Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea

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A SINGLE PROTEIN-RICH MEAL or an infusion of amino acids (AAs) are known to increase glomerular filtration rate (GFR) for a few hours, a phenomenon known as “hyperfiltration” or “functional reserve” (14, 52, 113, 119). In patients with chronic kidney disease (CKD), the hyperfiltration induced by a chronic protein-rich diet is assumed to contribute to the progressive decline in renal function by inducing a vicious circle that progressively deteriorates the “remaining” nephrons (59, 60). In diabetes mellitus (DM), hyperfiltration also develops in kidney disease (CKD), the hyperfiltration induced by a chronic protein-rich diet, or the diabetic condition all share a constellation of facts, putting them into a new perspective by proposing novel regulated regulations that were not previously given enough attention. We present evidence that glucagon and vasopressin are the earliest actors in the cascade of events leading to an increase in the GFR, and thus, probably in the resulting adverse consequences of hyperfiltration. Urea and/or other nitrogen-derived waste products probably also play a pivotal role in these processes. The ingestion of a protein meal, a sustained high-protein diet, or the diabetic condition all share a constel-
Nitrogen Excretion and Water Economy: A Challenge for the Kidney

Protein-induced hyperfiltration. Since almost a century, early scientists observed that a high protein intake induced a marked kidney hypertrophy in rats (163). When adequate markers of GFR became available, it was understood that not only kidney weight but also GFR was influenced by the level of protein intake. Moreover, even a single-protein meal was shown to induce a transient rise in GFR (14, 39, 52, 113, 119, 172, 194, 249).

The interesting study of Lew and Bosch (158) shows how protein intake is positively and strongly associated with GFR among healthy subjects (Fig. 2A). This is also true among subjects with CKD (158). Conversely, a reduction in daily

Among different healthy individuals, an increase in urea excretion (matching an increase in protein intake) is achieved by a rise in urea concentration in the urine without a change in daily urine volume (thus without increasing the daily water requirements). Figure is drawn from numerical data shown in Table 1 in Ref. 158 and additional data kindly provided by the authors in 1992. B: reevaluation of the results of the MDRD study, a clinical trial evaluating the decline in renal function in chronic kidney disease (CKD) patients switched to a moderately low protein diet for 3 yr. Note the initial GFR decline following the abrupt reduction in daily protein intake (B3 to F4), and the slower decline observed in the remaining months (up to F36). Reproduced from Ref. 157.
protein intake can be expected to reduce the GFR. Unfortunately, such a reduction was not anticipated in the design of the large clinical trial “Modification of Diet in Renal Disease” (MDRD) exploring the possible benefit of a reduction in protein intake in CKD patients (144). This ambitious trial led to inconclusive results, but in subsequent analyses, Levey et al. (157) considered separately two distinct phases in this study, as shown in Fig. 2B. The significant reduction in GFR that occurred in the first 4 mo was not the sign of a worsening of renal function, but was an adaptation to a lower protein intake. Once this initial decline had occurred, the remaining 32 wk of the study revealed a significant benefit, that is a 30% slower decline in GFR in the patients with the low protein diet. Thus, although imperfect, this study speaks in favor of a moderate reduction in protein intake in CKD patients, as also advised in several other reports (45, 115, 117, 161).

Besides the effect on kidney weight and GFR, a few studies in the 1950s showed that a high-protein diet increases the ability of the kidney to concentrate urine (97, 124). A further analysis of the data from the study of Lew and Bosch (158) (who kindly provided us with all numerical data obtained in their subjects, including the 24-h urine volumes and urea concentrations, not reported in their paper) illustrates this fact. As shown in Fig. 2, C and D, the human kidney adapts to wide differences in daily urea excretion (and thus in protein intake) by increasing the concentration of urea in urine without altering the urine volume, as also observed in another study (162). This confirms in humans the “economy of water” achieved in the excretion of nitrogen wastes already reported in animal studies (58, 124, 210). Moreover, urea is also responsible for an economy of water in the excretion of other solutes, as demonstrated more than 70 years ago by the elegant studies of Gamble et al. (108, 109) and recently confirmed in mice with invalidation of the urea transporters of the terminal inner medullary collecting duct (102). Using a mathematical model of the medulla, Layton et al. (154) reproduced Gamble’s observations by simulating the hypertrophy of the inner stripe (57) and the hyperfiltration observed after chronic high protein intake.

Waste products derived from protein catabolism. Interestingly, no hyperfiltration occurs after consumption of a protein-free meal (194). Why do only proteins induce hyperfiltration? The catabolism of carbohydrates and lipids provides only CO₂ and H₂O (metabolic water) that are easily excreted by the lungs and kidneys, respectively. In contrast, protein ingestion results, in addition to CO₂ and H₂O, in the production of a number of nitrogen wastes (urea, uric acid, ammonia, creatinine) and other metabolites (phosphates, sulfates, protons, etc.) that are all excreted by the kidney. Figure 3A shows the amount of several solutes excreted per day in the urine in healthy humans on a Western-type diet (27). Urea is by far the most abundant urinary solute (a fact not easily perceived when urea is expressed in mg/dl). Moreover, because of its low concentration in extra- and intracellular fluids (Fig. 3B), the daily urea excretion amounts to about twofold the total body urea pool (60% of a 70-kg body weight × 5 mmol/l = 210 mmol). In contrast, the daily excretion of sodium represents no more than one-fifteenth of the total body sodium pool, and about one-tenth of the extracellular sodium pool.

Because the plasma concentration of urea and other nitrogenous compounds is maintained at a low (a few mmol/l) or very low (<100 μmol/l) level in plasma and extracellular fluids in mammals, the excretion of large daily loads of these compounds in the urine might induce a dramatic diuresis, would the kidney not be able to perform a spectacular and solute-specific concentrating activity. Figure 3B illustrates the concentration of several solutes in plasma and urine. All solutes are not concentrated equally. Urea is usually concentrated about 50 times above its plasma level, ammonia about 500 times. In contrast, sodium is usually less concentrated in urine than in plasma. Too rarely emphasized in papers describing the urine concentrating mechanism, most of the “solute-free water” reabsorbed to concentrate urine is devoted to the concentration of urea and ammonia (27, 36).

Ammonia is by far the most toxic of all nitrogenous compounds. Although the daily excretion is far from negligible (~40 mmol/day), its concentration in plasma is maintained at a very low level. The intestine and liver do not release ammonia in the blood. Ammonia is produced in the kidney itself (most intensely in the medullary pars recta of the proximal tubule) by the hydrolysis of glutamine and is secreted into the nephron lumen. It then flows along the loops of Henlé, but is later prevented to flow through nephron segments located in the cortex because it is “shunted” within the outer medulla, between the medullary thick ascending limbs (where it is actively reabsorbed) and the nearby medullary collecting ducts (where it is secreted). This shunt limits the flow of ammonia in the cortical distal tubules and collecting ducts and thus prevents a too intense diffusion in the highly perfused cortex. Nevertheless, because of the intense ammonia production by the kidney, ammonia concentration is usually higher in renal
Urea, thought for many decades to be nontoxic, is now recognized to have significant toxicity, both by itself and by the formation of cyanate, responsible for carbamylation of proteins (94, 138). Carbamylation of certain proteins impairs several cell functions including the immunological potency of leukocytes, the electrolyte and gas transport capacity of erythrocytes (reviewed in Ref. 36), and compromises endothelial functions (reduction of nitric oxide synthase, etc.) (94). It also participates in oxidative stress (84, 149). In CKD patients, the fraction of carbamylated serum albumin at baseline is significantly associated with a higher risk of mortality during the following year (44). Moreover, in vitro studies show that urea levels observed in CKD favor the proliferation, in the intestinal microflora, of certain types of bacteria expressing urease activity (able to degrade urea into ammonia and CO2). This is assumed to favor the disruption of the intestinal barrier by affecting epithelial tight junctions and to increase local inflammation (242, 243). In dialyzed patients, chronic dialysis with 50 mmol/l urea in the dialysate produced malaise, headache, and vomiting (137). Patients with “familial azotemia without renal failure,” a genetic anomaly leading to a threefold increase in their plasma urea concentration (see a review in Ref. 37), suffer no obvious symptoms. Nevertheless, sustained elevations in plasma urea concentration may lead to a number of minor deficiencies in multiple regulations, that, altogether, may weaken the adaptability and defense ability of the organism.

Along with the sustained protein-induced hyperfiltration (107, 158), the kidney undergoes a significant hypertrophy (33) that probably represents an adaptation to the augmented reabsorption of solutes filtered in greater amounts, and to the need for excreting and concentrating greater amounts of protein-derived waste products. Before GFR measurements were possible, early scientists had observed that a high-protein diet induced an increase in the urine concentrating ability (97) along with a significant kidney hypertrophy (163). However, feeding experimental animals with an amount of urea equivalent to that produced by the catabolism of the protein-rich diet did not induce any hypertrophy of the kidney (164) and did not induce a rise in creatinine clearance (when this clearance was used as an index of GFR) (181). It was thus concluded that some stimulus resulting from protein intake or metabolism was indispensable for this hypertrophy, the improvement of urine concentrating ability, and the hyperfiltration. Urea alone induced only an osmotic diuresis with no extra “workload” to the kidney.

The two terms of this relationship are not independent because an increase in GFR could presumably induce a decline in plasma urea or at least attenuate the protein-induced rise in plasma urea. However, what about urine urea concentration? As indicated above, urine urea concentration actually rises markedly with increasing protein intake (Fig. 3D). The U/P ratio also rises (from ~60 to 80 for daily urea excretions of 200–500 mmol/day; personal calculation based on data from Lew and Bosch’s study) (158). Interestingly, a high-protein diet, or an infusion of urea after a period on a low protein diet, improves the kidney’s concentrating ability, and urea is known to play a major role in the urine concentrating process (58, 101, 102, 208, 210). Moreover, an increase in urine concentration is known to reduce the fractional excretion of urea (29, 36) because more urea is reabsorbed at low urine flow rates. This increased reabsorption could thus partially offset a possible GFR-dependent decline in plasma urea. The very selective localization of facilitated urea transporters along the nephron, the direct and indirect influence of vasopressin, and the resulting complex urea movements that take place in the renal medulla may affect urea concentration in diverse sites along the nephron and in the final urine (101, 253). Some specific situations (knockout of a facilitated urea transporter, dysfunction of the thick ascending limb, or disruption of the medullary architecture in polycystic kidney disease) may result in a “ureaselective concentrating defect” characterized by a large fall in the U/P urea ratio, and only a modest fall in overall urine concentrating ability (25, 140, 254). Less dramatic alterations in renal function may also induce changes in this ratio, even if less intense.

It is usually assumed that the rate of urea excretion by the kidney does not undergo any regulation, and that it only results from glomerular filtration of plasma urea minus urea reabsorption along the nephron. However, this is a too simplistic view. There is abundant evidence that urea excretion is regulated to some extent (21, 36, 179, 189). Urea is actively reabsorbed in ruminants and other herbivores (209), and even in rats when fed a low protein diet for several weeks (135), allowing the re-use of urea nitrogen after urea degradation by gut microflora expressing urease (see a review in Ref. 21). On the other hand, in carnivores and omnivores, urea is most probably actively secreted in the pars recta of the proximal tubule (37), and the fractional excretion of urea falls dramatically when this secretion is impaired (21, 36, 37, 153, 206). Like for uric acid, organic anions, xenobiotics, etc., this active urea secretion...
probably takes place most intensely in the medullary portion of the pars recta, surrounded by a high density of venous ascending vasa recta (36, 37). Because this process subtracts urea from the blood and adds it to the urine, it may significantly influence the U/P urea concentration ratio. This secretion might possibly be regulated by glucagon because glucagon has been shown to increase the fractional excretion of urea in several studies (3, 5, 147).

Thus, because of the complex handling of urea in the kidney, plasma urea and urine urea may be influenced either in parallel or in opposite ways, depending on complex regulations or alterations in tubular transport. We hypothesize that the U/P urea ratio is influenced by vasopressin and glucagon actions on the nephron and collecting duct. This ratio may represent an accessible marker reflecting the composition of the tubular fluid flowing at the inaccessible macula densa, the site of the tubuloglomerular feedback control of GFR. We suspect that the relative concentration of urea in the tubular fluid along the thick ascending limb may reciprocally influence the concentration of sodium and thus alter the level of tubuloglomerular feedback (TGF). This will be explained in more detail in the last section of this review. We want, first, to describe why vasopressin and glucagon are most likely pivotal elements in this complex regulation and how these two hormones contribute to set up the best compromise between efficient urea excretion and water economy.

Glucagon, Nitrogen Metabolism, and GFR

Glucagon and nitrogen metabolism. It is usually assumed that the main role of glucagon is to stimulate gluconeogenesis during periods of fast. However, in normal feeding conditions, when glucose is available in sufficient amounts, the main role of glucagon is likely associated with nitrogen handling and disposal (128, 171). Glucagon is a main stimulus for ureagenesis in the liver (221), in strict association with gluconeogen...
Glucagon also stimulates urea excretion by the kidney. Clearance experiments in rats showed that urea synthesis in the liver and urea excretion by the kidney are simultaneously induced by glucagon in a coordinated fashion (3, 5). This allows the disposal of the nitrogen atoms of ingested AAs because there is no way to store nitrogen in the body (48, 128). The newly formed glucose may be consumed in postprandial thermogenesis or enter other metabolic pathways. During fasting, glucagon also favors ureagenesis along with gluconeogenesis, thus ensuring the disposal of the amine groups when the carbon chains of AAs enter carbohydrate metabolism for the sake of glycemic control.

Actually, hypoglycemia per se is a weak stimulus for glucagon secretion compared with the high efficacy of a physiological AA mixture (185). Interestingly, Rocha et al. (201) showed that the glucagon-stimulating activity induced by AAs infused individually in normal dogs was greater with gluconeogenic AAs than with other AAs. Among the latter, the three branched-chain AAs (valine, leucine, isoleucine), which cannot be metabolized in the liver, failed to influence glucagon secretion (200). This suggests that the rise in glucagon secretion is associated with the use of AAs for gluconeogenesis and associated ureagenesis. One of glucagon’s important metabolic functions is thus to stimulate urea synthesis in the liver and urea excretion by the kidney, whether the AAs used come from endogenous stores (during fast) or from excess protein ingestion (36). Accordingly, hyperglucagonemia increases the rate of urea synthesis, decreases the blood AA concentration, and induces nitrogen loss from organs in both healthy and diabetic rats (9, 11, 12, 118). In patients with DM, the functional hepatic nitrogen clearance was found to correlate strongly with fasting glucagon concentration, independently of other factors (10).

**Glucagon and GFR.** Because glucagon secretion increases after meat ingestion, its role in postprandial hyperfiltration has been strongly suspected for a long time. Several studies in the 1970-1980s showed that glucagon infusion in animals and humans increases GFR (120, 126, 136, 186, 218). Others found correlations between the rise in GFR and the rise in plasma glucagon concentration (17). However, some authors denied a role for glucagon because the time course of the rise in GFR preceded the rise in glucagon (46). Others noted that the concentration of glucagon required in plasma to elicit a rise in GFR was far higher than that measured after a protein-rich meal (198). Smoyer et al. (220) showed, in healthy subjects, that an oral administration of arginine induced a greater rise in GFR than an equivalent intravenous infusion, but that the associated rise in plasma glucagon concentration was lower, thus suggesting that glucagon was not the primary mediator of arginine-induced hyperfiltration (220).

Conversely, several findings strongly supported a role for glucagon in the protein- or AA-induced hyperfiltration. In an elegant clinical investigation in healthy subjects, Giordano et al. (113) found a highly significant correlation between the rise in GFR and the simultaneous rise in plasma glucagon concentration in response to infusion of five graded doses of AAs administered on different days to the same subjects (at a 3- to 7-day interval) (Fig. 5, A and B). Insulin, growth hormone, or IGF-1 showed no such correlation (113). Branched-chain AAs, that do not stimulate glucagon secretion and are not metabo-

lized in the liver (see above), also did not induce a rise in GFR (73, 81) (except valine) (110), whereas gluconeogenic AAs, those involved in hepatic gluconeogenesis and ureagenesis, induced both a rise in glucagon secretion and a rise in GFR (73, 197). Claris-Appiani et al. (80) showed that a mixed AA infusion increased GFR in healthy subjects, a rise that was well correlated with that in urea excretion (Fig. 5, C–E), whereas the infusion in the same subjects of essential AAs, which escape splanchnic metabolism, induced no rise in GFR and no rise in urea excretion (80). Noteworthy, a meat meal or AA infusion did not increase GFR in pancreatectomized dogs or patients who can no longer secrete glucagon (89, 106, 199). However, after an intravenous infusion of glucagon, GFR rose in these dogs and patients, as it did in controls (106, 199).

Direct infusion of glucagon at a moderate rate into the renal artery of dogs or humans, raising intrarenal glucagon concentration about threefold, did not induce a rise in GFR (61, 106, 235). Several studies confirmed that the contribution of glucagon does not result from a direct action of this hormone on the kidney but requires glucagon to flow through the liver first (251), allowing selected AAs to be deaminated and produce urea (155, 156). Preman (195) showed that the rise in GFR in dogs occurred in response to an intraperitoneal infusion of glucagon, a site allowing the hormone to flow first through the liver, before reaching the general circulation. Accordingly, a liverborne mediator or metabolite, generated under the influence of glucagon, was suspected to be responsible for the renal vasodilation (13, 235). Attempts to characterize this compound, named “glomerulopressin,” were not successful (51), and its existence was not confirmed by other authors. Because glucagon stimulates gluconeogenesis and ureagenesis in hepatocytes, leading to glucose and urea release in the blood, other studies investigated the possible role of these two compounds in the induction of glomerular hyperfiltration, but provided negative results (4, 196). Thus the putative liver-borne hormone (or mediator) remained unidentified for several years.

**Importance of the glucagon/insulin ratio and of liver-borne cAMP**

One major explanation for the divergent results summarized above is that most authors failed to consider that the metabolic actions of glucagon in the liver are counteracted by insulin. As well explained by Parrilla et al. (185) in 1974, “it is the glucagon/insulin ratio, and not the absolute concentration of either hormone, that determines the metabolic events in the liver” because insulin blunts the glucagon-induced production of the second messenger cAMP by hepatocytes. This was well established in the 1970s (99, 185, 212, 234), but has rarely been remembered afterward, and is completely neglected nowadays.

cAMP is usually hydrolyzed and its constituents recycled in the cell where it is produced. However, at variance with most other peptidic hormones, glucagon stimulates cAMP production in hepatocytes to a much greater extent than do catecholamines although the two hormones increase glucose release similarly (Fig. 6, A and B) (99). The extra glucagon-induced cAMP diffuses out of the hepatocytes into the hepatic venous blood through an organic acid membrane transporter. This results in a well-described, significant, dose-dependent rise in the concentration of plasma cAMP in the general circulation (63, 125, 222, 225). Insulin blunts dose dependently
the glucagon-induced production and release of cAMP. Because of the energetic cost of this pathway, requiring the permanent de novo synthesis of adenine nucleotides, some authors assumed that cAMP release by the liver could not be futile and had to play a role in a distant organ (40, 139, 142). This assumption found direct support 30 years later (see a review in Ref. 23). As shown in Fig. 6C, Ahloulay et al. (4) showed that an intravenous infusion of a low dose of glucagon did not increase GFR unless cAMP was infused simultaneously, thus simulating the influence of glucagon on the liver. The likelihood that cAMP is an extracellular mediator between the liver and the kidney has been reviewed in detail elsewhere (23). The putative liver-borne glomerulopressin, released under the action of glucagon, is probably cAMP (although cAMP alone is not sufficient to raise GFR). Importantly, its release is under the control of the glucagon/insulin concentration ratio, not of glucagon concentration alone. Another confounding factor in the search for glucagon’s influence on GFR may be that the responsiveness of the liver to the glucagon-induced release of cAMP tends to be exhausted after awhile (88a, 125), possibly due to the shortage of substrates for cAMP synthesis. This and the kinetics of cAMP clearance may explain some discrepancies in the time course of hyperfiltration and of glucagon concentration in the blood.

The role of glucagon in hyperfiltration cannot be appropriately evaluated without taking into account the level of insulin because protein intake, or selected AAs, may differentially influence the secretion of the two hormones. Experiments in dogs showed that some AAs stimulate the secretion of both hormones in equal proportions while others stimulate preferentially the secretion of one or the other hormone (200). In normal subjects, the intravenous infusion of a mixed AA solution, containing about equal concentrations of essential and nonessential AAs, approximately doubled the plasma concentrations of both insulin and glucagon (71, 72). In subjects fed a single protein meal, soy protein or casein induced widely different insulin/glucagon ratios (207). Another study showed that a protein meal was a less potent secretagog for insulin than for glucagon. Interestingly, the addition of 50 g of glucose to a protein meal delayed and amplified the glucagon response (150).

Fig. 5. Effect of an amino acid (AA) infusion or ingestion of a protein meal on GFR and its relationship with plasma glucagon or glucagon/insulin ratio. A and B: results observed in healthy subjects who were infused, on 6 different days, with 5 different doses of AAs or no AA (basal). Plasma glucagon was highly correlated with GFR. Reproduced from Ref. 113. C–E: results observed in healthy subjects infused with a single dose of mixed AAs. C: GFR was well correlated with the simultaneous urea excretion rate before (black symbols) and after 1 and 2 h of AA infusion (crosses and open symbols, respectively). D and E: change in GFR observed in response to AA infusion was not correlated with plasma glucagon concentration but was significantly correlated with the glucagon/insulin concentration ratio ($r = 0.40, P < 0.05$). Redrawn after Ref. 80.
If a liver-borne compound is involved in the renal action of glucagon on GFR, it is logical to assume that the glucagon/insulin ratio, rather than glucagon alone, needs to be considered. This is indeed what Claris-Appiani et al. (80) confirmed. In their study, the rise in GFR seen after the infusion of a mixed AA solution was not significantly correlated with the plasma level of glucagon but was significantly correlated with the glucagon/insulin ratio (Fig. 4, D and E). In the Smoyer et al. (220) clinical investigation involving arginine-induced hyperfiltration, the fact that arginine stimulates both insulin and glucagon secretion (200) may explain why the rise in GFR was not well correlated with that in glucagon. The glucagon/insulin ratio should have been considered in this case, not just glucagon.

Note that an increase in plasma cAMP alone is not sufficient to induce a rise in GFR. In rat experiments, we showed that the changes in GFR were correlated with the simultaneous changes in plasma cAMP induced by a glucagon infusion but not significantly correlated with the glucagon/insulin ratio (Fig. 4, D and E). In the Smoyer et al. (220) clinical investigation involving arginine-induced hyperfiltration, the fact that arginine stimulates both insulin and glucagon secretion (200) may explain why the rise in GFR was not well correlated with that in glucagon. The glucagon/insulin ratio should have been considered in this case, not just glucagon.

Note that an increase in plasma cAMP alone is not sufficient to induce a rise in GFR. In rat experiments, we showed that the changes in GFR were correlated with the simultaneous changes in plasma cAMP induced by a glucagon infusion, but that GFR did not change when cAMP was infused alone (without glucagon), even if inducing very high plasma cAMP concentrations (4). Both cAMP and glucagon actions are simultaneously required to influence GFR (Fig. 6C). Because of the contemporary rise in the excretion rate of sodium, chloride, urea, and phosphate, and fall in the excretion rate of calcium and magnesium (5), we proposed that cAMP reduced fluid and solute reabsorption in the proximal tubule (a water-permeable segment), while glucagon stimulated sodium reabsorption in the thick ascending limb (a water-impermeable segment) (4). These combined influences should alter the composition of the tubular fluid at the macula densa (see below).

Initially, Ahloulay et al. (4) assumed that cAMP was influencing proximal tubule reabsorption by being taken up by proximal tubule cells through an organic acid transporter. Further information from the literature suggested that this renal action of cAMP might rather be mediated by a specific membrane receptor for cAMP, comparable to those well characterized in the unicellular organism Discoidum discoideum (23). Interestingly, the seven transmembrane-domain cAMP receptors of D. discoideum share significant homology with the human parathyroid hormone (PTH) receptor, and the effects of glucagon or cAMP on the proximal tubule mimic those of PTH (23). The possibility that the mammalian kidney expresses membrane cAMP receptors on the same protein as the PTH receptor needs further confirmation.

Vasopressin, Protein Intake, and GFR

**Protein intake, urea, urine concentration, and vasopressin.**

After a single protein meal, vasopressin secretion and urine osmolarity are enhanced for several hours in human subjects (Fig. 7, A and B) (119). A chronic high protein intake induces a
chronic rise in plasma vasopressin (75, 85) and improves urine concentrating ability in both rats and humans (58, 97). This high vasopressin level may thus participate in glomerular hyperfiltration and kidney hypertrophy. Figure 7, C and D, shows that, in two independent studies in healthy humans, the GFR in the hours following a protein meal was significantly correlated with the simultaneously observed urine osmolality. Of note, no rise in GFR and no kidney hypertrophy are observed in response to high-protein feeding in Brattleboro rats with hereditary central diabetes insipidus (unable to secrete vasopressin) (57) or in rats with lithium-induced urinary concentrating defect (79). Vasopressin and a normal urine concentrating ability are thus required for these protein-induced changes. Thomas Addis, in 1940, had already understood that the “osmotic work” related to urine concentration was a burden for the diseased kidney (1). When he advised his patients to reduce their protein intake, he intended “to put the kidney at rest of osmotic work.” Treatment with selective vasopressin V2 receptor antagonists (called vaptans) or voluntary increase in water intake match the same purpose.

A low protein diet, which reduces the need to excrete nitrogen wastes, reduces GFR in rats (58, 104, 215, 216) and humans (72, 144). Is urea itself responsible for the rise in GFR seen with a high-protein diet? Clearly, no. As recalled earlier, feeding urea in amounts equal to those brought by a high-protein diet does not induce kidney hypertrophy as does a high protein intake (164), and an intravenous infusion of urea does not trigger a rise in GFR. It increases urea excretion only by raising plasma urea concentration and thus urea filtration, without a change in urea fractional excretion (3, 4). In contrast, after a protein meal, a high-protein diet, or glucagon infusion, the fractional excretion of urea is increased, revealing a change in urea handling by the renal tubule (and plasma urea does not change or increases only modestly) (3). However, GFR exhibited a marked increase in rats (5.9 ± 0.4 vs. 3.4 ± 0.4 ml-min⁻¹-kg body weight⁻¹ in controls) when a highly concentrated urea solution (1 mol/l) was given chronically as the sole drinking fluid (203). This rise in GFR is probably mediated by vasopressin (see below). Although urea is a less potent stimulus for vasopressin secretion than sodium (257), drinking only a very hyperosmotic urea solution certainly stimulated vasopressin secretion. This observation as well as those showing that a high-protein diet does not raise GFR in rats with hereditary diabetes insipidus (57) suggest that a significant urea load and high vasopressin secretion are both required simultaneously to influence GFR and induce kidney hypertrophy.

In addition to its well-known action on aquaporin-2 (AQP2), increasing the permeability to water of the whole CD, vasopressin also increases the permeability to urea, selectively in the terminal inner medullary collecting duct (IMCD) through its action on urea transporters UT-A1 and UT-A3 (101, 146, 219). This allows the delivery of concentrated urea to the renal papillary interstitium, a critical step in the concentration of
urea in the urine. Transgenic mice lacking UT-A1/3 exhibit a significant urine concentrating defect and show a markedly reduced concentration of urea in the papilla, but a normal sodium concentration (103, 104). Their urine concentrating defect is thus “urea-specific” (253). When fed a low protein diet, resulting in a markedly low urea excretion rate, urine concentration in these knockout mice was similar to that in wild-type mice (103, 104). This observation demonstrates that vasopressin’s action on urea transporters is crucial for the water economy associated with the excretion of urea. Similarly, normal rats or human subjects suffering protein malnutrition are often said to exhibit a “urea concentrating defect.” Actually, it is not a real “defect;” their low urine osmolarity is due only to the low urea content in the urine. Their urine osmolarity rises appropriately after administration of either exogenous vasopressin or urea (133, 145).

Vasopressin’s action on intrarenal urea handling is also crucial for vasopressin’s influence on GFR. A reduction in urine concentrating activity induced by surgical or chemical papillectomy in rats leads to a significant 20–40% reduction in GFR (168, 227, 240, 248) (see Table 3 in Ref. 33). Papillectomy does not reduce vasopressin levels but precedes the usual action of vasopressin on urea permeability in the terminal IMCD.

Vasopressin and GFR: chronic studies. Several studies showed that a chronic infusion of vasopressin or its V2 receptor agonist dDAVP induces a marked rise in GFR (measured by inulin clearance) in Brattleboro rats with hereditary central diabetes insipidus (91, 111) (Fig. 8A) and in normal rats (53). Conversely, treatment for 3 wk with the selective V2 receptor antagonist tolvaptan in patients with autosomal-dominant polycystic kidney disease induced a significant decline in GFR (measured by the 125I-iothalamate infusion technique) (49). This was not the sign of a worsening of kidney function because this decline was fully reversible upon cessation of the treatment. This observation is in good agreement with experiments in rats with CKD induced by 5% nephrectomy. A threefold increase in fluid intake improved kidney function and reduced mortality (54), whereas infusing the V2 receptor agonist dDAVP worsened CKD (56).

Along with the sustained rise in GFR, chronic dDAVP infusion or chronic partial water deprivation induces anatomic and functional changes in the kidney that strictly mimic those induced by a high protein intake (increased heterogeneity between superficial and deep nephrons, thickening of the inner stripe of the inner medulla, increase in volume per unit length, and enzymatic activities of the medullary TAL) (28, 32, 55, 229–231) (see reviews in Refs. 30 and 33).

Bouby et al. (53) studied the chronic influence of different vasopressin levels in conscious normal rats, with GFR measurements based on 14C-labeled inulin infusion through implantable minipumps, and 24-h urine collection. Urine osmolality was altered in opposite directions for 1 wk by either an infusion of dDAVP or an increase in fluid intake achieved by feeding the rats a powered food mixed with a water-rich agar gel. A control group underwent no change (see Table 1). The wide differences in urine flow rate between groups did not perturbate inulin recovery, as shown by the measured 24-h inulin excretion. GFR was markedly higher in response to dDAVP and lower in response to additional fluid intake (that, respectively, doubled or reduced Uosm to half, compared with control rats). Plasma inulin concentration showed reciprocal changes. However, with a further rise in fluid intake and greater fall in Uosm, GFR went up again to the level seen in control rats (“Very high WI” in Table 1). As explained in a recent review (27), this was also observed in humans (15). The influence of vasopressin and/or urine concentration on GFR is biphasic; the relationship between GFR and Uosm exhibits a “J-shaped” curve (27). A rise in GFR with increasing Uosm occurs only above a certain threshold. Actually, a further decline in GFR in a lower range of Uosm, when solute-free water must be excreted, would be disadvantageous because the delivery of fluid and solutes to the diluting segment is a limiting factor for the maximum diluting capacity of the kidney (47).

Thus, in both humans and rats, GFR is positively influenced by vasopressin/low hydration through V2 receptor-mediated actions. Interestingly, the fractional excretion of urea and, to a lesser extent that of other solutes, is reduced when urine flow rate declines as urine osmolality goes up (29, 53). Thus the water economy associated with an improved urine concentrating ability is obtained at the expense of first, a higher GFR, possibly leading to the vicious circle described by Hostetter and Brenner (59, 131, 180), and second, a less efficient excretion of urea (Table 1) and other solutes including sodium (see a review in Ref. 26).
Table 1. Influence of chronic alterations in urine concentration on GFR (measured by 24-h inulin clearance) in normal Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>V, ml/day</th>
<th>Uosm, mosmol/kgH2O</th>
<th>GFR, µl/min</th>
<th>Plasma Inulin, cpm/100 µl</th>
<th>Inulin Excretion, ×10³ cpm/day</th>
<th>Urea Fractional Excretion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>dDAVP infusion</td>
<td>5.6 ± 0.2</td>
<td>3.023 ± 89</td>
<td>1.93 ± 0.10</td>
<td>186 ± 34</td>
<td>8,422 ± 436</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Control (normal WI)</td>
<td>11.1 ± 1.0</td>
<td>1.526 ± 139</td>
<td>1.62 ± 0.16</td>
<td>207 ± 18</td>
<td>9,311 ± 349</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>High WI</td>
<td>20.3 ± 1.5</td>
<td>762 ± 44</td>
<td>1.06 ± 0.10</td>
<td>296 ± 26</td>
<td>8,980 ± 549</td>
<td>118 ± 18</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.002</td>
<td>NS</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Very High WI (2)</td>
<td>41.9 ± 0.9</td>
<td>391 ± 12</td>
<td>1.65 ± 0.13</td>
<td>171 ± 13</td>
<td>8,328 ± 397</td>
<td>75 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE of results based on 2 successive 24-h urine collections on days 6 and 7 after initiation of the different dDAVP or water intake (WI) treatments. V. 24-h urine volume; Uosm, 24-h urine osmolality; GFR, glomerular filtration rate; cpm, counts/min. Inulin excretion refers to 24-h urinary inulin excretion. Data are from Ref. 53. 1, The ANOVA took into account only the first 3 groups; 2, The very high WI group was carried out during the same experiment as the other groups but was not presented in the original paper because it was realized that the influence of the vasopressin/hydration system on GFR and thus solute fractional excretions was biphasic (J-shaped curve; see text), but there were not enough data to support this finding in this experiment. This biphasic GFR-Uosm relationship was also observed later in an acute study in humans (15).

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Table 2. Influence of acute alterations in urine concentration on GFR (measured by inulin clearance) in normal anesthetized Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>V, µl/min</th>
<th>Uosm, mosmol/kgH2O</th>
<th>GFR (inulin), ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>dDAVP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33 ± 3</td>
<td>566 ± 61</td>
<td>2.38 ± 0.15</td>
</tr>
<tr>
<td>Experimental</td>
<td>14 ± 1</td>
<td>1,153 ± 96</td>
<td>2.80 ± 0.22 **</td>
</tr>
<tr>
<td>Dilute saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10 ± 1</td>
<td>1,427 ± 119</td>
<td>2.76 ± 0.27</td>
</tr>
<tr>
<td>Experimental</td>
<td>64 ± 9</td>
<td>301 ± 72</td>
<td>2.05 ± 0.14 *</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 × 20-min clearance periods before (control) and after (experimental) one of the following treatments. Rats exhibiting Uosm < 1,000 mosmol/kgH2O during the control period (n = 12) were infused with dDAVP to increase their Uosm. Rats exhibiting Uosm > 1,000 mosmol/kgH2O during the control period (n = 6) were infused with dilute saline to reduce their Uosm. Data were published in abstract form (34). *P < 0.05, **P < 0.01 by paired t-test.

Vasopressin and GFR: acute studies. The possible influence of acutely administered vasopressin on GFR is controversial (16, 66, 70, 105, 111). Vasopressin is often infused after a prior water load and/or dilute fluid infusion intended to “suppress” endogenous vasopressin (or in Brattleboro rats which lack endogenous vasopressin). However, the resulting high water diuresis completely washes out the urea accumulated in the inner medulla. Several hours are required to restore this medullary urea gradient. As explained below, we think that intrarenal movements of urea play an important role in the mechanism by which vasopressin influences GFR. After an intense water diuresis (or in Brattleboro rats), these urea movements cannot take place quickly enough to allow GFR to rise during short-term experiments.

In an attempt to reevaluate the influence of vasopressin level and/or urine concentration on GFR on a chronic protocol, we took advantage of the spontaneous variations in Uosm observed among normal rats. First, in 74 normal anesthetized Wistar rats prepared for classic clearance experiments, we observed a significant positive correlation between baseline GFR (inulin clearance) during the control period and the simultaneously measured Uosm (r = 0.371, P < 0.01) (22). Second, in anesthetized Wistar rats prepared for classic clearance experiments, we either infused dDAVP into those with a low baseline Uosm (< 1,000 mosmol/kgH2O) or infused dilute saline into those with a high baseline Uosm (> 1,000 mosmol/kgH2O). As shown in Table 2, along with the expected changes in urine flow rate and osmolality, we observed a significant rise in GFR (inulin clearance) in response to dDAVP (which stimulated V2 receptors), and a significant decline in GFR after infusion of dilute saline (which presumably reduced endogenous AVP concentration and thus V2 receptor stimulation) (34).

Figure 8B shows individual GFR values in healthy subjects after an intense water load and/or dilute fluid infusion intended to “suppress” endogenous vasopressin (or in Brattleboro rats which lack endogenous vasopressin). As shown in Table 2, along with the expected changes in urine flow rate and osmolality, we observed a significant rise in GFR (inulin clearance) in response to dDAVP (which stimulated V2 receptors), and a significant decline in GFR after infusion of dilute saline (which presumably reduced endogenous AVP concentration and thus V2 receptor stimulation) (34).
Vasopressin, Glucagon, and Water Economy

No other hormone can compensate the action of vasopressin on urine concentration. The lack of this hormone or the resistance of the kidney to its V2 receptor-mediated actions leads to diabetes insipidus. However, in addition to vasopressin, glucagon also contributes to some water economy. This influence is easily detectable in experimental studies when the level of vasopressin is kept constant (whether absent, low, or high). In elegant micropuncture experiments in rats in which several peptidic hormones acting on the nephron (PTH, glucagon, vasopressin, calcitonin) had been neutralized (creating a "hormone-deprived" model), the infusion of vasopressin or glucagon or both hormones together showed a dose-dependent additive effect of glucagon above that of vasopressin (94). Urine osmolality was 1,242 ± 49 mosmol/kgH2O with glucagon plus vasopressin vs. 936 ± 50 mosmol/kgH2O with vasopressin alone (P < 0.001). In another study, during clearance experiments in anesthetized rats, with vasopressin set at an either low or high level in two different groups (by infusion of dilute saline or dDAVP, respectively), glucagon infusion increased significantly urine osmolality in both conditions (by～340–400 mosmol/kgH2O) and solute-free water reabsorption, along with a marked rise in urea excretion (3). The stimulation of sodium reabsorption in the TAL may account for this effect (94) because an intravenous glucagon infusion in anesthetized rats was shown to enhance the concentration of electrolytes accumulated in the renal medulla (148). Finally, the CD is also a target site for glucagon. In the isolated perfused rat IMCD, glucagon increased water reabsorption in a dose-dependent way, an effect that was partially additive to that of vasopressin (255). Moreover, AQP2 abundance was increased by glucagon in membrane fractions of IMCD (255).

It may be important to recall that vasopressin’s action on the CD dramatically reduces the fractional excretion of urea by favoring urea reabsorption along the CD, both indirectly (due to longer contact time and more intense transepithelial concentration difference along the whole CD) and directly (by its action on UTs in the terminal IMCD) (36, 253). In contrast, glucagon increases the fractional excretion of urea, as described in the preceding section. Thus glucagon contributes to water economy in the excretion of urea and partially compensates the reduced efficacy of urea excretion induced by vasopressin’s action. Jointly, these two hormones ensure the best compromise between an efficient urea excretion and an adequate water economy.

The Case of DM

Plasma glucagon concentration is significantly elevated in both type 1 and type 2 DM (10, 116, 160). Moreover, the kidney of patients with type 1 diabetes exhibits an augmented hyperfiltration in response to an AA infusion (232). It is now well recognized that the metabolic perturbations of DM may be due, in large part, to inappropriate exaggerated glucagon actions (including protein wasting and disturbances in plasma AA concentrations) more than to the lack of insulin secretion or action on its target tissues (8, 19, 67, 129, 233). Accordingly, glucagon receptor antagonists have been proposed as a new potential antidiabetic treatment modality (7, 78, 160, 178, 193, 233, 241). Such antagonists might not only reduce hyperglycemia and other associated metabolic symptoms, they might also protect the kidney as suggested by the following experimental studies. Chronic hyperglucagonemia induced by a glucagon infusion in mice, in addition to producing a type 2 diabetes phenotype, induced a significant kidney hypertrophy and increased 24-h urinary albumin excretion, glomerular mesangial expansion, and extracellular matrix deposition (159). Seney (213) showed that glucagon infusion in normal rats reduced the responsiveness of the TGF feedback system, thereby diminishing TGF-induced restraint on GFR, and permitting GFR to rise (213). In another study, a single injection of a specific glucagon antibody in rats with streptozotocin-induced DM significantly reduced the GFR (without reducing glycemia) in the next 2 h from 4.67 ± 0.18 to 3.49 ± 0.20 ml/min (P < 0.001), whereas a nonspecific antibody had no effect (2). These results strongly suggest that the marked elevation in glucagon concentration seen in diabetic patients may participate in the glomerular hyperfiltration of DM.

Several studies showed that vasopressin plasma concentration is elevated in DM (24, 258). The cause of this elevation is not fully elucidated (211, 259). This elevated vasopressin level helps the diabetic kidney conserve water in the face of an increased urinary solute load due to the overt glycosuria (6). However, it also has adverse consequences in the long term by participating in hyperfiltration. When DM was induced by streptozotocin in Brattleboro rats (devoid of vasopressin), their creatinine clearance and urinary albumin excretion did not rise, as they did in control rats with normal vasopressin secretion, although glycemia increased to the same extent in both groups (43). In rats with normal vasopressin secretion, the selective inhibition of the V2 receptor-mediated actions of vasopressin prevented the rise in albuminuria seen in untreated diabetic rats. Moreover, in these untreated rats, a strong correlation was observed between the daily solute-free water reabsorption (an index of the osmotic work of the kidney) and both the urinary albumin excretion rate and the GFR (42). This shows that hyperfiltration and albuminuria are strongly associated with a more intense concentrating activity of the kidney in this rat model of diabetes.

In healthy humans, the infusion of dDAVP induced a significant rise in albuminuria (41). A similar rise was also observed in patients with either central or nephrogenic diabetes insipidus due to mutations of AQP2. In contrast, dDAVP did not induce any rise in albuminuria in patients with diabetes insipidus due to mutations of the V2 receptor (41). This clearly shows that vasopressin influences albuminuria by its antidiuretic V2 receptor-mediated action, not by the V1a receptors.

In 22 type 1 diabetic patients and 8 control subjects, the intradividual variations in GFR (radio-labeled iohalumate) were evaluated by comparing two clearance studies performed with a mean interval of 3 mo. The changes in GFR between these two measurements were positively correlated with the changes in plasma vasopressin concentration, suggesting that vasopressin may be of importance in the regulation of GFR (188). Several recent epidemiological studies showed that baseline plasma copeptin (a surrogate of vasopressin) (27) was positively associated with a faster decline in estimated GFR or a higher risk of severe renal outcome in patients with DM, independently of other relevant covariates (50, 244). In the DIABe tes, HYpertension, microalbuminuria or proteinuria, CARDiovascular events, and Ramipril (DIABHYCAR) trial, with a 6-yr follow-up, baseline copeptin was measured in 3,101 participants with type 2 diabetes and albuminuria, 729 of 117,679 cases (50).
in patients of

The yearly decline in estimated GFR was 2.5- to 3.0-fold faster provided into three tertiles according to their baseline copeptin. whom had macroalbuminuria at inclusion. Patients were di-

Fig. 9. Relationships between copeptin at baseline (in tertiles) and progression of kidney disease in diabetic patients with albuminuria of the DIABHYCAR study (see the text). A: change in GFR according to increasing copeptin tertiles (T1–T3) in the whole cohort (white bars) and in a subset of 729 patients with macroalbuminuria at baseline (grey bars). B: Kaplan-Meier survival (renal event-free) curves during follow-up by tertiles of plasma copeptin at baseline. Renal events were defined as the doubling of the serum creatinine level or dialysis initiation. Adapted from Ref. 244. *P < 0.05 compared with T1. †P < 0.05 compared with T2.

whom had macroalbuminuria at inclusion. Patients were divided into three tertiles according to their baseline copeptin. The yearly decline in estimated GFR was 2.5- to 3.0-fold faster in patients of tertile 3 than in those of tertile 1 (Fig. 9A). The risk for renal events (defined as the doubling of the serum creatinine levels or initiation of dialysis during follow-up) increased significantly with baseline copeptin (Fig. 9B). In the population-based Malmö Diet and Cancer study cohort, increasing quartiles of copeptin at baseline (the lowest quartile as reference) were significantly and independently associated with microalbuminuria after appropriate adjustments (odds ratios 1.05, 1.08, and 1.65; P for trend = 0.02). Although these epidemiological studies cannot prove a causality link, they suggest that vasopressin has adverse effects on renal function, not only in diabetic rats but also in diabetic patients.

Integrative View

The original findings reviewed in the preceding sections provide abundant evidence for an involvement of glucagon and vasopressin in the hyperfiltration observed in response to protein intake and in DM. Because the secretion of these two hormones is directly stimulated by the intake of proteins, they likely represent the earliest key components in the complex process leading to upregulation of GFR and to the ensuing adverse consequences. Experimental results in rats suggest that an intervention intended to inhibit their action is able to slow CKD progression and prevent or delay diabetic nephropathy (as detailed above). These findings also suggest that the impact of these hormones on kidney function is dependent upon the presence of a significant supply of urea by the liver. By which mechanism does this triad affect glomerular function? Are these two hormones indispensable for hyperfiltration, are they required simultaneously, and are their effects additive? These questions will be addressed below.

Glucagon and vasopressin are not likely to act directly on the glomerulus because a number of studies addressing the localization of their receptors in the kidney have not identified specific receptors in glomeruli nor in the afferent or efferent arterioles. Vasopressin V1a receptors are expressed in arteri-

lar smooth muscle cells and possibly in the mesangium (an issue under debate), but the influence of vasopressin on GFR and albuminuria is clearly dependent on V2 receptor-mediated actions because they are induced by the infusion of the V2 agonist dDAVP and are inhibited by a selective V2 antagonist (as detailed above). dDAVP has very little affinity for V1a receptors. Moreover, the water retention due to dDAVP induces a decline in plasma osmolarity, which lowers endogenous vasopressin secretion and thus makes any possible influence of V1a stimulation insignificant. An indirect action resulting from the tubular actions of these hormones is thus most likely.

TGF regulation of GFR and the possible indirect influence of urea. The GFR is permanently regulated by a TGF mechanism that limits the rate of filtration according to the luminal concentration of sodium and/or chloride perceived by the macula densa, a group of specialized cells located in the terminal portion of the TAL and in close contact with the glomerular arterioles and extraglomerular mesangium. In a series of elegant micropuncture studies, collecting tubular fluid in the late proximal and early distal tubules (Fig. 10A), Seney et al. (213–216) showed that rats on a high-protein diet (40 vs. 6% casein) or an infusion of glucagon have reduced concentrations of Na and Cl in the early distal tubule, leading to a diminished signal for TGF at the macula densa (Fig. 10, B and C), thereby diminishing the TGF-dependent restraint of GFR, thus permitting GFR to rise.

As shown in Fig. 10B, this was attributable to markedly reduced sodium and chloride concentrations in the early distal tubule, the closest accessible site to the macula densa in vivo (44 vs. 63 mmol/l; for sodium and 32 vs. 54 mmol/l for chloride). The lower concentration of NaCl at the exit of the loop of Henle likely results from an enhanced reabsorption of these solutes in the TAL. However, why would the kidney reabsorb more sodium when it needs to excrete more urea? Although not discussed by the authors, this is most probably related to the need for a more intense urine concentrating activity because sodium reabsorption in the TAL is powering the countercurrent multiplication process that creates and maintains the osmotic gradient in the medulla (122).
Another crucial observation in this study has not been given enough attention. The osmolality of the tubular fluid in the early distal tubule was identical in the high- and low-protein diet conditions, despite the marked difference in sodium and chloride concentrations (Fig. 10B). (215). This observation calls for two important comments. First, it shows that the TAL’s diluting ability may be limited by the transepithelial osmolarity difference (between lumen and peritubular fluids), rather than by the transepithelial NaCl concentration difference (as usually assumed). Indeed, when the transepithelial osmotic gradient increases above a certain limit, the TAL is no longer fully water-tight, as shown in several studies (see a review in Ref. 22). Second, it means that there must be another solute filling the “osmotic gap” observed between total fluid osmolality and sodium concentration. No other solute but urea could explain such a large difference, in the context of a high vs. low protein intake (Fig. 10B). We think that, on a high protein intake (Fig. 10A), the presence of more urea allows the “static head” for sodium concentration in the TAL lumen (as defined by Burg) (68) to be reduced to a greater extent. Urea thus represents an “osmotic buffer” in the tubule lumen that allows the TAL epithelium to generate a stronger transepithelial sodium chloride gradient.

This urea addition in the loop of Henle probably takes place in the pars recta of the proximal tubule, as recently reviewed (37). It is known to be quantitatively larger in so-called “antidiuretic” conditions (high vasopressin secretion) (18, 87, 88, 152, 202) and in desert-adapted rodents (239), probably because of more intense and efficient countercurrent exchanges in the medullary vasculature, initiated by the vasopressin-dependent delivery of concentrated urea to the deep inner medulla and by the reduction in medullary blood flow. Glucagon or liver-derived cAMP may also promote this urea addition by its action on the collecting duct and possibly also by stimulating directly urea secretion in the pars recta (see above) (37).

It is not possible to quantify the secretion and/or the recycling of urea in whole animal models or in human studies. However, as explained in the beginning of this review, we think that the U/P ratio of urea concentrations is a valid index of the situation prevailing upstream at the macula densa. This is supported by the strong correlations found between this ratio and the GFR in many independent acute and chronic studies in humans and rats, as shown in Fig. 4.

**Stimulation of TAL transport by glucagon and vasopressin.** As recalled earlier, the TAL is a target site for both glucagon and vasopressin (Fig. 1), where these hormones stimulate sodium reabsorption via the Na-K-2Cl cotransporter (68, 74, 122, 173, 175). They are thus both susceptible to influence the TGF control of GFR. What could be the physiological meaning of this action? The most obvious answer is that both hormones contribute to a faster excretion of water-soluble wastes by allowing a rise in GFR, while minimizing the water requirement necessary for this excretion. Chronic infusion of dDAVP, a selective agonist of V2 antidiuretic receptors, was shown to induce a marked hypertrophy and increased enzymatic activities of the medullary TAL, similar to those induced by a...
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high-protein diet (33). An infusion of glucagon was shown to increase the heat production in the inner stripe of the outer medulla, probably due to an enhanced metabolism in the medullary TAL, the nephron segment accounting for most of the metabolic rate in this zone (136).

The fact that chronic SGLT2 inhibition reduces GFR in diabetic patients (77, 86, 151) and prevents diabetes-induced hyperfiltration and/or albuminuria and mesangial expansion in animal models of diabetes (112, 236, 238) is compatible with the notion that diabetic hyperfiltration is mediated by a depressed TGF. As demonstrated by Vallon et al. (237) in diabetic rats, an increased filtration of glucose brings the kidney to reabsorb more glucose and sodium through the sodium-glucose cotransporter SGLT2 in the proximal tubule. Accordingly, more of the filtered sodium is reabsorbed in the proximal tubule, and the sodium delivery to the TAL is reduced, thus reducing the TGF signal at the macula densa (237). Inhibition of the glucose-associated sodium reabsorption by SGLT2 inhibitors induces a massive sodium overload to the TAL and thus induces an inverse situation.

Are glucagon, vasopressin, and urea simultaneously indispensable for inducing hyperfiltration? A few studies suggest that the two hormones are both together necessary for the protein-induced hyperfiltration. A protein meal does not increase GFR in pancreatectomized patients unable to secrete pancreatic glucagon (89, 106), and in healthy volunteers undergoing a water diuresis that suppresses vasopressin secretion (119). A high protein intake or the induction of DM does not induce an increase in creatinine clearance or a hypertrophy of the kidney in Brattleboro rats unable to secrete vasopressin (43, 57). On the other hand, each of these two hormones may influence GFR independently of one another. A glucagon infusion increases GFR even in the absence of vasopressin, as shown in Brattleboro rats (92, 93), in rats or dogs undergoing water diuresis (3, 5, 183), and in healthy subjects who received a water load before and/or during the investigation (186). dDAVP increases GFR in rats in which glucagon’s effects have been prevented by a somatostatin infusion (91). Of note, no rise in GFR is observed in response to experimental infusion of these two hormones when urea excretion is reduced by a low protein diet (see above).

In conclusion, the large body of data presented in this review show that the increased secretion of vasopressin and glucagon that follows the ingestion of a protein meal cooperate in the formation and excretion of urea and in the associated water economy via a complex series of tubular actions. These actions reduce the TGF signal at the macula densa, thus allowing GFR to rise. Figure 11 displays the main steps of these combined actions. Many studies have shown that a number of other mediators are involved in the protein-induced hyperfiltration (the renin-angiotensin system, prostaglandins, nitric oxide, dopamine, etc., to cite just a few) (64, 65, 126, 130, 170). However, their contribution most likely takes place at steps beyond the initial tubular actions of the two peptidic hormones.

Sustained hyperfiltration is associated with CKD and mortality in the general community (184). Despite major progress during the last two decades in evidence-based diabetes care, including blockade of the renin-angiotensin system, diabetes was the primary cause of renal disease in ~ 40% of the patients with new-onset kidney failure in 2011 in the United States, and hyperfiltration is a risk factor for progression of diabetic nephropathy (205). Besides dietary recommendations to reduce protein intake (114, 157), it is thus critical to explore new protective pathways. Selective blockers of the V2 receptors are being studied in several diseases (27, 191, 217, 228, 245). Their effects on diabetic nephropathy are also worthwhile to investigate. New antagonists of the glucagon receptor are being developed as antidiabetes drugs (Eli Lilly’s LY-2409021 and Ligand’s LGD-6972). According to the role of glucagon reviewed here, we can actually speculate on potential benefits for the preservation of kidney function, beyond the glucose-lowering effect of this new class of drugs, a major advantage in the fight against diabetic nephropathy, a devastating complication of diabetes.

Future Directions

This review has revisited the relationships between protein intake and kidney function, focusing on well-established facts that support a direct role of glucagon and vasopressin in the protein-induced hyperfiltration. However, a number of questions remain unanswered. For example, how does urea enter the loops of Henle, a massive addition well demonstrated to occur somewhere between the late proximal and the early distal tubule? Does it really undergo an active (or secondary active) secretion in the pars recta of the proximal tubule (as suggested by Bankir et al.) (37)? How does plasma cAMP reduce pars recta reabsorption? Does it bind to luminal CAMP receptors (as suggested by Bankir et al.) (23)? If not, are there glucagon receptors in the proximal tubule that are not coupled to adenylate cyclase (as suggested by Unwin and coworkers) (166)? How do cAMP and glucagon (at a physiological concentration) jointly influence GFR (as shown by the studies of Ahloulay et al.) (4)? Do urea and sodium really balance each other in the cortical thick ascending limb to reach a threshold of minimum osmolarity that is relatively the same in all situations (as suggested by the studies of Seney et al.) (213–216)? This would mean that the limiting step for dilution in the TAL is a transepithelial osmotic difference, not a transepithelial sodium concentration difference (as usually believed). Is the urine/plasma ratio of urea a valid index of the urea/plasma ratio at the macula densa and is GFR well correlated with this ratio in all circumstances?

We would like to draw attention here to a few technical points which may explain the previous difficulty to characterize some relevant facts. 1) A number of animal studies and clinical investigations are performed in fasting animals or subjects. Moreover, micropuncture experiments are often performed in rodents totally deprived of food during the preceding night. However, as explained in this review, the influence of glucagon and vasopressin is obviously related to the excretion of wastes that derive from protein intake and the associated water economy. Thus their influence is likely to be minimal in the fasting state. 2) A water load or a saline infusion is often administered before and during the experiments. This lowers the secretion of vasopressin and will prevent the disclosure of its possible influence on the kidney. 3) The infusion of saline, leading to increased sodium excretion, will markedly reduce the urea-to-sodium ratio in the urine, already lowered by fasting. However, several studies show that both vasopressin and a significant urea load are needed simultaneously to influence GFR. A reduced urea/sodium ratio will lessen the effect on GFR. 4) Most in vitro studies of isolated perfused TALs and
CDs use bath and perfusate fluids devoid of urea, despite the fact that urea is the dominant solute in the urine and reaches concentrations 10- to 100-fold higher in these tubule lumen than in the plasma when they are in situ. The possible influence of urea as an osmotic buffer in the lumen is thus totally excluded in the in vitro studies.

A number of previously carried investigations could be performed again, taking into account the concerns raised above. Studies of renal function in subjects bearing a mutation of the glucagon receptor (226) should also be informative if the protocol includes a protein meal or an AA infusion. In vitro perfusion of isolated pars recta of mice or rats (rather than rabbits, the only species studied so far) could reveal an active secretion of urea, especially after a high-protein diet or an acute stimulation by glucagon. Mice with selective knockout of the glucagon receptor are available and have been used for studies addressing the metabolic actions of glucagon (121, 247). Up to now, to our knowledge, no study in these mice addressed glucagon actions on the kidney. Experiments could especially use mice with conditional knockout of the glucagon or the vasopressin V2 receptor, or alternatively rats (in which GFR is easier to measure) to which selective antagonists of these receptors could be infused. For the role of urea, rats infused with selective UT-A antagonists (98) would also be useful.

In conclusion, we hope that this extensive review of the available literature will provide a useful background for future studies and will generate provocative new hypotheses. It should help investigators revisit these issues with new protocols and ideas.

APPENDIX A: INTERSPECIES COMPARISONS

When extrapolating experimental studies in laboratory animals to humans, one should take into account several important differences. First, rodents exhibit special morphological and functional adaptations that allow them to concentrate urine to a much higher level than do humans. Moreover, because of known allometric differences, these small animals (about 300 g for rats and 30 g for mice) have a much higher load of osmoles (including nitrogen wastes) to excrete per unit body weight and per unit kidney weight than do humans (36, 253). Dogs are carnivores naturally adapted to a high-protein diet and to infrequent large meals. They have 100% long looped-nephrons (important for the concentrating process in the inner medulla) compared with only 30–40% in rodents and probably only 20% in humans (31).
Finally, the protein content of the diet can be manipulated within a much larger range in experimental animals (typically 6 vs. 40 or even 50% protein in a rodent’s diet) (i.e., a more than a 6-fold difference) than in humans (for example, in the MDRD study, 0.58 vs. 1.3 g protein·kg body weight\(^{-1}\)·day\(^{-1}\), only a 2-fold difference) (144), making animal findings caricatural compared with what can be obtained in clinical investigations.

**APPENDIX B: VASOPRESSIN AND NITROGEN METABOLISM**

Nitrogen handling may be influenced by vasopressin not only in the kidney but also in the liver. In the kidney, vasopressin exerts its influence on urea handling mainly by its binding to V2 receptors. In the liver, hepatocytes express abundant V1a vasopressin receptors, and this is why the rat liver was used to clone the first vasopressin receptor by expression cloning in 1992 (174). However, only little attention was paid for many years to the possible influence of vasopressin on liver metabolism. In isolated hepatocytes or in the isolated perfused liver, vasopressin stimulates gluconeogenesis and ureagenesis from AAs, as does glucagon, but via another second messenger (90, 123, 143, 167, 187, 223). The effects are thus likely additive. An infusion of vasopressin in humans induces a transient rise in glycemia (223). However, to our knowledge, no study evaluated whether the plasma vasopressin level prevailing in usual life in response to osmotic stimuli is effective in liver metabolism. Recent epidemiological studies suggest it is the case. Some V1a receptor polymorphisms (95) or the plasma level of copeptin (96) (a surrogate marker for vasopressin) is associated with a greater prevalence or an increased risk of DM, respectively. A low water intake, presumably associated with a high vasopressin secretion, is associated with a higher risk of developing hyperglycemia in subjects from a general population over a 9-yt follow-up (204). These associations suggest that vasopressin may indeed influence urea metabolism in humans.

**APPENDIX C: ROLE OF ANP IN THE HYPERFILTRATION OF DIABETES**

Besides vasopressin and glucagon, another peptide hormone, atrial natriuretic peptide (ANP), may also participate in the hyperfiltration of DM. Some studies (but not all) (76) suggest that ANP is also elevated in DM (141, 182, 192), but the primary, physiological mechanism that could be responsible for this elevation is not elucidated. A protein meal does not seem to stimulate ANP secretion (177, 256). Studies in diabetic rats showed that abolishing ANP actions by either a specific antibody (182) or a specific receptor antagonist (141, 260) attenuates the hyperfiltration observed in the diabetic control group. ANP possesses specific receptors in the glomeruli and may thus influence GFR by a direct action, contrary to vasopressin and glucagon. However, interestingly, ANP receptors are also heavily expressed in the terminal IMCD. Although no double labeling study is available to our knowledge, there is a strong likelihood that these ANP receptors are colocalized in the terminal IMCD cells with the vasopressin-sensitive urea transporters UT-A1 and UT-A3 because a single cell type seems to be present in the terminal IMCD. Thus ANP might influence urea transport at this site, as previously discussed (38). Actually, Rocha and Kudo (201) showed in isolated perfused rat IMCD segments that ANP selectively inhibits vasopressin-stimulated lumen-to-bath urea transport (the direction in which this transport occurs in vivo) but not the bath-to-lumen urea transport or the basal urea permeability. Altogether, these results show that ANP probably plays a role in the hyperfiltration of DM and may interfere with urea handling in the renal medulla. However, whether there is a link between these two observations is unknown.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: L.B. provided conception and design of research; L.B. and N.B. analyzed data; L.B. prepared figures; L.B. drafted manuscript; L.B., R.R., and N.B. edited and revised manuscript; L.B., R.R., and N.B. approved final version of manuscript; R.R. and N.B. interpreted results of experiments.

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