Intrarenal regulation of sodium balance

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I should like to summarize for you some of our recent work with the micropuncture technique in the rat kidney, in order to demonstrate the probability that there exists an intrarenal regulating mechanism for the maintenance of sodium balance in the organism [1, 2].

Under normal conditions approximately 99% of the filtered sodium chloride is reabsorbed actively by the tubular epithelial cells. This process requires about $^2/_3$ of the renal oxygen uptake, far more than is required by any other function of the kidney. It is evident that the maintainance of the body sodium content depends upon the balance between the amount of sodium filtered at the glomerular site and the amount of sodium reabsorbed actively by the tubular epithelium.

A loss of sodium would occur if, simultaneous with a spontaneous increase in glomerular filtration rate (GFR), tubular sodium reabsorption would remain unchanged. Under these circumstances the excess filtered sodium would be excreted quantitatively. But it is known that such an increase in Na-excretion does not occur. It is, therefore, this adjustment of tubular sodium reabsorption which has gained the focus of attention in recent investigations. This type of balance is customarily described as an adjustment of the tubular sodium reabsorption to an increase in GFR or sodium load. Although the detailed mechanisms underlying this adjustment are still unknown, the hypothesis has been offered by Gertz [3] that an increase in intratubular volume, following a stepped-up rate of filtration, leads to an increase in tubular sodium reabsorption. This kind of tubular adjustment has been observed during hypertonic saline infusion in rats [4, 5] in which GFR increased, and fractional water and sodium reabsorption in the proximal convolution remained essentially unchanged. A similar behavior of the reabsorptive characteristics was found in both dogs and rats, when the tubular sodium load was lowered by constriction of the aorta above the origin of the renal artery [6, 7, 8]. In this case there was also found to be an unchanged fractional reabsorption in the proximal convolution. It should be noted that these results only describe the adjustment of the reabsorptive pattern in the proxi-

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mal convolution to a change in the GFR. The variations in GFR which occur normally with isotonic or hypertonic volume expansion are not explained, but are taken *prima factum*.

It is equally obvious that a balance between glomerular and tubular function exists also under those circumstances in which the primary variation is of reabsorptive capacity. For example, a primary decrease in tubular sodium reabsorption would result in urinary sodium loss, but only in the case that the tubular sodium load, i.e. GFR, does not lessen. A primary decrease in tubular reabsorptive capacity might occur during the action of saluretic drugs, or in adrenalectomized animals, or in kidneys with tubular damage.

In the following I will deal only with the latter case, in which urinary sodium loss at a reduced reabsorptive capacity is prevented by a compensatory reduction in GFR and in the sodium load. GOORMAGHTIGH [9], who first described the endocrine function of the juxtaglomerular apparatus, suggested just such a control of GFR by tubular function. This juxtaglomerular apparatus is a point of close contact between the tubular epithelium at the end of Henle's loop and the vascular pole of the glomerulum belonging to the same nephron. Because of this tubulo-vascular intimacy GOORMAGHTIGH came to believe that the composites of the tubular fluid flowing past the juxtaglomerular apparatus might influence its endocrine function. Here it is important to keep in mind that the tubular cells attached to the afferent arteriole (macula densa cells) are in contact at their luminal surface with the tubular fluid leaving the thick ascending limb of Henle's loop and on their peritubular side with the renin containing cells in the wall of the afferent arteriole. Micropuncture studies have pointed up the fact that the sodium concentration of tubular fluid at this part of the nephron is very low, much lower than in the peripheral plasma (4, 2, 10, 13). The reduction of sodium concentration occurs along the thick ascending limb of the loop of Henle because of its active sodium reabsorption at a restricted permeability for water. Normally, this sodium concentration varies little, or not at all, with the physiological changes in the concentrating ability of the kidney. You will recall that the rate of glomerular filtration, a process occurring on the other side of the juxtaglomerular apparatus, is relatively constant and is not influenced by the concentration of the final urine. It would appear to be evident that a low sodium concentration in the tubular segment (macula densa segment) depends upon the sodium reabsorption capacity of the thick ascending limb of the loop of Henle. The sodium concentration will increase toward plasma value when sodium reabsorption is diminished, and will meet plasma value when there is no longer any sodium reabsorption in the thick ascending limb [2]. This is in contrast to the changes which occur in the proximal convolution at a reduced sodium reabsorption. Here, only the intratubular flow rate increases without a change in sodium concentration, because the reabsorbate in that tubular segment is isotonic. To determine whether or not there exists an interdependency between sodium concentra-

tion in the tubular fluid at the macula densa cells and GFR of the same nephron, we changed tubular fluid composition at these cells by micropuncture technique and from there observed the effect on glomerular filtration [1].

Although the macula densa segment of the nephron is not accessible for direct micropuncture in the mammalian kidney, changes of the tubular fluid composition at the macula densa can be induced by retrograde injections of test solutions from a puncture site in the early distal convolution. The effect of this upon the single GFR was ascertained by observing the inner diameter of the proximal convolution belonging to the same nephron. A major reduction in GFR is followed by a decrease of the tubular inner diameter, and finally by a total collapse of the tubule when filtration rate is completely interrupted. It is clear that under the limitations of this technique we can only detect the major changes in GFR.

The results of the above experiments show that in kidneys with a high renin content an increase of sodium concentration to isotonic levels in the macula densa segment produces first a decrease and then a disappearance of the proximal tubular diameter in the same nephron. This indicates a reduction and a cessation of the singular glomerular filtrate. Recent results obtained by SCHNERMANN [11] show that this decrease in tubular diameter results from a decrease in filtration rate and not from an increase of proximal reabsorption of salt and water. SCHNERMANN raised the sodium concentration at the macula densa segment by increasing the perfusion rate through a single loop of Henle. He simultaneously collected the total fluid volume of the flow rate in the early proximal convolution of the same nephron, and thereby estimated the single GFR. A decrease of 50% or more of the proximal flow rate can be effected by an increase of sodium concentration at the macula densa. It is extremely improbable that an increase of salt and water reabsorption in the first 15–20% of the proximal convolution could account for these reductions of flow rate.

The injection of 150 mmol/l sodium bromide also interrupted GFR, but an interruption could not be obtained by increasing the osmolality at the macula densa without a concomitant increase in sodium concentration (300 mmol/l mannitol, cholinechloride 150 mmol/l). These data show that the sodium ion concentration is an essential parameter of such reaction. It should be noted that this increase in sodium concentration to plasma values is an abnormal situation, which, in its extreme intensity of concentration, mimics a complete reduction of the diluting function of the thick ascending limb of the loop of Henle. But it was demonstrated by SCHNERMANN through a series of graduated increases in sodium concentration from the normal low values of 20 mEq/l to plasma values that this feedback mechanism operates throughout the entire range of sodium concentrations.

There is evidence that the regulation of GFR by sodium concentration at the macula densa is mediated by the juxtaglomerular apparatus. Firstly, as it is known that the reduction in GFR is restricted to the individual

nephron unit in which the sodium concentration is varied at the macula densa, and that the cellular structures of the juxtaglomerular apparatus provide the sole anatomical connection between the macula densa and the glomerulus, it can be deduced that only the elements of the single tubule are involved. Secondly, the fact that GFR can be interrupted routinely in kidneys with a high renin content, but irregularly in kidneys with a low renin content is consistent with the previous assumption that the collapse reaction is propagated through the juxtaglomerular apparatus.

The sequence of events which leads to a reduction of GFR when sodium concentration is increased at the macula densa is unknown. It appears most likely that the filtration pressure is reduced by pre-glomerular vasoconstriction, which could result if angiotensin II is formed locally in the wall of the afferent arteriole. Implicit in this hypothesis is the suggestion that an important locus of renin activity is to be found at its site of release. This is supported by the finding of LEVER and PEART [12] that the renin concentration in the renal lymph might be 50 times greater than in renal venous blood. Which in turn suggests that renin is in fact released primarily into an interstitial compartment and not into the blood. At the present time it is customary to assess the activity of the juxtaglomerular apparatus by measuring renin concentration in renal venous blood. However, the interstitial volume in the afferent arteriole is much smaller than the total renal blood volume and therefore the renin concentration at the site of its action might be at least 3–6 orders of magnitude greater than in the blood draining the kidney. Hence, measurements of renin concentration in the renal venous blood must be at best a rather inaccurate index of juxtaglomerular activity.

In support of our theory an analogy may be drawn between this local action of angiotensin and another vaso-active peptite system, the bradykinin system, which is well known to act locally upon vascular resistance to blood flow in secretory glands.

As mentioned earlier, sodium balance of the organism depends upon the adjustment of filtered sodium chloride to the reabsorptive capacity of the tubular epithelium. The following arguments lead to the conclusion that GFR under many circumstances is adjusted to the tubular reabsorptive capacity, a conclusion deduced because a sodium sensitive feedback system has been seen to operate at the juxtaglomerular apparatus. 1. The macula densa cells are located at the end of the loop of Henle, a point where tubular fluid sodium concentration is usually less than in the systemic plasma regardless of the diuretic state of the animal. Sodium concentration in the later parts of the distal tubule depends upon the availability of the antidiuretic hormone and therefore is influenced by the water balance of the organism. 2. CORTNEY et al. and SCHNERMANN [13, 11] both showed that an increase of tubular flow rate entering the loop of Henle can cause an increase of early distal sodium concentration. Such a situation can either be a result of an increase in GFR or of a decrease in proximal salt and water reabsorption. Therefore it is predicated that it is the loop of Henle which

translates the changes in intratubular flow rate into changes of intratubular sodium concentration at the macula densa. 3. The operation of the macula densa cells in regard to their sensibility to sodium ion concentration at their site of direct contact with renin release and angiotensin formation in the afferent arteriolar wall, satisfies the requirements for a mechanism that adjusts glomerular filtration dynamics to the tubular reabsorptive capacity. In this light, the function of the juxtaglomerular apparatus is nothing less than the key stone of an intrarenal sodium conserving mechanism.

Results described in the literature concerning the effect of angiotensin on tubular sodium reabsorption present a discordant picture. If angiotensin changes tubular reabsorptive capacity for salt, the sodium feedback mechanism would become unstable because the peptide would then effect both the effector and the affector side of the feedback loop. To resolve this difficulty we studied the reabsorptive capacity for sodium chloride in the proximal convolution [14] with the split-droplet method of GERTZ. Reabsorptive half-time in the control experiments was 9.4 ± 0.4 sec. With an intra-tubular angiotensin concentration of 2.5 or 25×10^{-4} g/l, reabsorptive half-time was 9.5 ± 1.3 and 9.0 ± 1.0 sec. respectively. Neither average was statistically different from the control value. When the angiotensin concentration in the peritubular blood was elevated, reabsorptive half-time in the proximal convolution was 9.4 ± 1.3 sec, again not different from the controls. These data demonstrate that angiotensin has no direct effect on sodium reabsorption in the proximal convolution.

We also studied the effect of intratubular angiotensin on sodium reabsorption along the loop of Henle by including the peptide in concentrations of .5 or 5.0×10^{-5} g/l in solutions perfused with a microprofusion pump into the beginning of the loop of Henle. No effect was found. There is, therefore, no evidence from micropuncture studies which would permit the conclusion that the intrarenal action of angiotensin has other effects than those of its vasoactive action.

The operation of a sodium sensitive feedback mechanism can easily be detected in kidneys with reduced tubular reabsorptive capacity, as for example in the situation following renal ischemia. Sodium concentration at the macula densa is normally 25 mEq/l and can only be maintained at these low levels if the transport functions of the ascending limb are intact. Following a 30 min period of renal ischemia we found that the early distal sodium concentration was increased. This resulted in an inverse relationship to GFR and therefore also to the tubular sodium load. The importance of this adjustment of GFR to tubular reabsorptive capacity is stressed by a consideration of what would result if tubular sodium load remained normal with tubular reabsorption at 50%. Under these circumstances, urinary sodium excretion would be half of the filtered sodium load and urine volume would likewise be half of GFR. Without the replacement of salt and water loss, the kidney would excrete the last sodium ion of the organism approximately 3 hours after sustaining the lesion. It is obvious from this that the

reduced GFR and the vasoconstriction which usually occur in a damaged kidney are vital compensatory reactions which prevent this fatal sodium and water loss. The restoration of GFR then depends upon the rehabilitation of the tubular capacity to reabsorb salt, and thereby to lower sodium concentration at the macula densa.

Summary

It is concluded from micropuncture studies in the rat kidney that there exists a sodium sensitive feedback mechanism operating through the juxtaglomerular apparatus. The character of this feedback mechanism is an inverse correlation between sodium concentration in the tubular fluid of the macula densa and GFR. The physiological significance of this mechanism is to adjust the GFR, and by that also the tubular sodium load, to the tubular reabsorptive capacity for sodium chloride; it can therefore be specified as an intrarenal sodium conserving mechanism.

Zusammenfassung

Aus Mikropunktionsuntersuchungen an der Rattenniere wurde geschlossen, daß ein natriumempfindlicher «Feedback»-Mechanismus besteht, der durch den Juxtaglomerularapparat wirkt. Dieser «Feedback»-Mechanismus ist durch umgekehrte Korrelation zwischen der Natriumkonzentration in der tubulären Flüssigkeit der Macula densa und der Glomerulumfiltrationsrate charakterisiert. Seine physiologische Bedeutung besteht in der Anpassung der Glomerulumfiltrationsrate und somit des tubulären Natriumloads an die tubuläre Reabsorptionsfähigkeit von Natriumchlorid. Der «Feedback»-Mechanismus kann daher als ein intrarenaler, Natrium erhaltender Mechanismus bezeichnet werden.

Résumé

A la suite des résultats obtenus par microponctions rénales chez le rat on conclut à l'existence d'un mécanisme de feedback intéressant le sodium et opérant au moyen de l'appareil juxta-glomérulaire. Ce mécanisme de feedback est caractérisé par une corrélation inverse entre la concentration en sodium du liquide tubulaire de la macula densa et la filtration glomérulaire. La signification physiologique de ce mécanisme réside en l'ajustement de la filtration glomérulaire, par conséquent de la charge tubulaire en sodium, à la capacité de réabsorption tubulaire du chlorure de sodium. Il mérite ainsi l'appellation de mécanisme intrarénal conservateur de sodium.

Riassunto

Esperimenti di micropuntura nel rene del ratto portano alla conclusione che esista un meccanismo «feedback» sensibile al sodio, operante tramite

l'apparato ultraglomerulare. Questo meccanismo «feedback» è caratterizzato da una correlazione inversa tra la concentrazione di sodio nel fluido tubolare della macula densa e la quota della filtrazione glomerulare. L'importanza fisiologica di questo meccanismo è di adattare la quota della filtrazione glomerulare, e con ciò anche la quantità di sodio tubolare, alla capacità di riassorbimento tubolare del cloruro di sodio; può essere perciò specificato quale meccanismo intrarenale sodioconservante.

1. Thurau K. and Schnermann J.: Die Natriumkonzentration an den Macula-densa-Zellen als regulierender Faktor für das Glomerulumfiltrat. Klin. Wschr. *43*, 410 (1965).
2. Schnermann J., Nagel W. and Thurau K.: Die frühdistale Natriumkonzentration in Rattennieren nach renaler Ischämie und hämorrhagischer Hypotension. Pflügers Arch. ges. Physiol. *278*, 296 (1966).
3. Gertz K. H.: Transtubuläre Natriumchloridflüsse und Permeabilität für Nichtelektrolyte im proximalen und distalen Konvolut der Rattennieren. Pflügers Arch. ges. Physiol. *276*, 336 (1963).
4. Giebisch G., Klose R. M. and Windhager E. E.: Micropuncture study of hypertonic sodium chloride loading. Amer. J. Physiol. *206*, 687 (1964).
5. Lassiter W. E., Gottschalk C. W. and Mylle M.: Micropuncture study of net transtubular movement of water and urea in non-diuretic mammalian kidney. Amer. J. Physiol. *200*, 1139 (1961).
6. Glabman S., Aynedjian H. S. and Bank N.: Micropuncture study of the effect of acute reductions in glomerular filtration rate on sodium and water reabsorption by the proximal tubules of the rat. J. clin. Invest. *44*, 1410 (1965).
7. Dirks J. H., Cirksena W. J. and Berliner R. W.: The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J. clin. Invest. *44*, 1160 (1965).
8. Brunner F. P., Rector F. C. and Seldin D. W.: Mechanism of glomerulotubular balance. II: Regulation of proximal tubular reabsorption by tubular volume, as studied by stopped-flow microperfusion. J. clin. Invest. *45*, 603 (1966).
9. Goormaghtigh N.: Facts in favour of an endocrine function of the renal arterioles. J. Path. Bact. *57*, 392 (1945).
10. Hierholzer K., Wiederholt M., Holzgreve H., Giebisch G., Klose R. M. and Windhager E. E.: Micropuncture study of renal transtubular concentration gradients of sodium and potassium in adrenalectomized rats. Pflügers Arch. ges. Physiol. *285*, 193 (1965).
11. Schnermann J.: Microperfusion study on the response of single nephron filtrate to changing perfusion in the loop of Henle. III. int. Congr. Nephrol. 1966, p. 268.
12. Lever A. F. and Peart W. S.: Renin and angiotensin-like activity in renal lymph. J. Physiol. (Lond.) *160*, 548 (1962).
13. Cortney M. A., Nagel W. and Thurau K.: A micropuncture study of the relationship between flow rate through the loop of Henle and sodium concentration in the early distal tubule. Pflügers Arch. ges. Physiol. *287*, 286 (1966).
14. Horster M., Nagel W., Schnermann J. and Thurau K.: Zur Frage einer direkten Angiotensinwirkung auf die Natriumresorption im proximalen Tubulus und in der Henleschen Schleife der Rattenniere. Pflügers Arch. ges. Physiol. (in press).

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