PRODUCTION AND ACTION OF A NATRIURETIC HORMONE IN ISOLATED RAT KIDNEYS: NECESSITY OF PROSTAGLANDINS FOR ITS RELEASE

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Summary

A natriuretic factor prepared from plasma of salt loaded rats is active on a totally isolated cell free perfused rat kidney. The progressive increase in fractional sodium excretion with the amounts of injected factor shows a dose-response curve.

The inhibition of prostaglandin synthesis does not modify the kidney response to the natriuretic factor. The isolated kidney is able to produce this factor but the inhibition of prostaglandins suppresses its production. Therefore, we conclude that the presence of prostaglandins is a prerequisite for the production but not for the action of the natriuretic hormone.

Introduction

We have previously demonstrated the renal origin of a natriuretic hormone [1–5]. The disappearance of this hormone during experimental or human glomerulonephritis [5–8] explains the development of oedema occurring during such diseases.

It would be interesting to know whether this natriuretic hormone was acting alone or through a substrate produced by another organ. For this reason, we used the model of an isolated artificial cell-free perfused rat kidney as described by Bowman [9]. Moreover, as prostaglandins (Pg) could play a role in the regulation of Na excretion by the kidney, we looked for their relationship to natriuretic hormone.

Material and Methods

Action of a Natriuretic Hormone on Isolated Rat Kidneys

Ten rats fed a diet of 9 mEq NaCl a day were bled and plasma extracts used for testing the natriuretic activity were prepared by ultrafiltration on an Amicon
filter and passed through a Sephadex G-100 column in order to select a 45,000M wt fraction. We tested the natriuretic activity by administration of the extract, obtained from 5ml plasma and dissolved in 0.5ml saline, to isolated rat kidneys perfused by a Krebs-Henseleit solution to which was added albumin 6%, glucose 2g/L and lactate 5.10⁻³ M. Kidneys were removed from either normal rats (n = 11) or rats previously treated with Indomethacin (n = 8). For controls, we injected the extract solvent (0.5ml of 9% saline) into 5 normal kidneys and 6 Indomethacin-treated rat kidneys.

A series of experiments was performed using the same extract but prepared from 1, 5 and 10ml of plasma and dissolved in 0.5ml saline (n = 6, 11 and 6 respectively).

Production of a Natriuretic Hormone by the Isolated Rat Kidney

Kidneys isolated from 9 normal rats previously submitted to a salt depleted diet (<0.1 mEq Na/day) were perfused in vitro as described above. Perfusate samples were withdrawn before and 30 minutes after a saline load; these samples were prepared similarly to the plasma samples in order to detect their natriuretic activity. Nine other kidneys removed from rats previously injected with Indomethacin and also fed a salt poor diet were perfused similarly. The perfusate extracts were tested by administration to normal perfused rat kidneys (diet: 2.5 mEq Na/day); extracts were derived from 5ml of perfusate and were dissolved in 0.5ml saline.

Results

The natriuretic activity was expressed as the mean of the differences (± SEM) of fractional or absolute Na excretion between the 10 minute urine collection period before and the 20 to 30 minute urine collection period following the administration of extracts. Perfusate samples were withdrawn at the mid-point of urine collection.

Action of a Natriuretic Hormone on Isolated Rat Kidneys

The extracts obtained from the plasma of rats fed a salt rich diet induced a significant increase of absolute and fractional Na excretion when injected into the isolated kidney. The mean rise of fractional Na excretion was 6.01 ± 2.14% compared with 0.21 ± 2.15% obtained with the solvent alone (P<0.2) (Figure 1). No modification of GFR was observed. When the perfused kidney was removed from a rat previously treated with Indomethacin, a comparable rise in the fractional Na excretion of 4.09 ± 0.89% (P>0.1) was observed. A simple change in the absolute Na excretion was demonstrated, 6.01 ± 2.14%.

A dose-response curve was obtained when the injected plasma extracts were prepared from 1, 5 and 10ml of plasma. The fractional Na excretion was respectively 1.86 ± 1.62%, 6.01 ± 2.14% and 12.63 ± 2.05% (Figure 2).
Influence of Indomethacin on the natriuretic factor activity.

$\Delta$ FE Na %
(mean differences)

\[\begin{array}{ccc}
\text{C} & 5 & 6 \\
\text{FN} & 11 & 8 \\
\text{P < 0.02} & & \\
\end{array}\]

Figure 1. Mean changes ± SEM in fractional sodium excretion ($\Delta$ FE Na) in the isolated perfused rat kidney after the injection of the natriuretic factor (FN) compared with the injection of the extract solvent (C). Indo = kidneys isolated from Indomethacin pre-treated rats.

$\Delta$ F.E. Na %
(mean differences)

\[\begin{array}{c}
1 \\
5 \\
10 \text{ ml plasma} \\
\end{array}\]

Figure 2. Dose-response curve obtained by the injection into isolated rat kidneys of extracts proceeding from increasing samples of rat plasma. In ordinates are shown the mean changes in fractional sodium excretion ($\Delta$ FE Na)
Production of Natriuretic Hormone by Isolated Rat Kidneys

The extracts prepared from the fluid perfusing salt depleted rat kidneys induced a slight natriuresis when injected into another isolated rat kidney, comparable with the administration of the solvent alone. Conversely, the perfusate extracts obtained after a saline load led to a rise in fractional Na excretion, $6.05 \pm 1.92\%$, which was significantly different from the former ($P<0.02$) (Figure 3). When the

![Influence of Indomethacin on natriuretic factor production](image)

Figure 3. Mean changes ± SEM in fractional sodium excretion ($\Delta \text{FE Na}$) in the isolated perfused rat kidney after the injection of perfusate extracts from normal rats (N) before (Na-) and after saline load (Na+); and after the injection of perfusate extracts from Indomethacin (Indo) pretreated rats before (Na-) and after saline load (Na+)

perfused kidney was isolated from a salt deprived rat treated with Indomethacin, no natriuretic activity could be detected in the perfusate after the salt load (Figure 3). GFR was not modified by the extracts except by those coming from perfusates of Indomethacin pretreated and salt loaded kidneys in which GFR decreased moderately; absolute Na excretion changed with fractional excretion.
Conclusions

The production or the release of natriuretic hormone by an isolated dog [5] or rat kidney demonstrates that its origin could be renal or that it can be released after being stored in the kidney.

Nevertheless, our previous experiments have demonstrated that this factor disappears from plasma and urine during experimental [1–3,6,7] or human glomerulonephritis [8] where the only injury is renal and, moreover, that it was produced by cultured renal tubular cells [1,4]; therefore, we think that it really is synthesised by the kidney itself.

In this work, we demonstrate that this natriuretic factor is active on isolated rat kidneys perfused by an artificial cell free solution and that this factor does not need extra-renal substrate (unlike angiotensinogen in the renin-angiotensin system).

We have studied the action and production of the natriuretic factor in conditions of prostaglandin synthesis inhibition. Indomethacin inhibits natriuretic factor synthesis by the kidney but not its action on the isolated kidney.

Thus, it seems that the presence of prostaglandins is a prerequisite for the production but not for the action of the natriuretic factor.

Moreover, we have been able to show a dose-response curve for the natriuretic factor activity on the isolated rat kidney. Since previous work performed with Nizet [10], had demonstrated that this factor was active also on intestinal sodium transport, since we know its site of production and we have obtained a dose-response curve, we can assert that the natriuretic factor is a true hormone, necessary, at least for one part of the regulation of sodium transport.

References

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Open Discussion

KOKET (Chairman) Can you tell us something about the reproducibility of the method you used in your bio-assay for the estimation of the natriuretic factor. That is the first question.

As far as I remember last year, Dr Godon presented some results about the natriuretic factor in patients with acute glomerulonephritis. Is there any progress about the biochemical nature of this natriuretic factor of renal origin available now?
CAMBIER The isolated perfused rat kidney is considered a good model because GFR is about 0.5ml/min which is physiological. When we add the substrates, albumin, glucose and lactate, the sodium excretion is about 0.5 or 1%, so it can be used for this study. We have not continued the technical evaluation of the natriuretic hormone, and I don’t think anybody has done since last year.

GODON (Liège; co-author) This material is of proteic nature with a molecular weight 45,000 approximately, but now the study to identify the exact chemical structure is in progress with biochemists. I must add perhaps that the isolated rat kidney is a better bio-assay than the kidney in vivo, because the action of the natriuretic hormone is more potent on the isolated kidney than on the kidney in vivo.

KOPP (München) I wonder if you have any data about the variability of natriuretic action on the isolated model with variation of the pH of the perfusion fluid?

CAMBIER There was no modification of the pH.

KOPP What was the pH of the perfusion?

CAMBIER 7.4.

KOPP Thank you.