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Cyclic AMP Is a Hepatorenal Link Influencing Natriuresis and Contributing to Glucagon-induced Hyperfiltration in Rats

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Abstract

The effects of glucagon (G) on proximal tubule reabsorption (PTR) and GFR seem to depend on a prior action of this hormone on the liver resulting in the liberation of a mediator and/or of a compound derived from amino acid metabolism. This study investigates in anesthetized rats the possible contribution of cAMP and urea, alone and in combination with a low dose of G, on phosphate excretion (known to depend mostly on PTR) and GFR. After a 60-min control period, cAMP (5 nmol/min × 100 grams of body weight [BW]) or urea (2.5 μmol/min × 100 grams BW) was infused intravenously for 200 min with or without G (1.2 ng/min × 100 grams BW, a physiological dose which, alone, does not influence PTR or GFR). cAMP increased markedly the excretion of phosphate and sodium (+303 and +221%, respectively, P < 0.01 for each) but did not alter GFR. Confusion of cAMP and G induced the same tubular effects but also induced a 20% rise in GFR (P < 0.05). Infusion of urea, with or without G, did not induce significant effects on PTR or GFR. After G infusion at increasing doses, the increase in fractional excretion of phosphate was correlated with a simultaneous rise in plasma cAMP concentration and reached a maximum for doubling of plasma cAMP. These results suggest that cAMP, normally released by the liver into the blood under the action of G, (a) is probably an essential hepatorenal link regulating the intensity of PTR, and (b) contributes, in conjunction with specific effects of G on the nephron, to the regulation of GFR. (J. Clin. Invest. 1996. 98: 2251–2258.) Key words: liver • proximal tubule • phosphate • sodium • glomerular filtration rate

Introduction

Glucagon is a pancreatic hormone which stimulates several metabolic pathways in the liver, its first target organ. It plays an essential role in the maintenance of plasma glucose concentration by stimulating glycogenolysis and gluconeogenesis in situations of high energy needs or during fasting. On the other hand, and independent of the body's immediate needs in glucose, this hormone also plays a crucial role in disposal of nitrogen from the body. This is the case either after the ingestion of a large protein meal (or an amino acid infusion), when exogenous amino acids need to be metabolized and excess nitrogen excreted, or during periods of fast, when endogenous amino acids are used for gluconeogenesis. Actually, gluconeogenesis and ureagenesis are always linked (1), in order to ensure an appropriate disposal of the amine groups when the carbon chains of amino acids enter carbohydrate metabolism (2–4).

The kidney is also a target organ for glucagon, which influences solute transport in several nephron segments. As discussed in a previous paper (5), the tubular effects of glucagon on the distal segments of the nephron most probably result from a direct action of the hormone on specific receptors. This is the case for the stimulation of electrolyte reabsorption in the thick ascending limb of Henle. On the other hand, the effects of glucagon on the proximal tubule seem to be indirect and to require the previous action of glucagon on the liver (5).

In addition to its tubular effects, glucagon also influences renal hemodynamics. Several studies have established that this hormone participates in, or is even indispensable for, the post-prandial increase in GFR (6–11). However, this effect of glucagon is probably indirect and involves other mediators (12) because the plasma concentration of glucagon required to increase GFR exceeds the usual peripheral concentration seen after a protein meal (6, 10, 13). Moreover, direct infusion of glucagon into the renal artery fails to increase GFR in dogs (10, 14), humans (8), and rats (Ahloulay, M., personal observation). In previous experiments, we have confirmed that the rise in peripheral plasma glucagon concentration seen after a protein meal, and reproduced by glucagon infusion at a rate of 1.25 ng/min × 100 grams of body weight (BW), failed to increase GFR (5, 15). On the other hand, we observed that the rate of glucagon infusion necessary to increase GFR was only 3- to 10-fold higher. This higher rate of infusion induced in peripheral blood a concentration of glucagon that is physiologic for the liver, given the normal porto-peripheral concentration gradient for pancreatic hormones, due to their direct release in the portal vein (5, 15). This is in good agreement with the study of Premen (16) showing that a glucagon infusion, which failed to increase GFR when infused peripherally, did increase GFR when infused in the portal vein. Taken together, these findings

© The American Society for Clinical Investigation, Inc.
0021-9738/86/11/2251/08 $2.00
Volume 98, Number 10, November 1996, 2251–2258


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Received for publication 2 April 1996 and accepted in revised form 23 September 1996.

1. Abbreviations used in this paper: BW, body weight; C, control periods; dDAVP, desamino-arginine vasopressin; E, experimental periods; FE, fractional excretion; G1, glucagon infusion 1.25 ng/min × 100 grams BW; G10, glucagon infusion 12.5 ng/min × 100 grams BW; P, plasma.

established that glucagon action on the kidney requires a prior action on the liver. It has been proposed that glucagon could induce the release from the liver of a vasoactive hormone, gluceromelopressin, influencing the resistance of renal arterioles (17, 18, and for reviews see references 19 and 20). Other studies have suggested that the link between the liver and the kidney could be a compound(s) derived from the metabolism of amino acids by the liver (21–23). The nature of this compound has not yet been determined.

The intracellular second messenger mediating the hepatic actions of glucagon is cAMP. Intravenous administration of glucagon is known to induce a marked and prompt rise in plasma cAMP, due to a rapid exit of this nucleotide from hepatocytes (24–28). cAMP is excreted by the kidney as a result of both filtration and secretion. It has been well established that cyclic nucleotides are secreted in the proximal tubule by a carrier-mediated process involving the organic acid transport system (29). On the other hand, a few studies suggest that cAMP secretion in the proximal tubule might be responsible for the effects of glucagon on the excretion of phosphate (5, 30). Plasma cAMP thus could represent a significant interorgan mediator linking the liver and the kidney. Could this nucleotide also be involved in the hemodynamic effects of glucagon on the kidney? Alternatively, or in conjunction, could urea, which is produced by the liver and excreted by the kidney under the influence of glucagon (15), represent another component of the hepatorenal link?

This study was undertaken to evaluate (a) the influence of a rise in plasma cAMP or in plasma urea on renal function and (b) the possible contribution of cAMP and/or urea to glucagon-induced hyperfiltration. We hypothesized that rather than resulting from the influence of a single factor, the rise in GFR could depend on a combination of two or more factors acting jointly on the kidney.

Classical clearance experiments were conducted in normal, nonfasted rats in which a stable level of urinary concentrating activity was established to prevent uncontrolled changes in urinary flow rate and intrarenal urea recycling which could exert a confounding influence on GFR (5). Glucagon, cAMP, and urea were infused individually or in combination to evaluate the resulting changes in sodium, phosphate, urea, and water excretion, and in GFR.

**Methods**

**Animals and experimental protocols**

Adult male Wistar rats (Iffa-Credo, Lyon, France), 230–300 grams BW, fed on a standard rat chow, were allowed food and water ad libitum until the time of the experiment. Clearance experiments were conducted in two series of anesthetized rats (Inactin, 10 mg/100 grams BW, fed on a standard rat chow, were allowed food and water ad libitum until the time of the experiment. Clearance experiments were conducted in two series of anesthetized rats (Inactin, 10 mg/100 grams BW, after completion of the surgery and for the rest of the experiment, Inutest (Laevosan Gesellschaft, Linz, Germany) was infused at 0.75 mg/ml × 100 grams BW. To ensure a relatively stable renal concentrating activity (5), desamino d-arginine vasopressin (dDAVP; Minirin, Ferring, Malmö, Sweden), an antidiuretic analogue of vasopressin, was infused at 1.66 pg/ml × 100 grams BW. As shown in a previous study, this improves the stability of basal renal function and prevents the possible confounding influence of uncontrolled changes in urinary flow rate on solute excretion and renal hemodynamics (5).

**Protocol 1.** After surgery and 1 h of equilibration (t = 0), urine was collected in preweighed tubes for four 20-min control periods (C) after which the experimental infusion was started and urine was collected for six 20-min experimental periods (E). Blood samples (≈ 300 μl) were taken from the left femoral artery at the beginning and end of the equilibration period, and then every 40 min during the next 200 min (that is at the beginning of periods 1, 3, 5, 7, 9, and at the end of period 10). Plasma concentrations at the midpoint of each clearance period were interpolated from the values measured in these samples.

This series of experiments included seven groups of rats, one of which served as time control. In the other six groups, urea, cAMP (Sigma, St. Louis, MO), or glucagon (highly purified porcine glucagon; Novo Industry, Bagsvaerd, Denmark) were infused alone or in combination. The different experimental infusions and the corresponding abbreviations are shown in Table 1 (Table I also shows two groups of rats studied previously that received a glucagon infusion at two different rates, reproducing physiologic concentrations for either the kidney [G1] or the liver [G10]; see explanations in reference 5). Urea was infused at a rate close to that used in our previous study (15). Regarding cAMP, this nucleotide has rarely been infused in vivo. We chose to infuse 5 nmol/min × 100 grams BW, a rate 17-fold lower than that used by Lorenz (31), and comparable to that used in other studies (30, 32, 33). The glucagon infusion rate used in the present study (G1) was 1.2 ng/min × 100 grams BW, corresponding to the lowest infusion rate of our previous study (a rate which does not increase GFR).

**Protocol 2.** In our previous experiments, changes in plasma cAMP in response to glucagon infusions G1 or G10 had been measured in a few rats (5). To extend these observations, an additional series of rats was used to determine the concentrations of cAMP in peripheral blood and urine in different conditions, and the associated changes in GFR and phosphate excretion. Inutest and dDAVP were infused as in protocol 1, and the experiments included one C and one E period. During each period, urine was collected for 60 min (after an equilibration period of 60 min for C, or 40 min for E). During E, different rats received the following infusions. Three rats received glucagon at a rate of 120 ng/min × 100 grams BW (a rate 100-fold higher than in protocol 1), two rats received cAMP at a rate similar to that in protocol 1, and one rat served as control (no change during E).

**Analysis, calculations, and statistics**

Urinary flow rate was evaluated by gravimetry. Osmolality was measured with a freezing point osmometer (Roehling, Berlin, Germany).

**Table I. Experimental Infusions Given in the Different Groups (per 100 grams BW)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Glucagon (ng/min)</th>
<th>cAMP (nmol/min)</th>
<th>Urea (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G1</td>
<td>8</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G10*</td>
<td>7</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>cAMP</td>
<td>4</td>
<td>0</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>Urea</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>cAMP + urea</td>
<td>4</td>
<td>0</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>G1 + urea</td>
<td>4</td>
<td>1.2</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>G1 + cAMP</td>
<td>4</td>
<td>1.2</td>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>G1 + cAMP + urea</td>
<td>5</td>
<td>1.2</td>
<td>5.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

n, number of rats per group; * data from preceding study (5).
Table II. GFR (ml/min) Measured during C and E Periods in the Different Rat Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>C</th>
<th>E</th>
<th>Δ = (E - C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.05±0.31</td>
<td>2.99±0.29</td>
<td>-0.06±0.13</td>
</tr>
<tr>
<td>G1*</td>
<td>2.70±0.13</td>
<td>2.53±0.12</td>
<td>-0.20±0.10</td>
</tr>
<tr>
<td>G10*</td>
<td>2.57±0.13</td>
<td>3.13±0.23</td>
<td>+0.57±0.13</td>
</tr>
<tr>
<td>cAMP</td>
<td>2.35±0.18</td>
<td>2.45±0.14</td>
<td>+0.10±0.11</td>
</tr>
<tr>
<td>Urea</td>
<td>2.60±0.15</td>
<td>2.71±0.17</td>
<td>+0.11±0.11</td>
</tr>
<tr>
<td>cAMP + urea</td>
<td>3.08±0.28</td>
<td>3.18±0.46</td>
<td>+0.10±0.30</td>
</tr>
<tr>
<td>G1 + urea</td>
<td>2.38±0.19</td>
<td>2.23±0.09</td>
<td>-0.15±0.12</td>
</tr>
<tr>
<td>G1 + cAMP</td>
<td>2.32±0.11</td>
<td>2.76±0.24</td>
<td>+0.44±0.18</td>
</tr>
<tr>
<td>G1 + cAMP + urea</td>
<td>2.94±0.25</td>
<td>3.54±0.36</td>
<td>+0.60±0.14</td>
</tr>
</tbody>
</table>

Paired t test between E and C: †P < 0.05; ‡P < 0.01. § Data taken from a previous study (5).

Inutest concentration in plasma and urine was measured by the an- throne method (34). Inutest clearance was considered to represent GFR. The concentration of urea in plasma and urine was measured by a modification of the Berthelot method (Urea-Kit Biomérieux, Lyon, France) and that of phosphate by standard methods (RA 1000; Technicon Instruments Corp., Tarrytown, NY). cAMP was measured in plasma by radioimmunoassay after acetylation, using a commercial kit (RIA 1H cAMP, Amersham Corp., Arlington Heights, IL).

In protocol 1, the results obtained for each rat during the four control (1–4) and the last four experimental periods (7–10) were averaged to provide one C and one E value per rat. Means±SE for C and E were then calculated for each group. In protocols 1 and 2, the statistical significance of the differences observed between C and E in each group was evaluated by paired t test. In addition, the changes in GFR observed in the different groups in protocol 1 were compared by one-way ANOVA followed by the Fisher post-hoc test.

**Results**

The infusion of dDAVP in all rats during the experiments ensured a similar urine flow rate and osmolality in all groups (range 7.0–10.0 µl/min and 1,670–1,995 mosmol/kg H2O, respectively). No significant changes between control and experimental periods were observed in the time-control group except for a 36% increase in phosphate excretion (P < 0.01).

**GFR.** Table II and Fig. 1 show the results concerning GFR for the groups explored in protocol 1. Results obtained in a previous study for glucagon infusion at rates of 1.25 and 12.5 ng/min × 100 grams BW (G1 and G10, respectively) are also shown as a reference. G1, which reproduced a physiological concentration of glucagon in peripheral blood, failed to increase GFR, whereas a 10-fold higher infusion rate increased GFR by 21.5% (5). Urea alone or combined with cAMP or G1 was without effect. cAMP alone did not alter GFR, whereas cAMP + G1 increased GFR significantly (+18.5%). Coinfusion of urea with cAMP and G1 enhanced GFR somewhat further (+20.0%) (Fig. 1). When changes in GFR in the different experimental groups were analyzed by ANOVA, the only change which differed significantly from those in the control group were found in G10, cAMP + G1, and cAMP + G1 + urea (P < 0.01, P < 0.05 and P < 0.01, respectively). Moreover, G1 combined with cAMP induced a significantly different effect than G1 alone (P < 0.01).

**Water and electrolytes.** Fig. 2 depicts the influence of cAMP and glucagon (G10) on solute and water excretion. cAMP infusion increased urinary flow rate and excretion of the main urinary solutes. However, the magnitude of these changes was not uniform. Sodium and phosphate excretion was markedly increased (>200%), whereas excretion of water, potassium, and urea was increased more modestly (≈20–50%). (This effect was also observed in rats which did not receive dDAVP [34a].) These increased excretions were probably responsible for a decline in plasma concentration of urea (−7.5%) and phosphate (−14.0%), a decline which did not reach statistical significance, however. These changes induced by cAMP infusion are very similar to those observed after G10 infusion regarding both water and solute excretions (Fig. 2) and plasma composition (5).

Fig. 3 illustrates the influence of the different experimental infusions on fractional excretion of phosphate (FEPO4). As al-

![Figure 1](image-url) - Changes in GFR observed between E and C periods in response to the different infusions. Each point represents a single rat. Group means are shown by horizontal bars. Results of glucagon experiments G10 and G1 were obtained in a previous study (5). Paired t test (E vs. C): *P < 0.05; **P < 0.01.
ready described, glucagon increases FE$_{\text{PO}_4}$ in a dose-dependent fashion (absolute change: +5.5±1.6 and +12.3±2.5% of filtered load, for G1 and G10, respectively) (5). In all groups receiving cAMP, whether alone or in combination with urea and/or G1, a large and comparable increase in FE$_{\text{PO}_4}$ was observed (≈ 14%). Urea infusion induced a significant rise in FE$_{\text{PO}_4}$ (+8.8±2.3%) which was even greater when G1 was coinfused with urea (+11.8±1.0%).

Protocol 2 explored the relationship between changes in plasma cAMP ($P_{\text{cAMP}}$) and changes in GFR and in phosphate reabsorption induced by either glucagon or cAMP infusion. As observed in Fig. 4, for the seven rats in which the changes in $P_{\text{cAMP}}$ were moderate (< 40 nM, i.e., a doubling of the basal value), a highly significant correlation was observed between changes in FE$_{\text{PO}_4}$ and the simultaneous changes in $P_{\text{cAMP}}$ ($r = 0.995, P < 0.001$). For the five other rats in which higher changes in $P_{\text{cAMP}}$ occurred, FE$_{\text{PO}_4}$ reached a plateau probably corresponding to the maximal inhibition of phosphate reabsorption. Concerning GFR, the changes in the first seven rats also tended to be correlated with the simultaneous changes in $P_{\text{cAMP}}$ ($r = 0.680, 0.10 > P > 0.05$). Larger changes in $P_{\text{cAMP}}$ (< 200 nM) were associated with maximal changes in GFR when they resulted from a high rate of glucagon infusion. In contrast, they were not accompanied by any change in GFR when they resulted from cAMP infusion, as already observed in protocol 1 (Fig. 2).

**Urea.** As described earlier, the infusion of glucagon at a rate of 12.5 ng/min × 100 grams BW (G10) stimulates urea
synergistic rise in plasma cAMP resulting from glucagon action on the liver. Third, it suggests that the glucagon-induced increase in GFR depends on the combination of a physiological rise in peripheral plasma glucagon concentration and of a simultaneous rise in plasma cAMP resulting from glucagon action on the liver.

Influence of plasma cAMP on renal function. It is usually believed that circulating cAMP does not influence cellular functions throughout the body because most cells are impermeable to this nucleotide (35). This explains why few studies have investigated the effects of cAMP infusion on kidney function (30–33). However, in some cells, cAMP is transported by the organic acid transport system inhibitable by probenecid and para-aminohippuric acid (29, 36–38). This system is present in hepatocytes and cells of the proximal tubule, mostly in the pars recta (39, 40). As a result, cAMP generated in hepatocytes may be secreted in the blood (24–27) and taken up and secreted by proximal tubule cells (29, 36, 41).

The present results show that cAMP influences the excretion of the four major solutes with a predominant effect on sodium and phosphate. Because pars recta cells take up cAMP, cAMP infusion should increase their intracellular cAMP concentration, thereby increasing sodium reabsorption and the fractional excretion of urea.

Discussion

This study brings new information regarding the humoral control of renal function. First, it confirms that plasma cAMP exerts an influence on proximal reabsorption. Second, it suggests that the proximal effects of glucagon are likely dependent on the release in peripheral blood of liver-borne cAMP and on the subsequent uptake of this nucleotide by proximal cells. Third, it suggests that the glucagon-induced increase in GFR depends on the combination of a physiological rise in peripheral plasma glucagon concentration and of a simultaneous rise in plasma cAMP resulting from glucagon action on the liver.

Table III. Plasma Urea and Urea Excretion in the Different Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma urea (mM)</th>
<th>Urea excretion (µmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>Control</td>
<td>7.1 ± 0.4</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>G1*</td>
<td>7.6 ± 0.8</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>G10*</td>
<td>6.5 ± 0.6</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>cAMP</td>
<td>5.5 ± 0.5</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Urea</td>
<td>7.4 ± 0.4</td>
<td>8.9 ± 0.6</td>
</tr>
<tr>
<td>cAMP + ura</td>
<td>7.4 ± 0.8</td>
<td>9.5 ± 1.2</td>
</tr>
<tr>
<td>G1 + ura</td>
<td>6.6 ± 0.5</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>G1 + cAMP</td>
<td>6.2 ± 0.4</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>G1 + cAMP + ura</td>
<td>6.8 ± 0.4</td>
<td>8.3 ± 0.8</td>
</tr>
</tbody>
</table>

Paired t test between E and C: *P < 0.05; †P < 0.01; ‡P < 0.001. *Data taken from a previous study (5).
centration, thus inhibiting sodium phosphate and sodium bicarbonate cotransport, as cAMP generated intracellularly under the influence of PTH would do (42). Phosphate resulting from the breakdown of cAMP by phosphodiesterases probably also contributes to the rise in phosphate excretion (33, 43).

The fact that intravenously infused cAMP reduces late proximal reabsorption suggests that the plasma level of cAMP may permanently influence the intensity of late proximal tubule function. This influence is probably physiologically significant because small increments in \( P_{\text{AMP}} \) are accompanied by detectable changes in \( FE_{\text{PO}} \), and maximum response occurs for only doubling basal \( P_{\text{AMP}} \) (Fig. 4). Accordingly, cAMP might be an interorgan link between organs releasing cAMP in the blood (mainly the liver [24–27] and the heart [44]) and the kidney.

Interestingly, in the years after the discovery of the role of cAMP as a second messenger, a number of reports have emphasized that glucagon stimulates hepatic cAMP production and release in blood (or perfusate) \( \sim 40 \)-fold more than epinephrine, although they induce quantitatively similar metabolic effects (24, 25, 45, 46). Our results suggest that the extra amounts of cAMP produced by the liver in response to glucagon probably serve as an extracellular messenger acting on the kidney.

**Role of hepatic cAMP in the glucagon-induced changes in proximal reabsorption and in GFR.** Several previous studies have shown that glucagon exerts natriuretic and phosphaturic effects (5, 47–51) by reducing the reabsorption of sodium, phosphate, and water in the whole proximal tubule, with a predominant influence in the pars recta (52). However, no glucagon receptors (53) and no glucagon-sensitive adenylate cyclase activity (54) have been found in this nephron segment. The present results strongly suggest that the effects of glucagon on the proximal tubule are dependent on the uptake of hepatic cAMP by proximal tubule cells. However, the intensity of the cAMP-dependent reduction in pars recta reabsorption can be expected to vary with the glucagon/insulin concentration ratio in portal blood, and not with the absolute glucagon concentration observed in peripheral blood, because insulin decreases the glucagon-induced release of cAMP by the liver (55–57).

Several studies (including two from our laboratory) have shown that intravenous infusion of glucagon that induces increments in peripheral plasma glucagon within the physiological range does not increase GFR (5, 6, 10, 13, 15). In contrast, glucagon infusion increasing plasma concentration to 900 pg/ml or above (5) (a concentration usually achieved in the portal circulation) or infusion of lower amounts in the portal vein (10) does increase GFR. It has been proposed that the rise in GFR could depend on the liberation of the liver by a mediator or metabolite derived from amino acid metabolism (6, 15, 16, 21). A putative liver-derived vasoactive mediator glomerulopressin has not been well characterized to date (for review see reference 20). In this study, we evaluated whether two metabolites, cAMP and urea, released by the liver under the action of glucagon, could mediate the liver-dependent effects of glucagon on the kidney. Our results show that cAMP plays a crucial role but that the contribution of urea is only modest.

Although an infusion of cAMP is able to mimic the proximal effects of glucagon, it does not exert an influence on GFR. However, when cAMP and glucagon (G1) are combined, a distinct increase in GFR occurs. Actually, this combination reproduces for the kidney the conditions met physiologically when glucagon is normally released by the pancreas in the hepatic circulation. Accordingly, the present study shows that in normal conditions the combination of two factors acting simultaneously on the kidney is required for influencing GFR, namely the renal action of liver-derived cAMP and the direct renal action of glucagon. That cAMP is necessary but not sufficient to raise GFR is also suggested by the absence of any change in GFR when large increases in \( P_{\text{AMP}} \) occur without glucagon addition, contrasting with the parallel changes observed in \( P_{\text{AMP}} \) and in GFR after G1 or G10 infusions (Fig. 4). Note that the release of variable amounts of adenosine in the nephron lumen, due to the breakdown of cAMP, may partially counteract the increase in GFR, thus weakening the correlation shown in Fig. 4, because luminal adenosine has been shown to reduce GFR (58).

Because a high infusion rate of glucagon (G10) increases simultaneously urea synthesis by the liver and GFR (15), it was interesting to consider whether increased urea delivery to the kidney could play a role in the renal hemodynamic response to this hormone. Urea alone did not influence GFR (Table II). However, it seemed to have a modest additive effect above that due to the combination of G1 and cAMP. Notably, the increased excretion of urea in groups receiving an urea infusion occurred without any change in GFR and was solely due to a rise in \( P_{\text{urea}} \). In contrast, the similar increase in urea excretion seen after glucagon infusion (G10) resulted from a rise in GFR without any change in \( P_{\text{urea}} \), as already underlined (5, 15).

The contribution of glucagon to the rise in GFR seen after a protein meal or an amino acid infusion remains controversial (6–11, 13, 59). This might be due to the fact that the rise in GFR is also partly dependent on the release of hepatic cAMP, which itself depends on the balance between insulin and glucagon, not on glucagon alone.

How can the combination of cAMP and glucagon induce a rise in GFR? Glucagon (G1 level) is known to stimulate directly NaCl reabsorption in the thick ascending limb (60, 61). This could decrease NaCl concentration at the macula densa. On the other hand, glucagon (G10 or above) has been shown to depress tubulo-glomerular feedback (62). Because glucagon (G1) alone does not increase GFR, it is likely that this effect at the macula densa level becomes significant only when a prior cAMP-dependent reduction in late proximal reabsorption provides a higher fluid and solute delivery to the loop. This hypothesis is compatible with the observations that inhibition of reabsorption in either the proximal tubule (63) or the loop of Henle (64) prevents the protein-induced rise in GFR.

In this study, the intravenous cAMP infusion resulted in a plasma concentration which is much higher than that induced by glucagon administration (Fig. 4). Thus, further studies using lower infusion rates of cAMP are necessary to confirm the proposed influence of circulating cAMP on renal function. Alternatively, attempts to prevent cAMP secretion in the proximal tubule during glucagon administration (G10) should bring even more convincing evidence on this role. In preliminary experiments, we observed that infusion of para-aminohippuric acid (which competes with cAMP for secretion by the proximal tubule) prevents the rise in GFR induced by glucagon G10 infusion (Ahloulay, M., F. Machet, and L. Bankir, unpublished results).

**Possible involvement of plasma cAMP in several pathophysiologic conditions.** The fact that plasma cAMP could influence proximal reabsorption and contribute to glucagon-
induced hyperfiltration provides pertinent explanations for several clinical disorders. Impaired cAMP production by the diseased liver could explain the sodium and water retention of cirrhosis, and abnormally low cAMP production due to hyperinsulinemia could account for the antinatriuretic effect of insulin (65) and for the edema of Kwaishokor (malnutrition characterized by intake of food with high carbohydrate and low protein content [66]). Conversely, the natriuresis of fasting (67) could be due to an increased cAMP production under the influence of increased glucagon secretion.

That both glucagon and cAMP are required to increase GFR can account for the fact that a rate of glucagon infusion which increases GFR in normal dogs (48) fails to do so in cirrhotic dogs (68) in which the hepatic cAMP response to glucagon is blunted. The progressive oliguric renal failure characteristic of the hepatorenal syndrome might be due to a sustained defect in hepatic cAMP production. On the other hand, the hyperfiltration of diabetes mellitus could be due, at least in part, to the combination of elevated glucagon secretion (69, 70) and exaggerated cAMP release by the liver in the absence of insulin action (71). It is noteworthy that immunoneutralization of circulating glucagon induced a significant fall in GFR in rats with streptozotocin-induced diabetes mellitus (72).

In conclusion, this study brings a new insight into the nature of the hepatorenal link suspected in several physiologic and pathologic conditions and the mechanism by which glucagon influences renal hemodynamics. The present results suggest that liver-borne cAMP exerts a permanent influence on the intensity of proximal function and that glucagon and circulating cAMP must act simultaneously on the kidney to increase GFR after a protein meal. Alterations in cAMP production by the liver in either direction and/or marked changes in glucagon and insulin secretion could explain disorders in GFR and in sodium and water excretion observed in various pathologic conditions.

Acknowledgments

We thank Dr. Réokia Belahsen (Université Chouaïb Doukkali, El Jadida, Maroc) for her contribution to this study and Doreen Bronner for reviewing the English.

This work was supported, in part, by the Groupe Danone (Paris, France). M. Ahloulay received a scholarship from the Fondation pour la Recherche Médicale.

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