, Von Bayer et al (1972) have stressed that the apparent linkage of sodium and glucose transport could be due to the variations of tubular fluid contact time, the TmG being enhanced when proximal transit time is increased because of increased sodium and water reabsorption. Nevertheless, our experiments suggest strongly that one site of increased sodium reabsorption in glomerulonephritis is proximal. Our micropuncture data demonstrate also that it is proximal sodium and water reabsorption which is increased. Moreover, in G.N. rats, the glomerulo-tubular balance seems to be disrupted since TF_in/P_in ratio is always more elevated in G.N. than in normal animals for similar GFR values.

The increased sodium reabsorption and the disruption of glomerulo-tubular balance are not due to a redistribution of glomerular flow rate since the lowering of glomerulonephritic rats’ GFR, as compared to normal rats, is the same either calculated on the basis of SNFGR or on the basis of the total kidney GFR. Since we have demonstrated, in our previous works, that the sodium retention by the glomerulonephritic kidney was due to the disappearance of a renal natriuretic factor and that, in the present paper, the increased sodium reabsorption is located, at least partly, in the proximal tubule, the site of action of the renal natriuretic factor is likely also to be proximal.

References

Godon, JP (1974) Inserm, 30, 161
Godon, JP (1975a) Nephron, 14, 382

526
Hammer, DK and Dixon, FJ (1963) Journal of Experimental Medicine, 104, 151
Keys, JL and Swanson, RE (1971) American Journal of Physiology, 221, 1
Mazumder, DC, Crosson, JT and Lubowitz, H (1975) Journal of Laboratory and Clinical Medicine, 82, 292
Robson, AM, Strivastava, PL and Bricker, NS (1968) Journal of Clinical Investigation, 47, 329
Von Führ, J, Kaczmarczyk, J and Krüttgen, CG (1965) Klinische Wochenschrift, 33, 729

Open Discussion

CHAIRMAN (ANDREUCCI, Naples) We must be careful when we speak about a natriuretic factor as a new hormone. We should bear in mind that there are several hormones already known which have natriuretic properties, such as PTH, catecholamines and calcitonin. Extracellular volume expansion is known to increase sodium and phosphate excretion and recent evidence suggests that some, if not most, of the phosphaturic response to extracellular volume expansion is accounted for by the increased rate of PTH secretion. PTH is known to decrease proximal tubular reabsorption of sodium, β-adrenergic catecholamines also inhibit proximal reabsorption of sodium and calcitonin inhibits sodium reabsorption.

GODON Yes, but we have demonstrated in some previous work that the natriuretic factor that we described is of renal origin, and we have some proof. We have demonstrated that in tissue culture, tubular epithelial cells produce a natriuretic material tested in normal rats and the glomerular epithelial cells are not able to produce it. The same material prepared from glomerulonephritic animals does not induce natriuretic activity when injected in normal rats. When we prepare the same material from a totally isolated perfused kidney, and PTH and the adrenergic system cannot be involved, of course we observe the production of this material during the perfusion. I think that answers your objection.