Glucagon actions on the kidney revisited: possible role in potassium homeostasis

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GLUCAGON ACTIONS ON THE KIDNEY REVISITED.

POSSIBLE ROLE IN POTASSIUM HOMEOSTASIS

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Abstract

It is now recognized that the metabolic disorders observed in diabetes are not, or not only due to the lack of insulin or insulin resistance, but also to elevated glucagon secretion. Accordingly, selective glucagon receptor antagonists are now proposed as a novel strategy for the treatment of diabetes. However, besides its metabolic actions, glucagon also influences kidney function. The glucagon receptor is expressed in the thick ascending limb, distal tubule and collecting duct, and glucagon regulates the transepithelial transport of several solutes in these nephron segments. Moreover, it also influences solute transport in the proximal tubule, possibly by an indirect mechanism. This review summarizes the knowledge accumulated over the last 30 years about the influence of glucagon on the renal handling of electrolytes and urea. It also describes a possible novel role of glucagon in the short term regulation of potassium homeostasis. Several original findings suggest that pancreatic alpha-cells may express a "potassium sensor" sensitive to changes in plasma K concentration, and could respond by adapting glucagon secretion that, in turn, would regulate urinary K excretion. By their combined actions, glucagon and insulin, working in a combinatory mode, could ensure an independent regulation of both plasma glucose and plasma K concentrations. The results and hypotheses reviewed here suggest that the use of glucagon-receptor antagonists for the treatment of diabetes should take into account their potential consequences on electrolyte handling by the kidney.

Keywords

Sodium, Calcium, Cyclic AMP, Membrane receptor, Electrolytes, Gluconeogenesis

Abbreviations

AQP	Aquaporin
AVP	Vasopressin
CCD	Cortical collecting duct
CD	Collecting duct
CTAL	Cortical thick ascending limb
DCT	Distal convoluted tubule
FE_{urea}	Fractional excretion of urea
GFR	Glomerular filtration rate
GPCR	G protein-coupled receptor
IC	Intercalated cell (of the collecting duct)
IMCD	Inner medullary collecting duct
MTAL	Medullary thick ascending limb
NCC	Sodium-chloride cotransporter
OMCD	Outer medullary collecting duct
PC	Principal cell (of the collecting duct)
PDte	Transepithelial potential difference
Plasma-K	Plasma potassium concentration
PTH	Parathyroid hormone
TAL	Thick ascending limb
TGF	Tubulo-glomerular feedback
TPTX	Thyroparathyroidectomy
TTKG	Transtubular potassium gradient
UT	Facilitated urea transporter

Introduction

Glucagon has been a matter of great interest in the 1960-80s (see reviews (37, 101)). Glucagon's metabolic actions on the liver were shown to be mediated by cyclic AMP as a second messenger (145). During this period, most functional investigations in animals and humans included the measurement of both insulin and glucagon in plasma as it was understood that several metabolic regulations depended on the insulin/glucagon ratio (63, 135, 165, 192, 193). The interest in glucagon progressively declined and most clinical and experimental studies later focused only on insulin. This may be due to the fact that glucagon is not very stable *in vitro* and that antibodies, raised for the radio-immuno assay, are not always specific enough. Moreover, the deficit in glucagon secretion observed in pancreatectomized patients did not have significant functional consequences, contrary to the deficit in insulin. A recent comprehensive review addressed the history of glucagon discovery and its role in glucoregulation (5).

Besides its effects on the liver, glucagon was also known to influence kidney function. Several studies showed that it increases glomerular filtration rate (GFR) and natriuresis *in vivo* (84, 105, 137, 191), and stimulates adenylate cyclase in kidney homogenates *in vitro* (125) and nephrogenic cAMP release *in vivo* (162). François Morel and his group (118), using miniaturized techniques, provided detailed information about the sites of action of various peptidic hormones along the different nephron segments (118). In 1980, they revealed that the distal tubule and the collecting duct are specific target sites for glucagon (13). In subsequent years a number of functional studies explored the biological actions of glucagon on the nephron (see details further).

After 1996, glucagon-like peptide 1 became the focus of much attention to nephrologists while the interest in glucagon almost vanished. The two molecules originate from the same precursor (114), but are secreted by different organs, possess different receptors, and exert different actions on peripheral organs including the kidney (166, 171). The present review focuses only on glucagon.

A new interest in glucagon has arisen when it was rediscovered that the metabolic disorders observed in diabetes mellitus (DM) are not, or not only due to the lack of insulin or to insulin resistance of the target tissues, but to elevated glucagon levels (6, 66, 103, 106, 107, 164, 194). This adverse role of glucagon, already

identified a few decades ago (29, 62), was revived by new findings in mice with selective deletion of the glucagon receptor. These mice exhibit a reduced blood glucose concentration (74, 173) and fail to become hyperglycemic after streptozotocin administration (61, 100, 134, 198). New non-peptide glucagon receptor antagonists (56, 77, 108, 156, 170, 196, 205) brought significant improvement in animal models of diabetes or obesity (36, 85, 122, 123, 128, 202), and induced significant reduction of glycemia, HbA1c, or glucose production in healthy subjects or diabetic patients (88, 89, 143). This recent pharmacological breakthrough revived the interest in glucagon (12, 43, 103). However, none of these new studies and reviews addressed the effects of glucagon on the kidney. If glucagon antagonists may become new drugs for the treatment of DM, it is important to keep in mind glucagon actions on this other well-identified target organ, the kidney.

A review addressing the influence of glucagon on glomerular filtration rate (GFR) and its role in the hepatic synthesis of urea and disposal of nitrogenous endproducts has been published recently (19). The purpose of the present review is to summarize and synthetize the effects of glucagon on the renal tubule, especially on the transport of electrolytes, and to propose a new hypothesis about the possible role of the alpha cell and glucagon in the regulation of potassium homeostasis.

Glucagon receptors and glucagon actions along the different nephron segments and collecting system

1. Localization of glucagon receptors

The glucagon receptor is functionally linked to adenylyl cyclase (158). It belongs to the family of G-protein coupled receptors (GPCR) with the typical seven transmembrane domain structure (83), and no splice variants (30). The glucagon receptor mRNA is abundant in liver and is expressed to a lower extent in pancreas, heart, adipose tissue, adrenal glands, spleen and brain (44, 76, 182). The kidney is also an important site of glucagon receptor expression where it reaches 30-50% of the expression measured in liver (44, 76, 182). At the protein level, one study suggested that the canine renal glucagon receptor may be larger (12 kDa) than that expressed in the liver (82). Despite this high expression level, the kidney has been regarded at first as a modestly glucagon-sensitive organ. In homogenates of human kidney (cortex or medulla), the incubation of 1 µM glucagon resulted in a modest (a

mere 20%) stimulation of the adenylyl cyclase activity (90, 125). However, taking into account the high diversity of epithelial cell types along the nephron, Bailly et al. (13) measured the production of cAMP in well-identified segments of the nephron and collecting system isolated by microdissection to identify the sites of action of glucagon (**Figure 1A).** They found that the proximal tubule and thin limbs were insensitive to glucagon whereas in the medullary and cortical thick ascending limbs (MTAL and CTAL, respectively) glucagon led to a 35 to 60-fold increase of adenylyl cyclase activity. In the distal convoluted tubule (DCT) and cortical (CCD) and outer medullary collecting duct (OMCD), the sensitivity, although less intense, remained very significant (10 to 20-fold increase compared to untreated tubules) (13). The stimulation of adenylate cyclase by glucagon in the TAL and CD was also observed in Brattleboro rats with diabetes insipidus, devoid of vasopressin (189). Using a similar approach for studies of the inner medullary collecting duct (IMCD), contradictory results have been reported. Maeda et al. did not observe cAMP production after incubation of rat IMCD with glucagon (in the presence or absence of AVP) (109) whereas Yano et al. obtained an almost 60-fold increase of cAMP production after glucagon treatment of rat IMCD (206). Their study also showed a PKA-dependent glucagon-mediated increase in water permeability in this segment.

Experiments using a radiolabelled ligand (31) confirmed the functional localization of glucagon receptors along the rat nephron **(Figure 1B)**. The TAL and to a lesser extent distal nephron segments exhibited a strong specific binding whereas the proximal tubule remained negative, with however a strong non-specific binding. Of note, Marks et al. found glucagon receptor mRNA in the proximal tubule and showed that glucagon stimulates glucose uptake in proximal tubule brush-border membrane vesicles via a facilitative GLUT mediated transport process (112). They proposed that the glucagon receptor expressed in the proximal tubule may be coupled to a signaling pathway distinct from that expressed in more distal nephron segments.

Results inferred from transcriptomic approaches developed in human and mouse nephron segments confirmed the absence or very low expression of glucagon receptor mRNA in proximal tubules, its main expression in TAL and its presence in DCT and collecting duct (34, 35). Interestingly, among the hundreds of GPCR expressed in mouse distal and connecting tubule and collecting duct, the glucagon receptor is one of the most strongly expressed (147). The presence of the glucagon receptor, differentially expressed in precise nephron segments, argues for a role of this hormone in the regulation of segment-specific solute transport.

2. Effects of glucagon on the excretion of the different urinary solutes

The effects of glucagon on renal hemodynamics and on urinary ion and water excretion have been investigated very early after the discovery of this hormone. In the late fifties, Staub et al. described that GFR and excretion of different ions including K^+, Na^+, Cl^- and Pi were increased after glucagon infusion in dogs (50). These observations were confirmed a year later in humans (51). At that time, it was not clear whether the action of glucagon on renal electrolyte handling was a direct effect on the kidney or originated from regulation of tubular functions secondary to the increase in GFR. To start resolving this question, unilateral infusions of glucagon in one renal artery were performed in dogs (149). This protocol confirmed a direct effect of glucagon by demonstrating unilateral increases in Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} excretion in the treated *vs* non-treated kidney. However, the doses of glucagon used in many studies were supraphysiologic (0.1-5 µg per min) and a few subsequent works using more physiologic doses of glucagon failed to demonstrate an effect on renal Na^+, K^+, Cl^- and Pi excretion (25, 116, 169). These negative results may, in part, be due to the confounding influence of other hormones or to changes in urine flow rate.

De Rouffignac et al. studied the influence of a moderate dose of glucagon (5 ng/min per 100g body weight) in so-called "hormone deprived" rats (161). Because several hormones were known to stimulate adenylate cyclase in the same nephron segments, these authors used Brattleboro rats with hereditary central diabetes insipidus, (unable to secrete vasopressin due to single base deletion in the vasopressin gene), thyroparathyroidectomized (to suppress PTH) and infused with somatostatin (to inhibit glucagon and calcitonin secretion). As shown in **Table 1**, the glucagon infusion induced a modest decline in the fractional excretion of sodium, a massive decline in that of calcium and magnesium, and a very significant increase in the excretion of phosphates (161).

Ahloulay et al. (2, 4) investigated in normal rats the effects of 3 different doses of glucagon (1.20, 12 or 120 ng/min per 100g body weight). Plasma glucagon concentrations reached 2-3, 5-6 or 35-times the basal level, respectively, therefore remaining in the physiological range of peripheral (first dose) or portal blood concentrations. Interferences due to uncontrolled variations of urinary flow rate were

prevented by clamping the level of vasopressin. These well-controlled conditions allowed Ahloulay et al. to reveal two different modes of action of glucagon on the nephron (4). A direct and rapidly reversible effect on glucagon-sensitive nephron segments, influencing the excretion rate of of Ca^{2+} and Mg^{2+} (both being reabsorbed) **(Figures 2A, 2B)**. And a slowly reversible, and most likely indirect effect increasing Na^+, Cl^-, Pi **(Figures 2C, 2D, 2E),** likely secondary to a prior action of glucagon on the liver (release of cAMP in the blood) as shown in a later study (3, 4) (see review in (17)). Regarding K^+, glucagon infusion induced a rapid and promptly reversible secretion (4) that was also observed during micropuncture of the loop of Henle but not by microperfusion of isolated TAL **(Figure 3A-C), (**see details further**).** Interestingly, glucagon also increased urea excretion rate (**Figures 4A, 4B**) and promoted water reabsorption, inducing an increase in urine osmolality (**Figures 4C, 4D)** (2) (see details further).

To our knowledge, only one study investigated the influence of a chronic glucagon administration on urinary electrolyte excretion (18). Glucagon (100 µg/d) infused i.p. in normal rats by osmotic implantable minipumps for three weeks raised basal plasma glucagon concentration three-fold. Measurements during the last three days showed a 14 % lower creatinine clearance ($p < 0.05$), a 23 % higher urine osmolality and 24 % lower urine flow rate ($p = 0.05$), indicating a significant enhancement in water economy. The fractional excretion of Na^+, Cl^-, and K^+ was not significantly altered, but that of Ca^{2+} and Mg^{2+} was lowered by 29 and 27 %, respectively, in glucagon-treated rats than in control rats (18). In the whole, these results suggest that glucagon influences divalent ion excretion in the TAL and urine concentration chronically in the same way as it does acutely (see further).

Glucagon is also a significant player in the regulation of the acid-base balance. Paillard and coll. showed, in thyroparathyroidectomized (TPTX) rats subjected to hypotonic volume expansion, that an infusion of glucagon (inducing a 3- or 6-fold increase in its plasma level) induced a marked, dose-dependent increase in bicarbonate excretion (up to 4-fold) and decrease in titrable net acid secretion. As a result, urine alcalinization rose by 0.6 unit of pH (41, 116). These effects were not observed in non-TPTX rats or in TPTX rats subjected to isotonic volume expansion. These results show that physiological increments in plasma glucagon concentration decrease urinary acidification by affecting the tubular proton/bicarbonate transport, an effect not detectable in the presence of PTH and blunted by high circulating antidiuretic hormone. This highlights the pluri-hormonal control of acid-base balance (131).

The regulatory effects of glucagon on urinary solute excretion have also been observed in humans. Friedlander et al. studied the influence of glucagon, within a physiological range (i.e., a 4-fold increase in its plasma concentration), in 8 young healthy volunteers (59). As shown in **Figure 5A and 5B**, glucagon induced a significant decline in both the absolute and fractional excretion of calcium and magnesium. Simultaneously, urinary pH and bicarbonate excretion increased (by 0.5 units, and 4-fold, respectively, $p<0.001$ for both), resulting in a significant marked decline in net proton excretion ($p<0.02$). All these effects are similar to those observed in rat or dog studies.

Altogether these observations clearly established that glucagon exerts multiple effects on urinary solute excretion in experimental animals and humans. Because of the very specific localization of glucagon receptors along the nephron, this global influence may result from several independent effects on different nephron segments. Moreover, the absence of a global effect on a given solute may hide opposite effects of glucagon on different segments. Detailed information about the influence of glucagon on the different nephron segments and sub-segments has been obtained by two main techniques. 1. *In vivo* micropuncture allowing collection of tubular fluid with micro-pipettes inserted in different accessible sites along the nephron at the surface of the cortex or papilla. 2. *In vitro* microperfusion of well identified nephron and collecting duct segments or sub-segments isolated by microdissection. The main results of these studies are detailed below and summarized in **Figure 6**.

3. Effects of glucagon on the different nephron segments

Proximal tubule

In "hormone-deprived rats" (see above), glucagon infusion decreased Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-} and water reabsorption in the proximal convoluted tubule (**Table 1**), and strongly inhibited inorganic phosphate reabsorption in the loop of Henle (that is between the late proximal and early distal tubules), an effect that resulted in a three-fold increase in the fractional and absolute excretion of phosphate, and took place in the pars recta of the proximal tubule (161). The absence of glucagon-sensitive adenylyl cyclase stimulation in this segment suggest either the existence of another

type of glucagon receptor or a coupling of the receptor to another signaling pathway, as suggested by the observations of Marks et al. (112) (see above).

Another explanation for the *in vivo* action of glucagon on the proximal tubule has been provided by Ahloulay et al (3) (17). These authors hypothetized that the well demonstrated glucagon-induced cAMP release from the liver into the blood (26, 79, 175, 177) was responsible for the response of the proximal tubules. Indeed, an i.v. infusion of cAMP induced the same effects as did glucagon at a dose susceptible to reproduce the plasma concentration prevailing in the liver (about 10-fold higher than in peripheral blood). Bankir et al (17) then proposed that filtered cAMP, binding to the brush-border of proximal tubules (57, 81), could trigger the inhibitory effect on Na^+, Cl^-, phosphate and water reabsorption, effects similar to those induced by PTH. Actually, some similarity has been found between the human PTH receptor and G-protein coupled cAMP receptors expressed in unicellular organisms, suggesting that the PTH receptor could possess a specific binding site for cAMP (17). This hypothesis deserves further investigation.

Thick ascending limb

Although its two parts, the medullary and the cortical thick ascending limb (MTAL and CTAL, respectively), share some physiological functions, they display a different interstitial environment and different regulatory processes. For instance, parathyroid hormone (PTH) increases cAMP production (118) and active Ca^{2+} reabsorption in CTAL but not in MTAL (180). In terms of solute transport, these segments play a crucial role for ion transport through a transcellular (Na^+, Cl^-, K^+, HCO_3^-, NH_4^+) or a paracellular (Mg^{2+} and Ca^{2+}) pathway (see recent review (121)). Regulation of the transcellular pathway affects the transepithelial potential difference (PDte) as measured in isolated microperfused tubules. The incubation of isolated mouse MTAL with 1µM glucagon increased the PDte by 33% (183). This result was later confirmed in mouse MTAL and CTAL with a much lower concentration of glucagon (10 nM) (42, 203).

The modification of the PDte reflects a change in ion movements through TAL cells, indicating that glucagon interferes with the transcellular pathway. Using the same technology but performing fluxes measurements, Di Stefano et al. reported that glucagon (10 nM) reversibly increased Na^+ and Cl^- reabsorption in both MTAL (by 30-40%) and CTAL (by 10%) (**Figure 5D**) (42). A stimulated reabsorption of Cl^- has also been reported in rat MTAL after incubation with different doses of glucagon (8). This

reabsorption of Cl^- was shown to be sensitive to furosemide and ouabain, another evidence that glucagon stimulates the transcellular pathway. However, in this study, Ando et al. did not observe modifications of the PDte as shown in mouse MTAL (8). Moreover, despite the observation that glucagon induced intracellular cAMP production in rat MTAL, these authors proposed that the glucagon-induced stimulation of Cl^- reabsorption did not depend on cAMP because dibutyryl-cAMP or forskolin did not induce Cl^- reabsorption.

Regarding the paracellular pathway in mouse CTAL, Di Stefano et al. also reported a rather strong stimulation of the Mg^{2+} and Ca^{2+} reabsorption (by 60% and 140%, respectively) (**Figure 5C**) (42). Interestingly, mouse MTAL did not exhibit Ca^{2+} or Mg^{2+} fluxes either before or after incubation with glucagon, an observation similar to the effect of PTH. As for K^+, these authors did not observe any effect on K^+ flux whatever the segment, as illustrated in **Figure 3C**.

In vivo micropuncture experiments, comparing late proximal and early distal tubular fluids, preserve the physiological environment of the tubules but do not allow the precise identification of the nephron segment responsible for the observed effects because the proximal straight tubule, the thin limbs and the whole TAL are investigated simultaneously. Using this approach in hormone-deprived rats, several studies showed that an infusion of glucagon induced a dramatic increase in Ca^{2+} and Mg^{2+} reabsorption and a lesser increase in Na^+, Cl^- and K^+ reabsorption (14, 42, 161) (**Table 1 and Figures 3B, 5C**). These effects are consistent with those observed by *in vitro* microperfusion of TAL. However, the marked increase in K^+ reabsorption observed during perfusion of the entire loop of Henle (**Figure 3B**) cannot be attributed to the TAL (**Figure 3C**). It is therefore likely that the glucagon-dependent reabsorption of K^+ observed by micropuncture originates from the proximal straight tubule (161).

The TAL also participates in the regulation of acid-base homeostasis. By *in vitro* microperfusion, the rat CTAL was shown to reabsorb bicarbonate and ammonium through a transcellular pathway involving different transporters such as Na^+/H^+-exchangers (NHE3 and NHE4) and the Na^+,K^+,Cl^--cotransporter (NKCC2) (64). By *in vivo* micropuncture in the end-proximal and early distal tubules of the same nephrons in hormone-deprived rats, Mercier et al. showed that glucagon reduced bicarbonate reabsorption in Henle's loop, leading to a 45 %-increase in bicarbonate delivery to the early distal tubule (116). This effect was further confirmed and completed by David Good who showed that incubation of isolated microperfused rat MTAL with 2

nM of glucagon lead to a 35 % reduction of bicarbonate reabsorption (65). The CTAL was not studied in these experiments.

Distal convoluted tubule

To our knowledge only one study addressed the effects of glucagon on DCT. By micropuncture of early and late distal tubules in hormone-deprived rats, Bailly et al. compared the effects of glucagon and PTH on electrolyte transport in hormone-deprived rats (15). This study showed that glucagon stimulates Ca^{2+} and Mg^{2+} reabsorption in DCT independently of the loads delivered to the distal tubules or the plasma concentrations of Ca^{2+} or Mg^{2+}. But glucagon did not induce any significant effect on Na^+ and Cl^- transport. K^+ secretion was stimulated after glucagon treatment, however, it is not clear whether this effect was due to a direct action on DCT cells or to the lower K^+ load at the entry of the DCT and the higher tubular flow (111).

Collecting duct

CDs traverse the whole kidney along its cortico-medullary axis and are usually segmented into cortical, outer medullary, and inner medullary CDs (CCD, OMCD, and IMCD, respectively). These sub-segments are surrounded by different interstitial and vasculo-tubular environments. They display some similar and some specific functions. Noteworthy, the CD exhibits a distinct cellular heterogeneity with at least 3 different cell types, the principal cells (PC), the a-intercalated cells (a-IC) and the b-intercalated cells (b-IC). Here again, each cell type is involved in specific functions. Up to recently, the reabsorption of Na^+ and water was attributed to PC whereas a-IC were involved in acid excretion and b-IC in base excretion. This dogma has been revisited recently with the discovery of paracrine crosstalk between PC and IC (69), and also with the ability of IC to participate in Na^+ and Cl^- reabsorption (104).

To understand the role of glucagon on CD function, it is fundamental to identify the cell types expressing its receptor. However, to our knowledge, the actual cellular localization of the glucagon receptor (determined by immunolabelling, or *in situ* hybridization, etc…) has not been reported for the CD. The opposite responses induced by adrenergic or cholinergic agonists on the production of cAMP in the rat kidney provide some clue. The production of cAMP induced by vasopressin, but not that induced by glucagon, is antagonized by adrenergic agonists in the rat OMCD (33). Conversely, carbachol, a cholinergic receptor agonist, decreases the cAMP response to glucagon but not that to vasopressin (32). These results suggest that

vasopressin and glucagon receptors are expressed in PCs and ICs, respectively. However, searching the transcriptomic data obtained from mpkCCD, a model of murine cultured PC, we did not find the mRNA encoding the glucagon receptor (157). It is possible that these cultured cells no longer express the glucagon receptor (although they still express the vasopressin receptor). In isolated rat IMCD, a structure that comprises a single cell type, the IMCD cell (somewhat different from PC) (96), glucagon was found to modify water and urea permeabilities (206, 207),. More direct and unambiguous studies are clearly required to identify more precisely the cell type(s) responding to glucagon along the CD.

Regarding the functional effects of glucagon on transport properties of CCD or OMCD, the data available are also rather poor. There is apparently no report describing the effect of glucagon on Na^+, Cl^- or K^+ transports in isolated microperfused CCD or OMCD. Laroche-Joubert et al. demonstrated that the incubation of rat OMCD with 1 µM of glucagon activated the H,K-ATPase type 2 only when rats were fed a low potassium diet, i.e., a specific condition in which this transporter is expressed (98). This action of glucagon could promote K^+ reabsorption, at least during potassium restriction, but flux measurements that could confirm this possible effect are lacking.

More recently, the group of Magaldi investigated the action of glucagon on IMCD and more particularly, its role on urine concentration processes. They showed that water and urea transport in IMCD is inversely regulated by glucagon (water being more reabsorbed whereas urea being more excreted) (206, 207). These modifications are related to changes in AQP2 and urea transporter (UT-A1) protein abundance. In view of the short incubation time (30 min) with the hormone, glucagon probably affects the stability/degradation of AQP2 and UT-A1 and not their expression.

4. Effects of glucagon on ureagenesis, urea excretion, renal gluconeogenesis, and urine concentration

As explained in greater detail in a separate review (19), glucagon secretion is not only triggered by hypoglycemia for stimulating gluconeogenesis during fast. It is also triggered by the ingestion of a protein meal (or an infusion of amino acids) even if there is no need for increased gluconeogenesis (9, 27, 54, 86, 130). Actually, glucagon is a potent stimulus of the ornithine-urea cycle in the liver, that allows the

synthesis of urea, the end product of protein catabolism (95, 117, 120, 139, 172, 187, 197, 204). Glucagon markedly stimulates the excretion of urea that originates from the consumed amino acids (2, 4, 7), as illustrated in **Figures 4A and 4B.** Changes in plasma glucagon concentration in healthy humans are associated with inverse changes in plasma amino acid concentration (22) and patients with glucagonoma exhibit a marked hypoaminoacidemia (124). Adaptation to a high protein diet in rats induces a decrease in the insulin/glucagon ratio and a rise in the enzymes involved in gluconeogenesis (141). Actually, glucagon stimulates simultaneously in a coordinated fashion gluconeogenesis and ureagenesis from amino acids (110, 139, 168) to ensure disposal of the nitrogen atoms because there is no significant nitrogen storage in the body. Thus, in normal life, one of glucagon's main role is associated with nitrogen metabolism and excretion (19). In this situation, gluconeogenesis occurs even in the absence of a glucose need. The newly formed glucose can be metabolized for energy storage or consumed in postprandial thermogenesis.

Several observations suggest that urea could be actively secreted (a process requiring energy) in the pars recta of the proximal tubule, although the transporter responsible for this secretion is not yet identified (20); (99). Glucagon was shown to increase the absolute and fractional excretion of urea (FE_{urea}) in anesthetized rats (2, 4, 93) by a mechanism that has not been clarified yet. This effect might result from a glucagon-induced stimulation of this active secretion, or from a reduction of urea reabsorption in the proximal tubule due to the influence of liver-derived cAMP (see above). It may also result from a lesser urea reabsorption in the IMCD because glucagon has been shown to reduce the expression of the urea transporter UT-A1 in this segment (207).

Glucagon also participate in the concentrating activity of the kidney by an effect additive to that of the antidiuretic hormone vasopressin (AVP) (19). **Figures 4C and 4D** show that, in rats with experimentally-induced high or low vasopressin levels (corresponding to urine flow rates of 10 or 75 ml/min, respectively), glucagon increased urine osmolality and free water reabsorption along with a very significant increase in urea excretion rate (2). In a rat model devoid of vasopressin, the Brattleboro rat with hereditary central diabetes insipidus (see above), the infusion of glucagon at 1 or 10 ng/min, along with vasopressin, induced a dose-dependent rise in urine osmolality above that induced by vasopressin alone (47). This improvement in urine concentrating ability probably results from an increased accumulation of electrolytes in the inner medulla (94) resulting from the stimulation of NaCl reabsorption in the MTAL (see above) and an increase in AQP2 expression in the

IMCD (206). A more efficient intrarenal urea recycling and urea accumulation in the medulla may also be involved because a chronic infusion of glucagon was shown to double the abundance of UT-A2 mRNA, the urea transporter expressed in the TDL, without any change in other urea transporters expressed in the kidney (188). It may also involve an intrarenal Cori cycle (with glucose and lactate opposite movements between the outer and inner medulla) possibly stimulated by glucagon, as proposed recently, but not yet proven (20). In any case, the results observed after glucagon infusion in normal rats by different authors show that **glucagon promotes the excretion of urea in conjunction with a significant water economy**.

Because some gluconeogenesis occurs in the kidney, it was interesting to evaluate if glucagon stimulates this metabolic process in the kidney, as it does in the liver. Roobol et al found that, in rat kidney cortex slices, glucagon increased glucose formation by 50-80 % from various substrates, in the presence of 0.25 mM calcium (159). More recently, Mutel et al showed that glucagon-stimulated renal gluconeogenesis contributed to maintain plasma glucose concentration in mice in the absence of hepatic gluconeogenesis (126). However, no such effet had been observed in normal dogs (72). Glucagon influence on lipid metabolism in the kidney is poorly known. An old study showed that glucagon reduces the incorporation of acetate into fatty acids in the kidney, as it does in the liver and heart (92). Further studies are obviously required to evaluate if glucagon really influences proximal tubule metabolism in addition to its effect on electrolyte and urea transport.

Possible role of the apha cell and glucagon in potassium homeostasis

1. Possible contribution of glucagon to potassium homeostasis

The potassium concentration in plasma (plasma-K) and extracellular fluids is tightly regulated and promptly returns to a normal level after meals. In case of potassium deficiency or during the rest period of the circadian cycle, the renal excretion of potassium is reduced through inactivation of ROMK (for review see (199)) and progesterone-dependent activation of the H,K-ATPase type 2 (45, 163). Because of its low concentration in plasma and extracellular fluids, the amounts of potassium that can be ingested during a single meal may be equal to the whole extracellular potassium pool. Variations in plasma-K outside of relatively narrow limits are life-threatening. It is well-known that insulin stimulates potassium uptake by

hepatocytes and muscle cells, and thus prevents an excessive increase in plasma-K after oral intake (or experimental infusion) by ensuring a temporary storage of the excess potassium within the cellular compartment (39, 67, 71, 115, 127). But although potassium could not be stored in cells permanently, no explanation is provided on how potassium leaves the cells and is excreted after this temporary storage. Yet, potassium excretion is relatively fast compared to that of other electrolytes. In rats and humans, the excretion rate of potassium during daytime is about three times higher than that during nighttime, a much higher day/night ratio than that observed for water and sodium, while creatinine is excreted almost at the same rate during day and night (55, 70, 152, 176). Several studies also showed that potassium excretion increases rapidly after an acute intake of potassium, independently of any change in aldosterone level. Moreover, normal potassium homeostasis and circadian cycle are maintained in the absence of aldosterone (151, 152, 185, 200, 201). The rise in potassium excretion after the ingestion or infusion of a potassium load occurs with a much faster time course than what could be expected from a steroid-dependent mechanism. These observations point to the existence of an aldosterone-independent kaliuretic factor and of a potassium "sensor". Because the rise in potassium excretion occurs with no or minimal change in plasma-K, a few authors proposed, already in 1991 (150), and again more recently (71, 129) that the potassium sensor should reside at some point prior to the systemic circulation, thus, in the splanchnic area or the gut. However, this sensor and the (probably peptidic) hormone promoting acute potassium excretion are so far unknown.

During clearance experiments in rats, we observed that an infusion of glucagon induced a marked, dose-dependent and quickly reversible rise in potassium excretion rate, when the possible confounding influence of vasopressin on urine flow rate was prevented (**Figure 3A**) (4). This effect did not result from a rise in GFR or in urine flow rate. It was due to an increased potassium secretion because glucagon significantly increased the transtubular potassium concentration gradient (TTKG) (4). This result suggests that glucagon increased urinary potassium excretion by a direct stimulation of potassium secretion and that glucagon could play a role in the regulation of potassium homeostasis. A few old studies had already explored the possible influence of glucagon on plasma-K and potassium excretion, but provided ambiguous results, possibly because of the confounding influence of plasma insulin, glucose and amino acid concentrations (40, 49, 113, 144), and of simultaneous changes in urine flow rate that also influence potassium handling in the collecting duct (111, 142). Actually, a possible contribution of insulin to the glucagon-induced rise in potassium excretion cannot be excluded. Glucagon is known to stimulate

insulin secretion, and insulin has been shown in vitro, with the split-open rat CCD preparation, to increase the activities of transporters directly involved in potassium secretion, namely ROMK and the Na-K-ATPase (60). A mathematical model suggests that such changes might result in a significant stimulation of potassium secretion by the CD (60).

If glucagon is indeed involved in the regulation of potassium homeostasis by promoting urinary potassium excretion, it implies that the alpha cell, responsible for glucagon secretion in pancreatic islets, should be sensitive to extracellular potassium concentration and should stimulate glucagon secretion in response to increases in plasma-K. Several studies 20-40 years ago explored the hypothesis that changes in potassium concentration could stimulate glucagon secretion in several species including man, or in isolated perifused pancreas (40, 49, 52, 97, 113, 144). They provided conflicting results. Two papers clearly showed a concomitant rise in glucagon and insulin secretion by the isolated dog pancreas after addition of potassium at physiological concentrations (58, 133). But the most interesting study is that of Santeusanio et al who showed marked elevations in plasma glucagon and insulin concentrations in conscious dogs during an infusion of KCl, as illustrated in **Figure 7**. Glucose concentration did not change (167). Altogether, these results strongly suggest that a rise in plasma-K stimulates not only insulin secretion but also glucagon secretion.

Glucagon and insulin are known to act in a coordinated fashion to regulate glucose homeostasis. It is attractive to think that they could also act in a coordinated fashion to regulate potassium homeostasis. However, this would require two conditions. 1. That the alpha cells could "sense" potassium concentration. 2. That a "combinatory" mode of action would allow the **simultaneous and independent regulation** of both plasma glucose and plasma potassium concentrations. We will explain below how this may be possible.

2. Putative role of the alpha cell as a potassium sensor

Could alpha cells be able to sense variations in splanchnic plasma-K? If yes, how could this occur at the cellular level and lead to an increased glucagon secretion? At least, three different scenarios may be proposed. The first one has been proposed by Santeusanio et al (167) and involves insulin. Indeed, an increase in plasma-K induced by an i.v. infusion of KCl in anesthetized dogs induced marked, significant, and parallel increases in plasma insulin and glucagon without affecting

glycemia (**Figure 7**). When the production of insulin was impeded by treatment with alloxane, the tolerance towards potassium infusion was dramatically reduced, and both plasma glucagon and glucose concentrations rose. The authors concluded from these experiments that the simultaneous increase in insulin and glucagon in response to potassium infusion helps resolving two issues. First, the increase of insulin allows potassium to be stored, but induces some hypoglycemia. This hypoglycemic state then triggers the secretion of glucagon that will restore normoglycemia. This conclusion may be challenged because it does not take into account three factors: 1. the direct effect of glucagon on renal K^+ secretion described above, indicating that glucagon also contributes to potassium handling; 2. the fact that, in the absence of insulin, potassium infusion stimulates glucagon secretion even more than in normal conditions (300 pg/ml vs 80 pg/ml) (167), indicating that rapid elevation of plasma-K rather than insulin triggers glucagon secretion; and 3. the inhibition of glucagon secretion by insulin, mediated by the insulin receptor expressed in alpha cells, and leading to an inhibition of cytosolic Ca^{2+} oscillation (87, 154). These last two observations point to a direct effect of extracellular K^+ concentration on the secretion of glucagon, however, partially attenuated by insulin.

A second scenario can be proposed to explain how extracellular K^+ concentration, *per se*, could trigger glucagon secretion. This secretion is highly dependent on the cell membrane potential that is under the control of extra- and intracellular K^+ concentrations. Pancreatic mouse alpha cells have been reported to be electrically active with a membrane potential around -55 mV. If the secretion of glucagon upon plasma glucose variation remains debated (160), it is however tempting to assume that an elevation in extracellular K^+, that directly depolarizes the plasma membrane, may stimulate the exocytosis of glucagon. Indeed, Rorsman et al showed that an experimental depolarization of alpha cells activated the P/Q-type Ca^{2+} channel present at the cell surface (160). This entry of Ca^{2+} into the cells may, then promote the exocytosis of glucagon granules after binding to synaptogamin-7, a Ca^{2+}-binding protein expressed in these cells (72).

A third scenario could involve a specific K^+ sensor expressed on the cytoplasmic membrane of alpha cells. For a long time, membrane receptors were assumed to bind organic molecules, i.e., hormones or mediators, not minerals. But because calcemia is tightly regulated, Brown, Hebert et al. hypothetized that a specific membrane receptor might be sensitive to the plasma calcium concentration and should accordingly influence the rate of PTH secretion, the hormone that is mostly responsible for calcium homeostasis. In 1993, these authors cloned the

calcium receptor Ca-SR (28), the first ion sensor. These authors later predicted that sensors for other ions should also exist (78). In 2010, a proton sensor, GPR4 has been identified and shown to play a role in the maintenance of acid-base balance (181). The rationale behind the search of a calcium sensor is also valid for potassium. Because plasma-K level is tightly regulated, the existence of a putative "potassium sensor" and of a peptidic hormone responding to its stimulation may be postulated. It is therefore possible that alpha cells express a K^+-receptor that could induce the exocytosis of glucagon through the activation of a second messenger, for instance, a cAMP-dependent pathway, as does adrenaline via beta adrenergic receptors (68). This putative K^+ sensor remains to be identified.

3. Independent regulation of plasma glucose and potassium concentrations by insulin and glucagon in a combinatory mode

If glucagon really contributes to regulate potassium excretion, how can the two hormones, glucagon and insulin, regulate **independently** glucose and potassium homeostasis? This is probably achieved in a "combinatory" mode. As already emphasized for their metabolic actions on the liver, the effects of each hormone cannot be evaluated without taking into account the simultaneous influence of the other hormone (53, 63, 135, 153, 193, 195). Their concentrations can either vary in parallel or in opposite directions. This creates a combination of situations described in **Table 2**. During a prolonged fast, glucagon goes up, but insulin remains low. In contrast, carbohydrate feeding increases insulin but decreases glucagon. A protein meal or amino acids increase both glucagon and insulin. Glucagon stimulates gluconeogenesis and insulin favors cellular glucose uptake resulting in stable glycemia. Glucagon also stimulates urea synthesis, thus contributing to dispose of nitrogen. When both insulin and glucagon are increased after potassium intake, they both contribute to bring back plasma-K to normal by promoting an intracellular storage of K and an accelerated urinary excretion. Their combination ensures a stable glycemia, as observed in the experiments of Santeusanio et al. in conscious dogs (**Figure 7**) (167).

It is interesting to emphasize the relations between potassium and urea excretion. Glucagon was shown to stimulate in parallel ureagenesis in the liver and urea excretion by the kidney so that plasma urea does not vary in spite of a two-fold increase in both urea synthesis and excretion (2, 4). It is possible to assume that

plasma-K may also remain quite stable during large changes in K movements in muscles and kidneys. Moreover, as explained by Halperin and coll, urea and potassium excretions may be closely interrelated (73). Because nitrogen and potassium are often found in the same foods, this coordination is potentially advantageous.

In experimental studies, a potassium infusion was shown to reduce NCC phosphorylation and drive kaliuresis (140, 155, 174). Because of the known influence of glucagon on the DCT (13, 42) where NCC is expressed, it is conceivable that the potassium infusion induced an increase in glucagon secretion, and that glucagon mediated the observed effects on NCC.

The physiological and pathophysiological roles of glucagon: an integrative view

Although it was known for several decades that glucagon secretion is stimulated by the ingestion of proteins, it is most often considered that its main role is associated with maintenance of normoglycemia. More broadly, the multiple actions of glucagon on the liver and kidney can be interpreted as a coordinated response to acute perturbations of the milieu interieur induced by the intake of some foods. These actions are not vital. In pancreatectomized patients, it is crucial to replace insulin, but there is no apparent obvious consequence of the lack of glucagon. Mice with complete loss of alpha cells show that glucagon is not required for the general health (74). However, mice lacking the glucagon receptor (mimicking the human Mahvash disease due to inactivating mutations of the glucagon receptor) became progressively hypoglycemic, lethargic and cachexic after 12 months, and exhibited a much lower survival rate than heterozygous or wild-type mice (208). Thus, glucagon is required for long term survival.

1. Physiological aspects

During fast, glucagon plays its well-know role of stimulating gluconeogenesis but this also implies to excrete the nitrogen from the endogenous amino acids used as a substrate. **After a meal**, the endproducts of carbohydrates and lipids (CO_2 and H_2O = metabolic water) are easily excreted by the lungs and kidneys, respectively. In

contrast, after a protein-rich meal, there is a need to excrete the nitrogen derived from exogenous amino acids, mostly in the form of urea and ammonia. Moreover, a protein meal also brings in the milieu interieur potassium, strong acids, protons, phosphates, sulfates, uric acid, etc.… Glucagon exerts a coordinated action on GFR (19) and on solute transport in the different segments of the nephron and CD to help dispose of these compounds faster and thus, to limit the rise in their concentration in plasma.

Some of the effects of glucagon on the kidney are direct, and some others involve an intermediate circulating compound, cAMP, issued from glucagon's action on the liver, thus participating in a "pancreato-hepato-renal cascade" (17). **Figure 8** illustrates these multiple coordinated actions. In addition, following a few pioneer investigators, we reactivate here the concept that glucagon could play a role in the disposal of potassium. We also propose that the glucagon-producing alpha cells could express a potassium sensor protein that would stimulate glucagon secretion and participate, in conjunction with insulin, to potassium homeostasis, as depicted in **Figure 9**.

The role of glucagon is obviously related to the nutrition status. Thus, it may vary according to the type of diet. In **carnivores**, protein intake is high, resulting in an intense need for urea, potassium, phosphate, etc.… excretion. The diet of **herbivores** brings much less proteins but is rich in potassium. Thus, glucagon should play a significant role in both carnivores and herbivores. But it is important to note that carnivores eat unfrequent large protein meals, and may undergo long periods of fast between meals, whereas herbivores eat small amounts of food for hours long. This should result in much larger peaks and valleys in blood composition and hormone secretion in carnivores than in herbivores. Omnivores probably show an intermediate situation.

2. Pathophysiological aspects

The biological half life of glucagon is short (a few minutes) (10, 146). Thus, the effects of glucagon are prompt to occur and are rapidly reversible. It is mostly degraded by the liver and kidneys (102). In CKD, plasma glucagon concentration is increased (21) and glucagon could thus contribute to muscle wasting by favoring the catabolism of endogenous amino acids, in addition to the decreased influence of insulin (148). In **diabetes mellitus**, glucagon is elevated and its actions no longer counteracted by insulin. Moreover, glucagon effects for a given plasma concentration

are more intense in diabetic patients than in healthy subjects (136). It is conceivable that this glucagon elevation in DM may influence solute and fluid handling in the renal tubule and calcium/magnesium homeostasis, in addition to influencing GFR (19).

A **mutation of the glucagon receptor** (Gly40Ser) has been identified in humans. It leads to a significant reduction in cAMP release by the liver and a lesser rise in glycemia after glucagon infusion (75, 186). In some ethnic groups, this mutation is associated with elevated blood pressure (24, 119) an effect that might be due to an increased reabsorption of sodium in the proximal tubule (178). Note that this effect on sodium reabsorption, observed in fasted subjects might have been more intense if studied after a rise in glucagon induced by a protein meal or an amino acid infusion. Moreover, it would be interesting to study the influence of this mutation on potassium, calcium and magnesium renal handling.

Several drugs or other hormones may affect glucagon secretion and actions. It has recently been shown that **inhibition of the glucose transporter SGLT2** triggers glucagon secretion by alpha cells in patients with Type 2 DM and in diabetic mice (23). This increase in plasma glucagon represents a possible concerning side effect, especially in a patient population already affected by hyperglucagonemia. Some studies suggest that **thiazides** could directly stimulate alpha cell secretion. Glucagon levels were found to be elevated in hypertensive patients treated by thiazide diuretics and to decline upon withdrawal of the treatment (48). *In vitro* perfusion of isolated pancreas of control or diabetic dogs with a thiazide diuretic (but not with a loop diuretic) increased glucagon secretion dose-dependently (80). Thiazide diuretics are known to induce some potassium wasting (140). It is tempting to propose that this effect might be be secondary to a diuretic-induced increase in glucagon secretion. **Vasopressin** has been shown to influence either glucagon or insulin secretion (depending on ambient glycemia) through activation of V1b receptors expressed in pancreatic alpha and beta cells (1). It is thus possible that disorders of water metabolism and/or vasopressin secretion may interfere with glucagon-dependent regulations.

Future directions

The recent development of specific glucagon receptor antagonists and their possible use in humans leads to consider all possible side effects of these drugs. The actions of glucagon on the kidney, although not given much attention up to now,

should be reevaluated in this context. Some possible side effects have not yet been considered. For example, by blocking the glucagon-dependent ion transport in the distal nephron, these antagonists may induce alterations in the Ca^{2+} and/or Mg^{2+} balance and may lead to hypercalciuria and development of kidney stones. Experimental studies and clinical investigations using glucagon receptor antagonists should evaluate not only the metabolic effects of these drugs but also their effects on kidney function and especially electrolyte handling. Marked ethnic differences in potassium homeostasis have been well documented, especially between African American and Caucasian populations (11, 132, 179) and these differences seem to be influenced by the DASH diet (190). The possible contribution of glucagon to these ethnic differences in potassium handling desserves to be evaluated.

More generally, in clinical investigations and epidemiological studies related to diabetes or hypertension, measuring glucagon (and cAMP), in parallel with insulin and variables related to insulin resistance, should be encouraged. Considering the balance between insulin and glucagon may bring more interesting results than just looking at insulin alone, as done too often since a few decades. Note that glucagon should not be measured only in the morning after a night's fast in humans, or during day-time that is the resting period in rodents. It is important to evaluate its elevation above basal state in the 2-3 hours following a standardized amino acid or potassium ingestion, as is performed for insulin with an oral glucose tolerance test. In clinical trials (such as trial # NCT02669524) intended to evaluate the possible benefits of glucagon receptor antagonists in diabetic patients, the influence of these drugs on renal function should be considered in addition to the classical metabolic endpoints.

There are a number of other unresolved questions. Is the renal glucagon receptor the same as the hepatic receptor? Which cell type in the distal tubule and CD express glucagon receptors? Are glucagon receptors expressed in the proximal tubule (112)? Is extracellular cAMP really influencing proximal tubule transport, possibly by cAMP receptors (3, 17)? Are the effects of glucagon on the TAL similar to those induced by vasopressin (38)? A great number of studies have been devoted to the effects of vasopressin on the TAL but almost none considered the possible effects of glucagon although both hormones stimulate adenylate cyclase in the same way in this nephron segment (118). The effects of vasopressin seem to require relatively high levels of vasopressin (16, 46, 91). In contrast, the effects of glucagon on the TAL may probably be effective after each protein meal in order to achieve the best compromise between optimal urea excretion and efficient water economy.

Future studies should take advantage of mice with deletion of the glucagon receptor, compared to wild-type mice, to evaluate the effects of glucagon on plasma composition and kidney function. More specifically, mice with kidney-specific or even TAL- or CD-specific deletion of the glucagon receptor should be studied, similar to what has been done for the insulin receptor (138, 184). Clearance studies in KO mice and WT mice after an amino acid load or a potassium load, in a setting maintaining stable glycemia and vasopressin concentrations, or during DM, should reveal if glucagon secretion is increased in response to these loads and how it contributes to nitrogen and potassium balance (with calculation of urea fractional excretion and TTKG). In chronic studies with high and low potassium intake, too low potassium diets should be avoided because they induce a loss of appetite that reduces dramatically food intake, thus leading to confounding metabolic effects.

In vitro studies of glucagon secretion in isolated Langerhans islet or cultured alpha cells should not only use hypoglycemia as a stimulus but also evaluate the influence of increased amino acid or potassium extracellular concentration. *In vitro* microperfusion studies in isolated distal tubule and different sub-segments of the CD (without or with pretreatment by aldosterone) could confirm if glucagon indeed stimulates potassium secretion, and investigate the intracellular pathway at the molecular level. Mathematical models may help understand how glucagon and insulin secretion can combine their respective influences in order to regulate independantly potassium and glucose concentrations in plasma and extracellular fluids.

In summary, in addition to its well-known role in glucose homeostasis, glucagon plays important roles in electrolyte and nitrogen handling. Its multiple actions in normal situations and in various pathological states deserve more attention, especially in situations known to involve an increase in its secretion and an imbalance between glucagon and insulin plasma concentrations. The use of selective glucagon antagonists in animal studies and clinical investigations/trials, as well as mice with deletion of the glucagon receptor should provide new knowledge about the secretion of the hormone and its various actions on the metabolism and on kidney function.

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Figure Legends

Figure 1. A, Adenylate cyclase activity, and B, binding of radio-labeled glucagon (B) in different segments of the rat nephron and collecting duct. A. Basal (black columns) and stimulated (hatched columns) adenylate cyclase activity. B. Specific binding is the difference between total (hatched columns) and non-specific binding (black columns). PCT = proximal convoluted tubule; PR = pars recta; DTL = descending thin limb; ATL = ascending thin limb; MTAL and CTAL = medullary and corticial thick ascending limb, respectively; DCT = distal convoluted tubule; CCD, OMCD and IMCD = cortical, outer medullary, and inner medullary collecting duct, respectively. NT = not tested. A adapted from (13) and B adapted from (31).

Figure 2. *In vivo* clearance experiments in normal anesthetized rats showing changes in the fractional excretion (ΔFE) of several electrolytes induced by glucagon at different infusion rates. C, E, R = Control, experimental and recovery periods, respectively, i.e., before, during and after glucagon infusion (mean of 3 x 20 min, each), respectively (40 min equilibration between C and E and between E and R, not shown). The effects of glucagon on Ca and Mg were rapidly reversible whereas those on Na, Cl and phosphate were not, or only partially reversible during the experiment. Three different glucagon infusion rates are shown in ng.min^{-1}.100 g BW^{-1}, and 0 = time-control). Redrawn and adapted from (4).

Figure 3. Influence of glucagon on potassium handling in the rat or mouse kidney. C, E, R = Control, experimental and recovery periods, respectively. **A.** During *in vivo* clearance experiments in normal anesthetized rats. Infusion rates as in Figure 2. **B.** Results obtained during *in vivo* micropuncture study of Henle's loops (based on the difference in tubular fluid flow rate and composition between the late proximal tubule and the early distal tubule accessible at the kidney surface in hormone-deprived rats (see text) infused or not with glucagon (Glu and C, respectively). **C.** Results of isolated perfused mouse CTAL and MTAL before (C) during (Glu) and after (R) glucagon application. **A**, adapted from (4). **B** and **C**, adapted from (42).

Figure 4. Influence of glucagon on urinary urea excretion rate (A and B) and on urine concentration (C and D) in anesthetized rats during clearance experiments.

Measurements were made in separate groups of rats, with either a low diuresis induced by dDAVP infusion (Low-D), or a high diuresis induced by infusion of dilute saline (High-D). C and Glu = control and glucagon infusion periods. Adapted from [2]. Data shown in B was obtained in Low-D condition. Urea excretion was significantly correlated with urea filtration in both groups, and slopes of regression lines differed significantly. In C, the dotted line indicates plasma osmolality. In D, the double arrows indicate the amount of water reabsorbed under the influence of glucagon.

Figure 5. Influence of glucagon on calcium and magnesium handling in humans and mouse. A and B. Clearance study in healthy humans showing the concentration and the fractional excretion of calcium and magnesium. * = significant difference with the basal period. Redrawn and adapted from (59). **C**. *In vitro* microperfusion experiment of isolated microdissected mouse mTAL and cTAL during control (C), glucagon infusion (Glu) and recovery (R) periods. * = significant difference with the pre- and post-treatment periods. **D**. *In vivo* micropuncture experiments of the loop of Henle in "hormone-deprived" rats (see text and Figure 3) untreated (C) or treated with glucagon (Glu). *** = significant difference with the untreated group (unpaired t-test, $p<0.001$). Redrawn and adapted from (42).

Figure 6. Diagram showing the effects of glucagon on solute transport along the different nephron segments. In the upper part, arrows indicate glucagon-activated reabsorption. In the lower part, continuous arrows indicate glucagon-activated secretion, whereas dashed arrows indicate glucagon-induced inhibition of reabsorption. Effects on K^+ transport are highlighted in yellow and the lack of information for the CCD is displayed by black boxes. Abbreviations as in Figure 1.

Figure 7. Influence of a KCl infusion upon plasma glucagon, insulin and glucose in normal dogs. Open circles indicate significant differences compared to mean baseline values. Reproduced from (167).

Figure 8. Proposed view of the direct and indirect effects of glucagon (combined with liver-borne cAMP) on GFR and solute handling by the nephron.

Figure 9. Proposed view of the combined effects of insulin and glucagon on potassium homeostasis.

References

1. **Abu-Basha EA, Yibchok-Anun S, and Hsu WH**. Glucose dependency of arginine vasopressin-induced insulin and glucagon release from the perfused rat pancreas. *Metabolism* 51: 1184-1190, 2002.

2. **Ahloulay M, Bouby N, Machet F, Kubrusly M, Coutaud C, and Bankir L**. Effects of glucagon on glomerular filtration rate and urea and water excretion. *Am J Physiol* 263: F24-F36, 1992.

3. **Ahloulay M, Dechaux M, Hassler C, Bouby N, and Bankir L**. Cyclic AMP is a hepatorenal link influencing natriuresis and contributing to glucagon-induced hyperfiltration in rats. *J Clin Invest* 98: 2251-2258, 1996.

4. **Ahloulay M, Dechaux M, Laborde K, and Bankir L**. Influence of glucagon on GFR and on urea and electrolyte excretion: direct and indirect effects. *Am J Physiol* 269: F225-F235, 1995.

5. **Ahren B**. Glucagon--Early breakthroughs and recent discoveries. *Peptides* 67: 74-81, 2015.

6. **Almdal TP**. Importance of glucagon for nitrogen loss in diabetes--via an accelerated hepatic conversion of amino nitrogen to urea nitrogen. *Dan Med Bull* 38: 113-120, 1991.

7. **Almdal TP, and Vilstrup H**. Loss of nitrogen from organs in rats induced by exogenous glucagon. *Endocrinology* 123: 2182-2186, 1988.

8. **Ando Y, Tabei K, Furuya H, and Asano Y**. Glucagon stimulates chloride transport independently of cyclic AMP in the rat medullary TAL. *Kidney Int* 36: 760-767, 1989.

9. **Assan R, Attali JR, Ballerio G, Boillot J, and Girard JR**. Glucagon secretion induced by natural and artificial amino acids in the perfused rat pancreas. *Diabetes* 26: 300-307, 1977.

10. **Authier F, and Desbuquois B**. Glucagon receptors. *Cell Mol Life Sci* 65: 1880-1899, 2008.

11. **Aviv A, Hollenberg NK, and Weder A**. Urinary potassium excretion and sodium sensitivity in blacks. *Hypertension* 43: 707-713, 2004.

12. **Bagger JI, Knop FK, Holst JJ, and Vilsboll T**. Glucagon antagonism as a potential therapeutic target in type 2 diabetes. *Diabetes Obes Metab* 13: 965-971, 2011.

13. **Bailly C, Imbert-Teboul M, Chabardes D, Hus-Citharel A, Montegut M, Clique A, and Morel F**. The distal nephron of rat kidney: a target site for glucagon. *Proc Natl Acad Sci U S A* 77: 3422-3424, 1980.

14. **Bailly C, Roinel N, and Amiel C**. PTH-like glucagon stimulation of Ca and Mg reabsorption in Henle's loop of the rat. *Am J Physiol* 246: F205-212, 1984.

15. **Bailly C, Roinel N, and Amiel C**. Stimulation by glucagon and PTH of Ca and Mg reabsorption in the superficial distal tubule of the rat kidney. *Pflugers Arch* 403: 28-34, 1985.

16. **Bankir L**. Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. *Cardiovasc Res* 51: 372-390, 2001.

17. **Bankir L, Ahloulay M, Devreotes PN, and Parent CA**. Extracellular cAMP inhibits proximal reabsorption: are plasma membrane cAMP receptors involved? *Am J Physiol Renal Physiol* 282: F376-F392, 2002.

18. **Bankir L, and Bouby N**. Effects of chronic glucagon infusion on renal function in rats (Abstract 578-17)). *FASEB Journal* 13: A720, 1999.

19. **Bankir L, Roussel R, and Bouby N**. Protein- and diabetes-induced glomerular hyperfiltration : role of glucagon, vasopressin, and urea. *Am J Physiol Renal Physiol* 309: F2-F23, 2015.

20. **Bankir L, and Yang B**. New insights into urea and glucose handling by the kidney, and the urine concentrating mechanism. *Kidney Int* 81: 1179-1198, 2012.

21. **Bastl C, Finkelstein FO, Sherwin R, Hendler R, Felig P, and Hayslett JP**. Renal extraction of glucagon in rats with normal and reduced renal function. *Am J Physiol* 233: F67-71, 1977.

22. **Boden G, Rezvani I, and Owen OE**. Effects of glucagon on plasma amino acids. *J Clin Invest* 73: 785-793, 1984.

23. **Bonner C, Kerr-Conte J, Gmyr V, Queniat G, Moerman E, Thevenet J, Beaucamps C, Delalleau N, Popescu I, Malaisse WJ, Sener A, Deprez B, Abderrahmani A, Staels B, and Pattou F**. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. *Nat Med* 21: 512-517, 2015.

24. **Brand E, Bankir L, Plouin PF, and Soubrier F**. Glucagon receptor gene mutation (Gly40Ser) in human essential hypertension: the PEGASE study. *Hypertension* 34: 15-17, 1999.

25. **Briffeuil P, Thu TH, and Kolanowski J**. A lack of direct action of glucagon on kidney metabolism, hemodynamics, and renal sodium handling in the dog. *Metabolism* 45: 383-388, 1996.

26. **Broadus AE, Kaminsky NI, Northcutt RC, Hardman JG, Sutherland EW, and Liddle GW**. Effects of glucagon on adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in human plasma and urine. *J Clin Invest* 49: 2237-2245, 1970.

27. **Brouhard BH, LaGrone LF, Richards GE, and Travis LB**. Somatostatin limits rise in glomerular filtration rate after a protein meal. *J Pediatr* 110: 729-734, 1987.

28. **Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, and Hebert SC**. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 366: 575-580, 1993.

29. **Burcelin R, Katz EB, and Charron MJ**. Molecular and cellular aspects of the glucagon receptor: role in diabetes and metabolism. *Diabetes Metab* 22: 373-396, 1996.

30. **Burcelin R, Li J, and Charron MJ**. Cloning and sequence analysis of the murine glucagon receptor-encoding gene. *Gene* 164: 305-310, 1995.

31. **Butlen D, and Morel F**. Glucagon receptors along the nephron: [125I]glucagon binding in rat tubules. *Pflugers Arch* 404: 348-353, 1985.

32. **Chabardes D, Firsov D, Aarab L, Clabecq A, Bellanger AC, Siaume-Perez S, and Elalouf JM**. Localization of mRNAs encoding Ca2+-inhibitable adenylyl cyclases along the renal tubule. Functional consequences for regulation of the cAMP content. *J Biol Chem* 271: 19264-19271, 1996.

33. **Chabardes D, Montegut M, Imbert-Teboul M, and Morel F**. Inhibition of alpha 2-adrenergic agonists on AVP-induced cAMP accumulation in isolated collecting tubule of the rat kidney. *Mol Cell Endocrinol* 37: 263-275, 1984.

34. **Chabardes-Garonne D, Mejean A, Aude JC, Cheval L, Di Stefano A, Gaillard MC, Imbert-Teboul M, Wittner M, Balian C, Anthouard V, Robert C, Segurens B, Wincker P, Weissenbach J, Doucet A, and Elalouf JM**. A panoramic view of gene expression in the human kidney. *Proc Natl Acad Sci U S A* 100: 13710-13715, 2003.

35. **Cheval L, Pierrat F, Dossat C, Genete M, Imbert-Teboul M, Duong Van Huyen JP, Poulain J, Wincker P, Weissenbach J, Piquemal D, and Doucet A**. Atlas of gene expression in the mouse kidney: new features of glomerular parietal cells. *Physiol Genomics* 43: 161-173, 2011.

36. **Dallas-Yang Q, Shen X, Strowski M, Brady E, Saperstein R, Gibson RE, Szalkowski D, Qureshi SA, Candelore MR, Fenyk-Melody JE, Parmee ER, Zhang BB, and Jiang G**. Hepatic glucagon receptor binding and glucose-lowering in vivo by peptidyl and non-peptidyl glucagon receptor antagonists. *Eur J Pharmacol* 501: 225-234, 2004.

37. **de Duve C**. Born-again glucagon. *Faseb j* 8: 979-981, 1994.

38. **De Rouffignac C, Di Stefano A, Wittner M, Roinel N, and Elalouf JM**. Consequences of differential effects of ADH and other peptide hormones on thick ascending limb of mammalian kidney. *Am J Physiol* 260: R1023-1035, 1991.

39. **DeFronzo RA, Felig P, Ferrannini E, and Wahren J**. Effect of graded doses of insulin on splanchnic and peripheral potassium metabolism in man. *Am J Physiol* 238: E421-427, 1980.

40. **DeFronzo RA, Sherwin RS, Dillingham M, Hendler R, Tamborlane WV, and Felig P**. Influence of basal insulin and glucagon secretion on potassium and sodium metabolism. Studies with somatostatin in normal dogs and in normal and diabetic human beings. *J Clin Invest* 61: 472-479, 1978.

41. **Delahousse M, Mercier O, Bichara M, Paillard M, Leviel F, and Assan R**. Glucagon inhibits urinary acidification in the rat. *Am J Physiol* 254: F762-769, 1988.

42. **Di Stefano A, Wittner M, Nitschke R, Braitsch R, Greger R, Bailly C, Amiel C, Elalouf JM, Roinel N, and de Rouffignac C**. Effects of glucagon on Na+, Cl-, K+, Mg2+ and Ca2+ transports in cortical and medullary thick ascending limbs of mouse kidney. *Pflugers Arch* 414: 640-646, 1989.

43. **Djuric SW, Grihalde N, and Lin CW**. Glucagon receptor antagonists for the treatment of type II diabetes: current prospects. *Curr Opin Investig Drugs* 3: 1617-1623, 2002.

44. **Dunphy JL, Taylor RG, and Fuller PJ**. Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression. *Mol Cell Endocrinol* 141: 179-186, 1998.

45. **Elabida B, Edwards A, Salhi A, Azroyan A, Fodstad H, Meneton P, Doucet A, Bloch-Faure M, and Crambert G**. Chronic potassium depletion increases adrenal progesterone production that is necessary for efficient renal retention of potassium. *Kidney Int* 80: 256-262, 2011.

46. **Elalouf JM, Di Stefano A, and de Rouffignac C**. Sensitivities of rat kidney thick ascending limbs and collecting ducts to vasopressin in vivo. *Proc Natl Acad Sci U S A* 83: 2276-2280, 1986.

47. **Elalouf JM, Sari DC, and de Rouffignac C**. Additive effects of glucagon and vasopressin on renal Mg reabsorption and urine concentrating ability in the rat. *Pflugers Arch* 407 Suppl 2: S66-S71, 1986.

48. **Eldridge JC, Strandhoy J, and Buckalew VM, Jr.** Endocrinologic effects of antihypertensive therapy with guanabenz or hydrochlorothiazide. *J Cardiovasc Pharmacol* 6 Suppl 5: S776-780, 1984.

49. **Ellis S, and Beckett SB**. MECHANISM OF THE POTASSIUM MOBILIZING ACTION OF EPINEPHRINE AND GLUCAGON. *J Pharmacol Exp Ther* 142: 318-326, 1963.

50. **Elrick, Springs V, Staub A, and Stoll F**. A renal action of glucagon. *Proc Soc Exp Biol Med* 94: 57-60, 1957.

51. **Elrick H, Huffman ER, Hlad CJ, Jr., Whipple N, and Staub A**. Effects of glucagon on renal function in man. *J Clin Endocrinol Metab* 18: 813-824, 1958.

52. **Epstein G, Fanska R, and Grodsky GM**. The effect of potassium and valinomycin on insulin and glucagon secretion in the perfused rat pancreas. *Endocrinology* 103: 2207-2215, 1978.

53. **Felig P, Gusberg R, Hendler R, Gump FE, Kinney JM, and Mulrow PJ**. Concentrations of glucagon and the insulin:glucagon ratio in the portal and peripheral circulation. *Proc Soc Exp Biol Med* 147: 88-90, 1974.

54. **Ferlito S, Indelicato G, Di Vincenzo S, Del Campo F, La Vignera A, and Fichera C**. Effect of clonidine on glucose, insulin and glucagon responses to a protein meal in type 2 diabetics. *J Endocrinol Invest* 8: 185-187, 1985.

55. **Fezeu L, Bankir L, Hansel B, and Guerrot D**. Differential circadian pattern of water and Na excretion rates in the metabolic syndrome. *Chronobiol Int* 31: 861-867, 2014.

56. **Filipski KJ**. Small molecule glucagon receptor antagonists: a patent review (2011 - 2014). *Expert Opin Ther Pat* 25: 819-830, 2015.

57. **Forte LR, Chao WT, Walkenbach J, and Byington KH**. Studies of kidney plasma membrane adenosine-3',5'-monophosphate-dependent protein kinase. *Biochim Biophys Acta* 389: 84-96, 1975.

58. **Frankel BJ, Heldt AM, and Grodsky GM**. Effects of K+ and arginine on insulin, glucagon, and somatostatin release from the in vitro perfused rat pancreas. *Endocrinology* 110: 428-431, 1982.

59. **Friedlander G, Blanchet-Benqué F, Bailly C, Assan R, and Amiel C**. Tubular effects of glucagon in humans [Effets tubulaires rénaux du glucagon chez l'homme] (in French]. *Médecine/Sciences* 1: 100-103, 1985.

60. **Frindt G, and Palmer LG**. Effects of insulin on Na and K transporters in the rat CCD. *Am J Physiol Renal Physiol* 302: F1227-1233, 2012.

61. **Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, and Charron MJ**. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A* 100: 1438-1443, 2003.

62. **Gerich JE**. Physiology of glucagon. *Int Rev Physiol* 24: 243-275, 1981.

63. **Gesser CA, Muller-Hess R, and Felber JP**. The insulin:glucagon ratio and the secretion of growth hormone after intravenous administration of amino acids and carbohydrates in healthy subjects. *Infusionstherapie* 1: 483-489, 1974.

64. **Good DW**. Sodium-dependent bicarbonate absorption by cortical thick ascending limb of rat kidney. *Am J Physiol* 248: F821-829, 1985.

65. **Good DW**. Inhibition of bicarbonate absorption by peptide hormones and cyclic adenosine monophosphate in rat medullary thick ascending limb. *J Clin Invest* 85: 1006-1013, 1990.

66. **Gosmain Y, Masson MH, and Philippe J**. Glucagon: the renewal of an old hormone in the pathophysiology of diabetes. *J Diabetes* 5: 102-109, 2013.

67. **Greenlee M, Wingo CS, McDonough AA, Youn JH, and Kone BC**. Narrative review: evolving concepts in potassium homeostasis and hypokalemia. *Ann Intern Med* 150: 619-625, 2009.

68. **Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renstrom E, and Rorsman P**. Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca2+ current and the number of granules close to the L-type Ca2+ channels. *J Gen Physiol* 110: 217-228, 1997.

69. **Gueutin V, Vallet M, Jayat M, Peti-Peterdi J, Corniere N, Leviel F, Sohet F, Wagner CA, Eladari D, and Chambrey R**. Renal beta-intercalated cells maintain body fluid and electrolyte balance. *J Clin Invest* 123: 4219-4231, 2013.

70. **Gumz ML, and Rabinowitz L**. Role of circadian rhythms in potassium homeostasis. *Semin Nephrol* 33: 229-236, 2013.

71. **Gumz ML, Rabinowitz L, and Wingo CS**. An Integrated View of Potassium Homeostasis. *N Engl J Med* 373: 60-72, 2015.

72. **Gustavson SM, Chu CA, Nishizawa M, Neal D, Farmer B, Yang Y, Donahue EP, Flakoll P, and Cherrington AD**. Effects of hyperglycemia, glucagon, and epinephrine on renal glucose release in the conscious dog. *Metabolism* 53: 933-941, 2004.

73. **Halperin ML, Gowrishankar M, Mallie JP, Sonnenberg H, and Oh M**. Urea recycling: an aid to the excretion of potassium during antidiuresis. *Nephron* 72: 507-511, 1996.

74. **Hancock AS, Du A, Liu J, Miller M, and May CL**. Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. *Mol Endocrinol* 24: 1605-1614, 2010.

75. **Hansen LH, Abrahamsen N, Hager J, Jelinek L, Kindsvogel W, Froguel P, and Nishimura E**. The Gly40Ser mutation in the human glucagon receptor gene associated with NIDDM results in a receptor with reduced sensitivity to glucagon. *Diabetes* 45: 725-730, 1996.

76. **Hansen LH, Abrahamsen N, and Nishimura E**. Glucagon receptor mRNA distribution in rat tissues. *Peptides* 16: 1163-1166, 1995.

77. **Hasegawa F, Niidome K, Migihashi C, Murata M, Negoro T, Matsumoto T, Kato K, and Fujii A**. Discovery of furan-2-carbohydrazides as orally active glucagon receptor antagonists. *Bioorg Med Chem Lett* 24: 4266-4270, 2014.

78. **Hebert SC, and Brown EM**. The extracellular calcium receptor. *Curr Opin Cell Biol* 7: 484-492, 1995.

79. **Hendy GN, Tomlinson S, and O'Riordan JL**. Impaired responsiveness to the effect of glucagon on plasma adenosine 3':5'-cyclic monophosphate in normal man. *Eur J Clin Invest* 7: 155-160, 1977.

80. **Hermansen K, Schmitz O, and Mogensen CE**. Effects of a thiazide diuretic (hydroflumethiazide) and a loop diuretic (bumetanide) on the endocrine pancreas: studies in vitro. *Metabolism* 34: 784-789, 1985.

81. **Insel P, Balakir R, and Sacktor B**. The binding of cyclic AMP to renal brush border membranes. *J Cyclic Nucleotide Res* 1: 107-122, 1975.

82. **Iwanij V**. Canine kidney glucagon receptor: evidence for a structurally-different, tissue-specific variant of the glucagon receptor. *Mol Cell Endocrinol* 115: 21-28, 1995.

83. **Jelinek LJ, Lok S, Rosenberg GB, Smith RA, Grant FJ, Biggs S, Bensch PA, Kuijper JL, Sheppard PO, Sprecher CA, and et al.** Expression cloning and signaling properties of the rat glucagon receptor. *Science* 259: 1614-1616, 1993.

84. **Johannesen J, Lie M, and Kiil F**. Effect of glycine and glucagon on glomerular filtration and renal metabolic rates. *Am J Physiol* 233: F61-F66, 1977.

85. **Johnson DG, Goebel CU, Hruby VJ, Bregman MD, and Trivedi D**. Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist. *Science* 215: 1115-1116, 1982.

86. **Kabadi UM**. Dose-kinetics of pancreatic alpha- and beta-cell responses to a protein meal in normal subjects. *Metabolism* 40: 236-240, 1991.

87. **Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL, Herrera PL, Polonsky KS, McGuinness OP, and Kulkarni RN**. Insulin signaling in alpha cells modulates glucagon secretion in vivo. *Cell Metab* 9: 350-361, 2009.

88. **Kazda CM, Garhyan P, Kelly RP, Shi C, Lim CN, Fu H, Landschulz WH, and Deeg MA**. A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of the Glucagon Receptor Antagonist LY2409021 in Patients With Type 2 Diabetes. *Diabetes Care* 2015.

89. **Kelly RP, Garhyan P, Raddad E, Fu H, Lim CN, Prince MJ, Pinaire JA, Loh MT, and Deeg MA**. Short-term administration of the glucagon receptor antagonist LY2409021 lowers blood glucose in healthy people and in those with type 2 diabetes. *Diabetes Obes Metab* 17: 414-422, 2015.

90. **Kim JK, Frohnert PP, Hui YS, Barnes LD, Farrow GM, and Dousa TP**. Enzymes of cyclic 3',5'-nucleotide metabolism in human renal cortex and renal adenocarcinoma. *Kidney Int* 12: 172-183, 1977.

91. **Kim JK, Summer SN, Erickson AE, and Schrier RW**. Role of arginine vasopressin in medullary thick ascending limb on maximal urinary concentration. *Am J Physiol* 251: F266-270, 1986.

92. **Klain GJ**. In vivo effects of glucagon on fatty acid synthesis in fasted and refed rats. *J Nutr* 107: 942-948, 1977.

93. **Knepper MA, Gunter CV, and Danielson RA**. Effects of glucagon on renal function in protein-deprived rats. *Surg Forum* 27: 29-31, 1976.

94. **Kompanowska-Jezierska E, Sadowski J, and Walkowska A**. Glucagon increases medullary interstitial electrolyte concentration in rat kidney. *Can J Physiol Pharmacol* 73: 1289-1291, 1995.

95. **Krishna MG, Coker RH, Lacy DB, Zinker BA, Halseth AE, and Wasserman DH**. Glucagon response to exercise is critical for accelerated hepatic glutamine metabolism and nitrogen disposal. *Am J Physiol Endocrinol Metab* 279: E638-645, 2000.

96. **Kriz W, and Bankir L**. A standard nomenclature for structures of the kidney. The Renal Commission of the International Union of Physiological Sciences (IUPS) (also published in Am. J. Physiol. 254:F1-F8, 1988). *Kidney Int* 33: 1-7, 1988.

97. **Kuzuya T, Kajinuma H, and Ide T**. Effect of intrapancreatic injection of potassium and calcium on insulin and glucagon secretion in dogs. *Diabetes* 23: 55-60, 1974.

98. **Laroche-Joubert N, Marsy S, and Doucet A**. Cellular origin and hormonal regulation of K(+)-ATPase activities sensitive to Sch-28080 in rat collecting duct. *Am J Physiol Renal Physiol* 279: F1053-1059, 2000.

99. **Layton AT, and Bankir L**. Impacts of Active Urea Secretion into Pars Recta on Urine Concentration and Urea Excretion Rate. *Physiol Rep* 1: 2013.

100. **Lee Y, Wang MY, Du XQ, Charron MJ, and Unger RH**. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. *Diabetes* 60: 391-397, 2011.

101. **Lefebvre PJ**. Early milestones in glucagon research. *Diabetes Obes Metab* 13 Suppl 1: 1-4, 2011.

102. **Lefebvre PJ, and Luyckx AS**. Plasma glucagon after kidney exclusion: experiments in somatostatin-infused and in eviscerated dogs. *Metabolism* 25: 761-768, 1976.

103. **Lefebvre PJ, Paquot N, and Scheen AJ**. Inhibiting or antagonizing glucagon: making progress in diabetes care. *Diabetes Obes Metab* 17: 720-725, 2015.

104. **Leviel F, Hubner CA, Houillier P, Morla L, El Moghrabi S, Brideau G, Hassan H, Parker MD, Kurth I, Kougioumtzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, and Eladari D**. The Na+-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na+ reabsorption process in the renal cortical collecting ducts of mice. *J Clin Invest* 120: 1627-1635, 2010.

105. **Levy M, and Starr NL**. The mechanism of glucagon-induced natriuresis in dogs. *Kidney Int* 2: 76-84, 1972.

106. **Li XC, Liao TD, and Zhuo JL**. Long-term hyperglucagonaemia induces early metabolic and renal phenotypes of Type 2 diabetes in mice. *Clin Sci (Lond)* 114: 591-601, 2008.

107. **Li XC, and Zhuo JL**. Current insights and new perspectives on the roles of hyperglucagonemia in non-insulin-dependent type 2 diabetes. *Curr Hypertens Rep* 15: 522-530, 2013.

108. **Madsen P, Knudsen LB, Wiberg FC, and Carr RD**. Discovery and structure-activity relationship of the first non-peptide competitive human glucagon receptor antagonists. *J Med Chem* 41: 5150-5157, 1998.

109. **Maeda Y, Terada Y, Nonoguchi H, and Knepper MA**. Hormone and autacoid regulation of cAMP production in rat IMCD subsegments. *Am J Physiol* 263: F319-327, 1992.

110. **Mallette LE, Exton JH, and Park CR**. Control of gluconeogenesis from amino acids in the perfused rat liver. *J Biol Chem* 244: 5713-5723, 1969.

111. **Malnic G, Berliner RW, and Giebisch G**. Flow dependence of K+ secretion in cortical distal tubules of the rat. *Am J Physiol* 256: F932-941, 1989.

112. **Marks J, Debnam ES, Dashwood MR, Srai SK, and Unwin RJ**. Detection of glucagon receptor mRNA in the rat proximal tubule: potential role for glucagon in the control of renal glucose transport. *Clin Sci (Lond)* 104: 253-258, 2003.

113. **Massara F, Martelli S, Cagliero E, Camanni F, and Molinatti GM**. Influence of glucagon on plasma levels of potassium in man. *Diabetologia* 19: 414-417, 1980.

114. **Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, and Drucker DJ**. International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 55: 167-194, 2003.

115. **McDonough AA, Thompson CB, and Youn JH**. Skeletal muscle regulates extracellular potassium. *Am J Physiol Renal Physiol* 282: F967-974, 2002.

116. **Mercier O, Bichara M, Delahousse M, Prigent A, Leviel F, and Paillard M**. Effects of glucagon on H(+)-HCO3- transport in Henle's loop, distal tubule, and collecting ducts in the rat. *Am J Physiol* 257: F1003-1014, 1989.

117. **Miller LL**. Glucagon: a protein catabolic hormone in the isolated perfused rat liver. *Nature* 185: 248, 1960.

118. **Morel F**. Sites of hormone action in the mammalian nephron. *Am J Physiol* 240: F159-F164, 1981.

119. **Morris BJ, Jeyasingam CL, Zhang W, Curtain RP, and Griffiths LR**. Influence of family history on frequency of glucagon receptor Gly40Ser mutation in hypertensive subjects. *Hypertension* 30: 1640-1641, 1997.

120. **Morris SM, Jr.** Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* 22: 87-105, 2002.

121. **Mount DB**. Thick ascending limb of the loop of Henle. *Clin J Am Soc Nephrol* 9: 1974-1986, 2014.

122. **Mu J, Jiang G, Brady E, Dallas-Yang Q, Liu F, Woods J, Zycband E, Wright M, Li Z, Lu K, Zhu L, Shen X, Sinharoy R, Candelore ML, Qureshi SA, Shen DM, Zhang F, Parmee ER, and Zhang BB**. Chronic treatment with a glucagon receptor antagonist lowers glucose and moderately raises circulating glucagon and glucagon-like peptide 1 without severe alpha cell hypertrophy in diet-induced obese mice. *Diabetologia* 54: 2381-2391, 2011.

123. **Mu J, Qureshi SA, Brady EJ, Muise ES, Candelore MR, Jiang G, Li Z, Wu MS, Yang X, Dallas-Yang Q, Miller C, Xiong Y, Langdon RB, Parmee ER, and Zhang BB**. Anti-diabetic efficacy and impact on amino acid metabolism of GRA1, a novel small-molecule glucagon receptor antagonist. *PLoS One* 7: e49572, 2012.

124. **Muller WA, Cuppers HJ, Zimmermann-Telschow H, Micheli H, Wyss T, Renold AE, and Berger M**. Amino acids and lipoproteins in plasma of duodenopancreatectomized patients: effects of glucagon in physiological amounts. *Eur J Clin Invest* 13: 141-149, 1983.

125. **Mulvehill JB, Hui YS, Barnes LD, Palumbo PJ, and Dousa TP**. Glucagon-sensitive adenylate cyclase in human renal medulla. *J Clin Endocrinol Metab* 42: 380-384, 1976.

126. **Mutel E, Gautier-Stein A, Abdul-Wahed A, Amigo-Correig M, Zitoun C, Stefanutti A, Houberdon I, Tourette JA, Mithieux G, and Rajas F**. Control of blood glucose in the absence of hepatic glucose production during prolonged fasting in mice: induction of renal and intestinal gluconeogenesis by glucagon. *Diabetes* 60: 3121-3131, 2011.

127. **Nguyen TQ, Maalouf NM, Sakhaee K, and Moe OW**. Comparison of insulin action on glucose versus potassium uptake in humans. *Clin J Am Soc Nephrol* 6: 1533-1539, 2011.

128. **O'Harte FP, Franklin ZJ, and Irwin N**. Two novel glucagon receptor antagonists prove effective therapeutic agents in high-fat-fed and obese diabetic mice. *Diabetes Obes Metab* 16: 1214-1222, 2014.

129. **Oh KS, Oh YT, Kim SW, Kita T, Kang I, and Youn JH**. Gut sensing of dietary K(+) intake increases renal K(+)excretion. *Am J Physiol Regul Integr Comp Physiol* 301: R421-429, 2011.

130. **Ohneda A, Parada E, Eisentraut AM, and Unger RH**. Characterization of response of circulating glucagon to intraduodenal and intravenous administration of amino acids. *J Clin Invest* 47: 2305-2322, 1968.

131. **Paillard M, and Bichara M**. Peptide hormone effects on urinary acidification and acid-base balance: PTH, ADH, and glucagon. *Am J Physiol* 256: F973-985, 1989.

132. **Palacios C, Wigertz K, Martin BR, Braun M, Pratt JH, Peacock M, and Weaver CM**. Racial differences in potassium homeostasis in response to differences in dietary sodium in girls. *Am J Clin Nutr* 91: 597-603, 2010.

133. **Panzig E, Besch W, Rosenbaum KD, Tietz W, Kiene S, Wolf E, and Paul W**. The effect of potassium, calcium and magnesium concentration on insulin and glucagon secretion of the perfused dog pancreas. *Exp Clin Endocrinol* 86: 61-68, 1985.

134. **Parker JC, Andrews KM, Allen MR, Stock JL, and McNeish JD**. Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochem Biophys Res Commun* 290: 839-843, 2002.

135. **Parrilla R, Goodman MN, and Toews CJ**. Effect of glucagon: insulin ratios on hepatic metabolism. *Diabetes* 23: 725-731, 1974.

136. **Parving HH, Christiansen JS, Noer I, Tronier B, and Mogensen CE**. The effect of glucagon infusion on kidney function in short-term insulin-dependent juvenile diabetics. *Diabetologia* 19: 350-354, 1980.

137. **Parving HH, Noer J, Kehlet H, Mogensen CE, Svendsen PA, and Heding L**. The effect of short-term glucagon infusion on kidney function in normal man. *Diabetologia* 13: 323-325, 1977.

138. **Pavlov TS, Ilatovskaya DV, Levchenko V, Li L, Ecelbarger CM, and Staruschenko A**. Regulation of ENaC in mice lacking renal insulin receptors in the collecting duct. *Faseb j* 27: 2723-2732, 2013.

139. **Penhos JC, Wu CH, Daunas J, Reitman M, and Levine R**. Effect of glucagon on the metabolism of lipids and on urea formation by the perfused rat liver. *Diabetes* 15: 740-748, 1966.

140. **Penton D, Czogalla J, and Loffing J**. Dietary potassium and the renal control of salt balance and blood pressure. *Pflugers Arch* 467: 513-530, 2015.

141. **Peret J, Foustock S, Chanez M, Bois-Joyeux B, and Assan R**. Plasma glucagon and insulin concentrations and hepatic phosphoenolpyruvate carboxykinase and pyruvate kinase activities during and upon adaptation of rats to a high protein diet. *J Nutr* 111: 1173-1184, 1981.

142. **Perucca J, Bichet DG, Bardoux P, Bouby N, and Bankir L**. Sodium excretion in response to vasopressin and selective vasopressin receptor antagonists. *J Am Soc Nephrol* 19: 1721-1731, 2008.

143. **Petersen KF, and Sullivan JT**. Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans. *Diabetologia* 44: 2018-2024, 2001.

144. **Pettit GW, Vick RL, and Kastello MD**. The contribution of renal and extrarenal mechanisms to hypokalemia induced by glucagon. *Eur J Pharmacol* 41: 437-441, 1977.

145. **Pohl SL, Birnbaumer L, and Rodbell M**. Glucagon-sensitive adenyl cylase in plasma membrane of hepatic parenchymal cells. *Science* 164: 566-567, 1969.

146. **Pontiroli AE, Calderara A, Perfetti MG, and Bareggi SR**. Pharmacokinetics of intranasal, intramuscular and intravenous glucagon in healthy subjects and diabetic patients. *Eur J Clin Pharmacol* 45: 555-558, 1993.

147. **Pradervand S, Zuber Mercier A, Centeno G, Bonny O, and Firsov D**. A comprehensive analysis of gene expression profiles in distal parts of the mouse renal tubule. *Pflugers Arch* 460: 925-952, 2010.

148. **Price SR, Du JD, Bailey JL, and Mitch WE**. Molecular mechanisms regulating protein turnover in muscle. *Am J Kidney Dis* 37: S112-114, 2001.

149. **Pullman TN, Lavender AR, and Aho I**. Direct effects of glucagon on renal hemodynamics and excretion of inorganic ions. *Metabolism* 16: 358-373, 1967.

150. **Rabinowitz L**. Do splanchnic potassium receptors initiate a kaliuretic reflex? *News in Physiological Sciences* 6: 166-169, 1991.

151. **Rabinowitz L**. Aldosterone and potassium homeostasis. *Kidney Int* 49: 1738-1742, 1996.

152. **Rabinowitz L, Wydner CJ, Smith KM, and Yamauchi H**. Diurnal potassium excretory cycles in the rat. *Am J Physiol* 250: F930-941, 1986.

153. **Raheja KL, Linscheer WG, Coulson R, Wentworth S, and Fineberg SE**. Elevated insulin/glucagon ratios and decreased cyclic AMP levels accompany the glycogen and triglyceride storage syndrome in the hypothyroid chick. *Horm Metab Res* 12: 51-55, 1980.

154. **Ravier MA, and Rutter GA**. Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells. *Diabetes* 54: 1789-1797, 2005.

155. **Rengarajan S, Lee DH, Oh YT, Delpire E, Youn JH, and McDonough AA**. Increasing plasma [K+] by intravenous potassium infusion reduces NCC phosphorylation and drives kaliuresis and natriuresis. *Am J Physiol Renal Physiol* 306: F1059-1068, 2014.

156. **Rivera N, Everett-Grueter CA, Edgerton DS, Rodewald T, Neal DW, Nishimura E, Larsen MO, Jacobsen LO, Kristensen K, Brand CL, and Cherrington AD**. A novel glucagon receptor antagonist, NNC 25-0926, blunts hepatic glucose production in the conscious dog. *J Pharmacol Exp Ther* 321: 743-752, 2007.

157. **Robert-Nicoud M, Flahaut M, Elalouf JM, Nicod M, Salinas M, Bens M, Doucet A, Wincker P, Artiguenave F, Horisberger JD, Vandewalle A, Rossier BC, and Firsov D**. Transcriptome of a mouse kidney cortical collecting duct cell line: effects of aldosterone and vasopressin. *Proc Natl Acad Sci U S A* 98: 2712-2716, 2001.

158. **Rodbell M, Krans HM, Pohl SL, and Birnbaumer L**. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. 3. Binding of glucagon: method of assay and specificity. *J Biol Chem* 246: 1861-1871, 1971.

159. **Roobol A, and Alleyne GA**. Regulation of renal gluconeogenesis by calcium ions, hormones and adenosine 3':5'-cyclic monophosphate. *Biochem J* 134: 157-165, 1973.

160. **Rorsman P, Ramracheya R, Rorsman NJ, and Zhang Q**. ATP-regulated potassium channels and voltage-gated calcium channels in pancreatic alpha and beta cells: similar functions but reciprocal effects on secretion. *Diabetologia* 57: 1749-1761, 2014.

161. **Rouffignac-de C, Elalouf JM, and Roinel N**. Glucagon inhibits water and NaCl transports in the proximal convoluted tubule of the rat kidney. *Pflügers Arch* 419: 472-477, 1991.

162. **Rubinger D, Wald H, Friedlaender MM, Silver J, and Popovtzer MM**. Effect of intravenous glucagon on the urinary excretion of adenosine 3',5'-monophosphate in man and in rats. Evidence for activation of renal adenylate cyclase and formation of nephrogenous cAMP. *Miner Electrolyte Metab* 14: 211-220, 1988.

163. **Salhi A, Centeno G, Firsov D, and Crambert G**. Circadian expression of H,K-ATPase type 2 contributes to the stability of plasma K(+) levels. *Faseb j* 26: 2859-2867, 2012.

164. **Sammons MF, and Lee EC**. Recent progress in the development of small-molecule glucagon receptor antagonists. *Bioorg Med Chem Lett* 25: 4057-4064, 2015.

165. **Sanchez A, and Hubbard RW**. Plasma amino acids and the insulin/glucagon ratio as an explanation for the dietary protein modulation of atherosclerosis. *Med Hypotheses* 36: 27-32, 1991.

166. **Sandoval DA, and D'Alessio DA**. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. *Physiol Rev* 95: 513-548, 2015.

167. **Santeusanio F, Faloona GR, Knochel JP, and Unger RH**. Evidence for a role of endogenous insulin and glucagon in the regulation of potassium homeostasis. *J Lab Clin Med* 81: 809-817, 1973.

168. **Schutz Y**. Protein turnover, ureagenesis and gluconeogenesis. *Int J Vitam Nutr Res* 81: 101-107, 2011.

169. **Schwartz Sorensen S, Eiskjaer H, Orskov H, and Bjerregaard Pedersen E**. Effect of intravenous glucagon infusion on renal haemodynamics and renal tubular handling of sodium in healthy humans. *Scand J Clin Lab Invest* 53: 25-34, 1993.

170. **Shen DM, Lin S, and Parmee ER**. A survey of small molecule glucagon receptor antagonists from recent patents (2006 - 2010). *Expert Opin Ther Pat* 21: 1211-1240, 2011.

171. **Skov J**. Effects of GLP-1 in the kidney. *Rev Endocr Metab Disord* 15: 197-207, 2014.

172. **Snodgrass PJ, Lin RC, Muller WA, and Aoki TT**. Induction of urea cycle enzymes of rat liver by glucagon. *J Biol Chem* 253: 2748-2753, 1978.

173. **Sorensen H, Winzell MS, Brand CL, Fosgerau K, Gelling RW, Nishimura E, and Ahren B**. Glucagon receptor knockout mice display increased insulin sensitivity and impaired beta-cell function. *Diabetes* 55: 3463-3469, 2006.

174. **Sorensen MV, Grossmann S, Roesinger M, Gresko N, Todkar AP, Barmettler G, Ziegler U, Odermatt A, Loffing-Cueni D, and Loffing J**. Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. *Kidney Int* 83: 811-824, 2013.

175. **Sovik O, Heiervang E, Aksnes L, and Selvig S**. Responses of plasma adenosine 3',5'-monophosphate, blood glucose and plasma insulin to glucagon in humans. *Scand J Clin Lab Invest* 41: 669-674, 1981.

176. **Steele A, deVeber H, Quaggin SE, Scheich A, Ethier J, and Halperin ML**. What is responsible for the diurnal variation in potassium excretion? *Am J Physiol* 267: R554-560, 1994.

177. **Strange RC, and Mjos OD**. The sources of plasma cyclic AMP: studies in the rat using isoprenaline, nicotinic acid and glucagon. *Eur J Clin Invest* 5: 147-152, 1975.

178. **Strazzullo P, Iacone R, Siani A, Barba G, Russo O, Russo P, Barbato A, D'Elia L, Farinaro E, and Cappuccio FP**. Altered renal sodium handling and hypertension in men carrying the glucagon receptor gene (Gly40Ser) variant. *J Mol Med (Berl)* 79: 574-580, 2001.

179. **Suh A, DeJesus E, Rosner K, Lerma E, Yu W, Young JB, and Rosa RM**. Racial differences in potassium disposal. *Kidney Int* 66: 1076-1081, 2004.

180. **Suki WN, Rouse D, Ng RC, and Kokko JP**. Calcium transport in the thick ascending limb of Henle. Heterogeneity of function in the medullary and cortical segments. *J Clin Invest* 66: 1004-1009, 1980.

181. **Sun X, Yang LV, Tiegs BC, Arend LJ, McGraw DW, Penn RB, and Petrovic S**. Deletion of the pH sensor GPR4 decreases renal acid excretion. *J Am Soc Nephrol* 21: 1745-1755, 2010.

182. **Svoboda M, Tastenoy M, Vertongen P, and Robberecht P**. Relative quantitative analysis of glucagon receptor mRNA in rat tissues. *Mol Cell Endocrinol* 105: 131-137, 1994.

183. **Takeda K, Torikai S, Asano Y, and Imai M**. Modulation by verapamil of hormonal action on the Henle's loop of mice. *Kidney Int* 29: 863-869, 1986.

184. **Tiwari S, Sharma N, Gill PS, Igarashi P, Kahn CR, Wade JB, and Ecelbarger CM**. Impaired sodium excretion and increased blood pressure in mice with targeted deletion of renal epithelial insulin receptor. *Proc Natl Acad Sci U S A* 105: 6469-6474, 2008.

185. **Todkar A, Picard N, Loffing-Cueni D, Sorensen MV, Mihailova M, Nesterov V, Makhanova N, Korbmacher C, Wagner CA, and Loffing J**. Mechanisms of renal control of potassium homeostasis in complete aldosterone deficiency. *J Am Soc Nephrol* 26: 425-438, 2015.

186. **Tonolo G, Melis MG, Ciccarese M, Secchi G, Atzeni MM, Maioli M, Pala G, Massidda A, Manai M, Pilosu RM, Li LS, Luthman H, and Maioli M**. Physiological and genetic characterization of the Gly40Ser mutation in the glucagon receptor gene in the Sardinian population. The Sardinian Diabetes Genetic Study Group. *Diabetologia* 40: 89-94, 1997.

187. **Triebwasser KC, and Freedland RA**. The effect of glucagon on ureagenesis from ammonia by isolated rat hepatocytes. *Biochem Biophys Res Commun* 76: 1159-1165, 1977.

188. **Trinh-Trang-Tan MM, and Bankir L**. Regulation of UT-A2 mRNA by chronic glucagon administration (abstract A0129). *J Am Soc Nephrol* 10: 25A, 1999.

189. **Trinh-Trang-Tan MM, Bankir L, Doucet A, el Mernissi G, Imbert-Teboul M, Montegut M, Siaume S, and Morel F**. Influence of chronic ADH treatment on adenylate cyclase and ATPase activity in distal nephron segments of diabetes insipidus Brattleboro rats. *Pflugers Arch* 405: 216-222, 1985.

190. **Turban S, Thompson CB, Parekh RS, and Appel LJ**. Effects of sodium intake and diet on racial differences in urinary potassium excretion: results from the Dietary Approaches to Stop Hypertension (DASH)-Sodium trial. *Am J Kidney Dis* 61: 88-95, 2013.

191. **Ueda J, Nakanishi H, Miyazaki M, and Abe Y**. Effects of glucagon on the renal hemodynamics of dogs. *Eur J Pharmacol* 41: 209-212, 1977.

192. **Unger RH**. Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. *Diabetes* 20: 834-838, 1971.

193. **Unger RH**. Insulin-glucagon ratio. *Isr J Med Sci* 8: 252-257, 1972.

194. **Unger RH, and Cherrington AD**. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest* 122: 4-12, 2012.

195. **Unger RH, and Dallas MD**. Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. *Diabetes* 20: 834-838, 1971.

196. **Vater A, Sell S, Kaczmarek P, Maasch C, Buchner K, Pruszynska-Oszmalek E, Kolodziejski P, Purschke WG, Nowak KW, Strowski MZ, and Klussmann S**. A mixed mirror-image DNA/RNA aptamer inhibits glucagon and acutely improves glucose tolerance in models of type 1 and type 2 diabetes. *J Biol Chem* 288: 21136-21147, 2013.

197. **Vilstrup H, Hansen BA, and Almdal TP**. Glucagon increases hepatic efficacy for urea synthesis. *J Hepatol* 10: 46-50, 1990.

198. **Vuguin PM, and Charron MJ**. Novel insight into glucagon receptor action: lessons from knockout and transgenic mouse models. *Diabetes Obes Metab* 13 Suppl 1: 144-150, 2011.

199. **Wang WH**. Regulation of ROMK (Kir1.1) channels: new mechanisms and aspects. *Am J Physiol Renal Physiol* 290: F14-19, 2006.

200. **West ML, Sonnenberg H, Veress A, and Halperin ML**. The relationship between the plasma potassium concentration and renal potassium excretion in the adrenalectomized rat. *Clin Sci (Lond)* 72: 577-583, 1987.

201. **Wingo CS, Seldin DW, Kokko JP, and Jacobson HR**. Dietary modulation of active potassium secretion in the cortical collecting tubule of adrenalectomized rabbits. *J Clin Invest* 70: 579-586, 1982.

202. **Winzell MS, Brand CL, Wierup N, Sidelmann UG, Sundler F, Nishimura E, and Ahren B**. Glucagon receptor antagonism improves islet function in mice with insulin resistance induced by a high-fat diet. *Diabetologia* 50: 1453-1462, 2007.

203. **Wittner M, and Di Stefano A**. Effects of antidiuretic hormone, parathyroid hormone and glucagon on transepithelial voltage and resistance of the cortical and medullary thick ascending limb of Henle's loop of the mouse nephron. *Pflugers Arch* 415: 707-712, 1990.

204. **Wolfe BM, Culebras JM, Aoki TT, O'Connor NE, Finley RJ, Kaczowka A, and Moore FD**. The effects of glucagon on protein metabolism in normal man. *Surgery* 86: 248-257, 1979.

205. **Xiong Y, Guo J, Candelore MR, Liang R, Miller C, Dallas-Yang Q, Jiang G, McCann PE, Qureshi SA, Tong X, Xu SS, Shang J, Vincent SH, Tota LM, Wright MJ, Yang X, Zhang BB, Tata JR, and Parmee ER**. Discovery of a novel glucagon receptor antagonist N-[(4-{(1S)-1-[3-(3, 5-dichlorophenyl)-5-(6-methoxynaphthalen-2-yl)-1H-pyrazol-1-yl]ethyl}phenyl)carbo nyl]-beta-alanine (MK-0893) for the treatment of type II diabetes. *J Med Chem* 55: 6137-6148, 2012.

206. **Yano Y, Cesar KR, Araujo M, Rodrigues AC, Jr., Andrade LC, and Magaldi AJ**. Aquaporin 2 expression increased by glucagon in normal rat inner medullary collecting ducts. *Am J Physiol Renal Physiol* 296: F54-59, 2009.

207. **Yano Y, Rodrigues AC, Jr., de Braganca AC, Andrade LC, and Magaldi AJ**. PKC stimulated by glucagon decreases UT-A1 urea transporter expression in rat IMCD. *Pflugers Arch* 456: 1229-1237, 2008.

208. **Yu R, Ren SG, and Mirocha J**. Glucagon receptor is required for long-term survival: a natural history study of the Mahvash disease in a murine model. *Endocrinol Nutr* 59: 523-530, 2012.

Table 1. Influence of glucagon on GFR and solute handling studied in "hormone-deprived" rats (to avoid interferences with other hormones)

	Control	Glucagon	Gluc/Control
GFR			
(µl/min per g kidney weight)	726 ± 55	899 ± 39 *	1.24
Fractional excretion (amount excreted in the urine in % of the filtered load)			
Na+	1.21 ± 0.29	0.93 ± 0.24	0.77
K+	17.9 ± 2.0	25.6 ± 2.9	1.43
Pi	2.9 ± 1.4	12.6 ± 2.0 *	4.34
Ca++	8.12 ± 0.40	0.44 ± 0.08 ***	0.05
Mg++	17.8 ± 2.1	3.6 ± 0.9 ***	0.20
Relative concentration in early distal tubule (by micropuncture)			
(= ratio of tubular fluid-to-plasma or tubular fluid-to-plasma ultrafiltrate)			
Inulin	5.03 ± 0.20	3.88 ± 0.24 **	0.77
Na^+	0.38 ± 0.01	0.31 ± 0.01 **	0.82
K^+	0.66 ± 0.02	0.37 ± 0.03 ***	0.56
Pi	0.61 ± 0.18	0.89 ± 0.09	1.46
Ca^{++}	0.61 ± 0.07	0.31 ± 0.02 **	0.51
Mg^{++}	1.66 ± 0.16	0.49 ± 0.08 ***	0.30

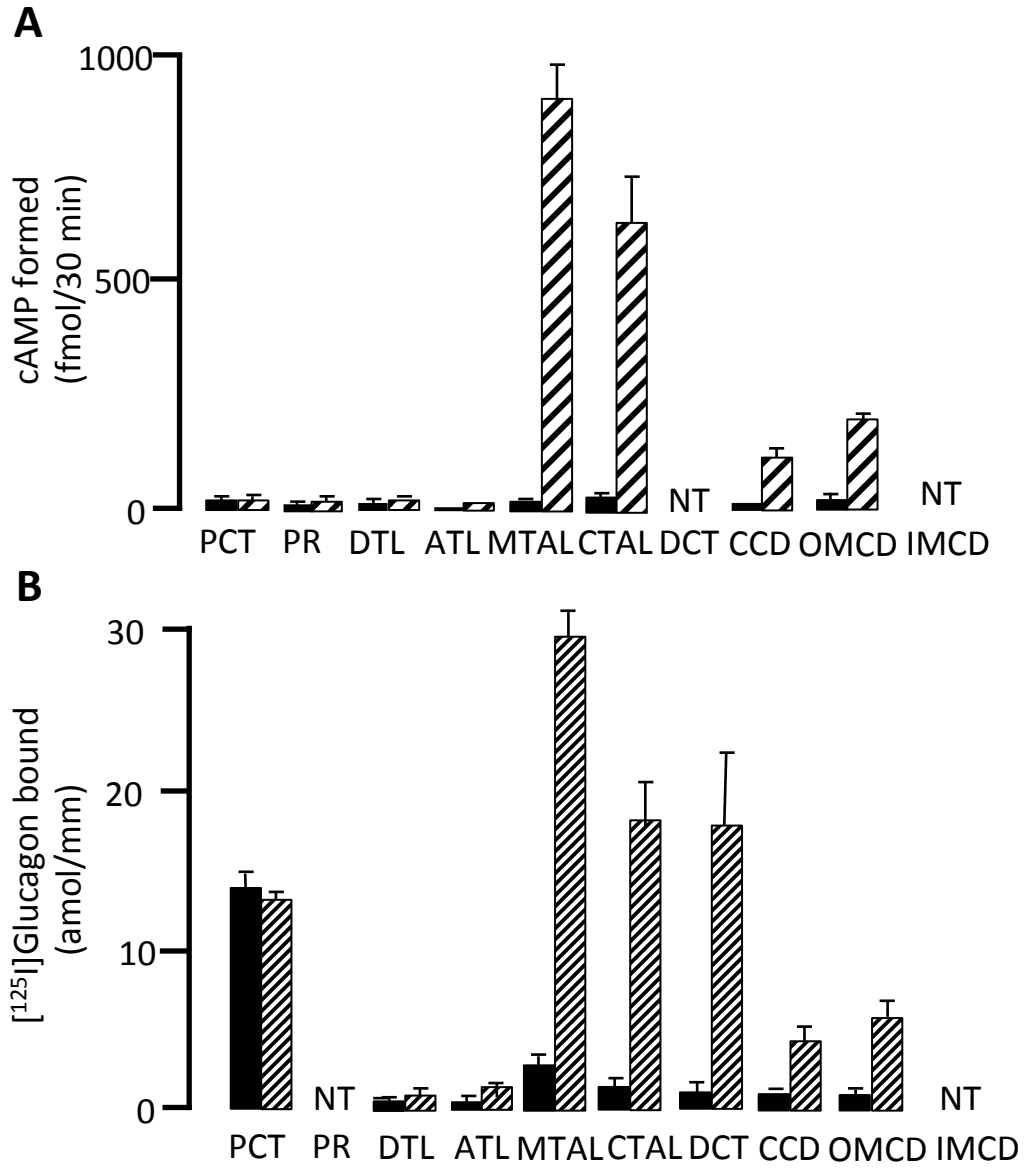
Adapted from Tables 2 and 5 in de Rouffignac et al [158]
Means ± SEM of 5 rats/group. Student's t test: *. p<0.05; **. p<0.01; ***. p<0.001.

Table 2. Simultaneous regulation of glucose, nitrogen and potassium handling by insulin and glucagon, acting on the liver and kidney in a combinatory mode (all intermediate situations between "high" and "low" levels of each hormone are possible).

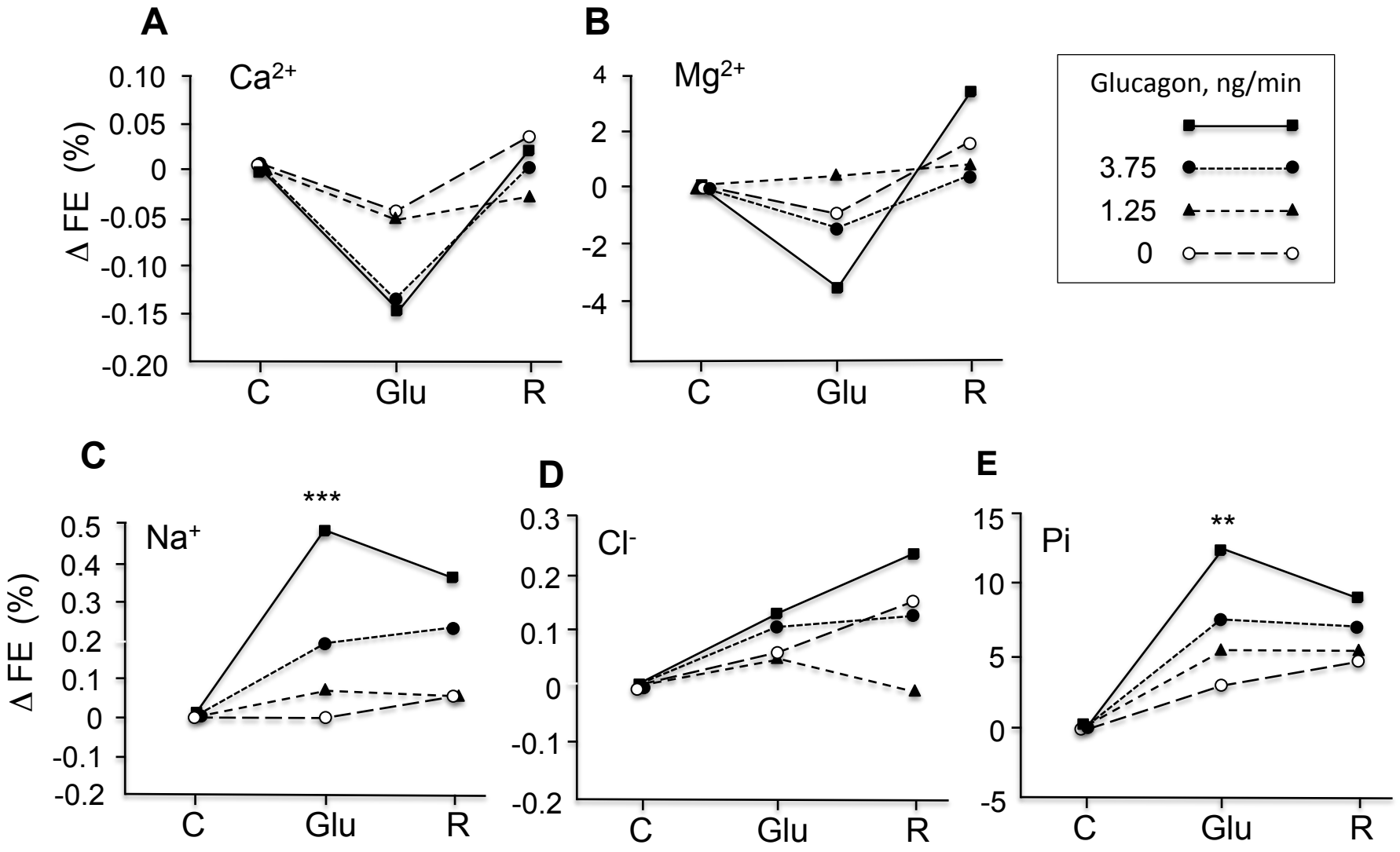
Condition	***Insulin***	***Glucagon***	***Consequence on glucose metabolism***	***Consequence on nitrogen handling***	***Consequence on potassium handling***
Postprandial state (several hours after a meal)	Low	Low	Only modest gluconeogenesis providing glucose for basal metabolism	No significant effect	No significant effect
Fast (exceeding the normal interval between meals)	Low	**High**	Gluconeogenesis from endogenous AAs for sustaining glucose needs of the body	Ureagenesis from endogenous AAs. Excretion of newly synthetized urea	Excretion of potassium issued from the cells from which AAs were catabolized
Carbohydrate-rich meal	**High**	Low	Metabolism and/or storage of the ingested glucose	No significant effect	No significant effect
(a) Meat meal (rich in proteins and potassium) **Or** **(b)** Potassium load or potassium-rich meal	**High**	**High**	**(a)** Increased gluconeo-genesis from ingested AAs (even if no additional glucose is needed). Metabolism and/or storage of the newly-formed glucose	**(a)** Increased ureagenesis from ingested AAs. Increased glucagon-dependent urea excretion	**(a)** and **(b)** Insulin-dependent storage of potassium in cells. Followed by progressive release resulting from glucagon-induced increase in urinary potassium excretion

AAs: amino acids

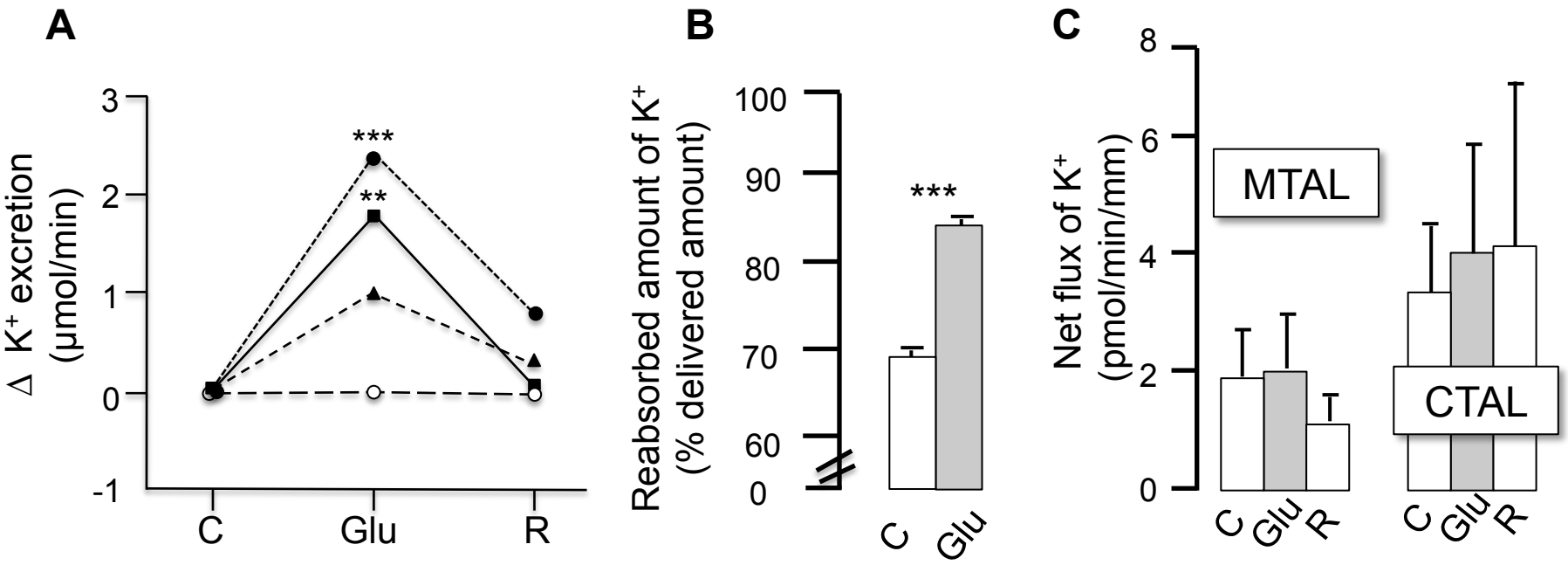
Bankir et al, Figure 1



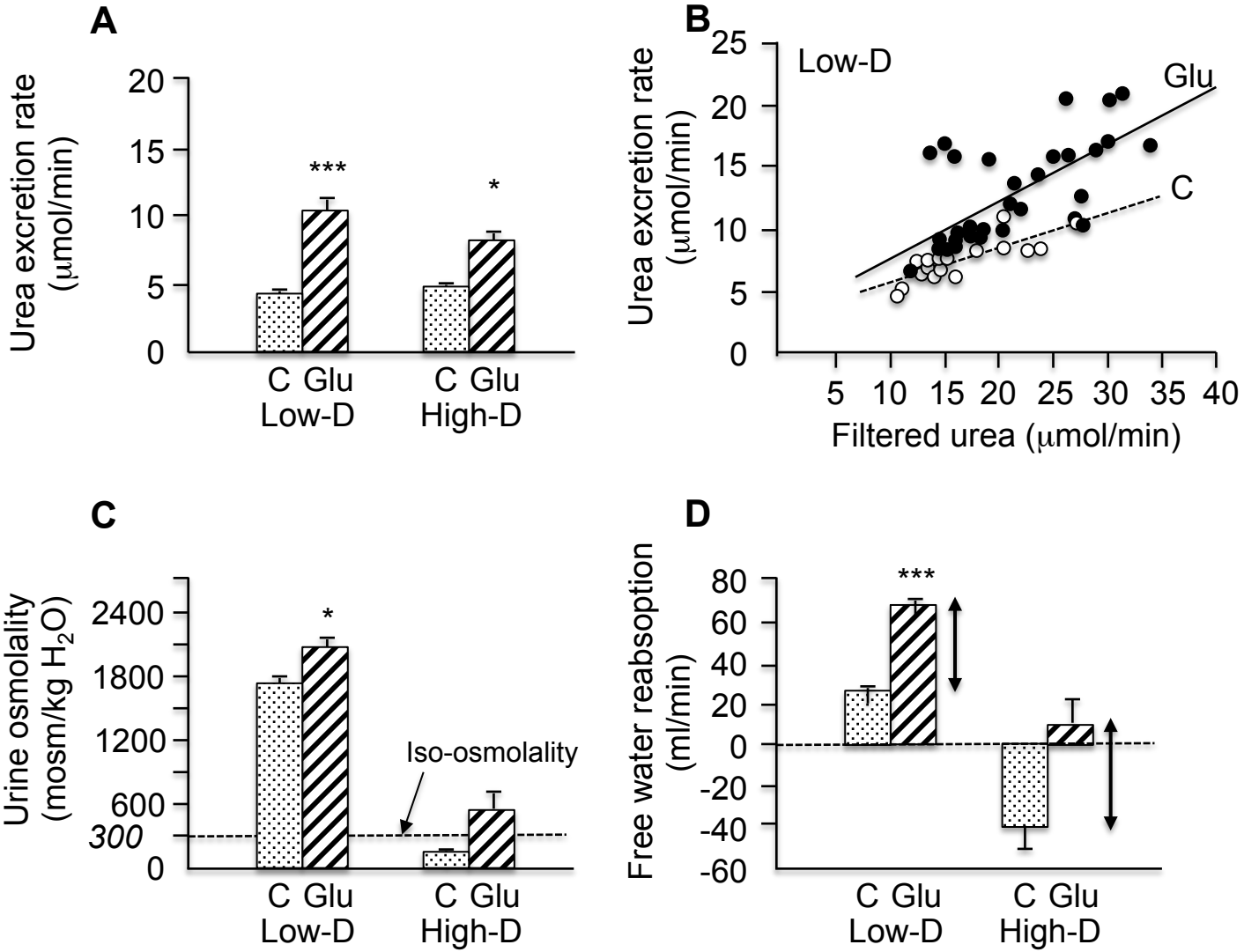
Bankir et al, Figure 2



Bankir et al, Figure 3



Bankir et al, Figure 4



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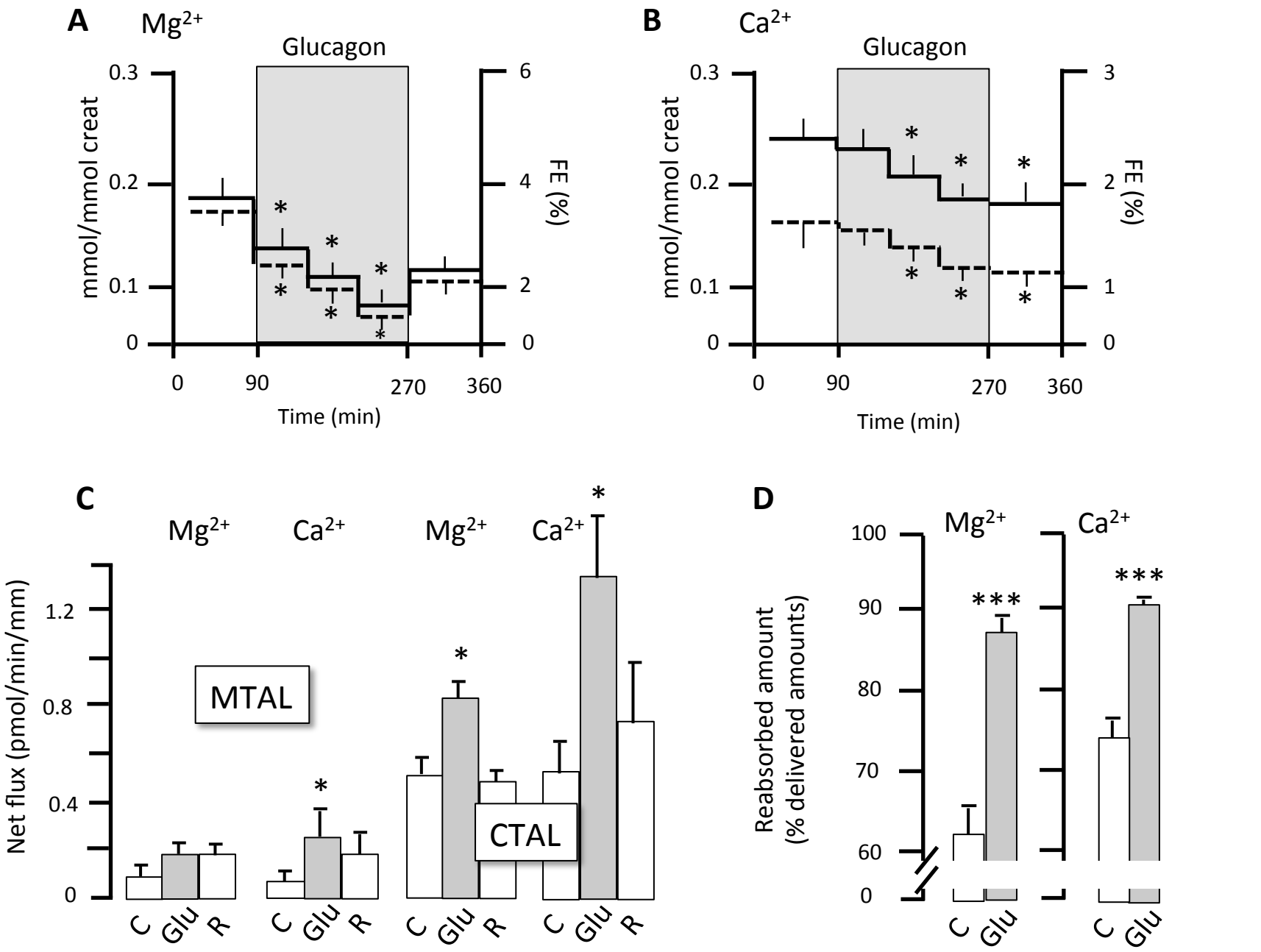
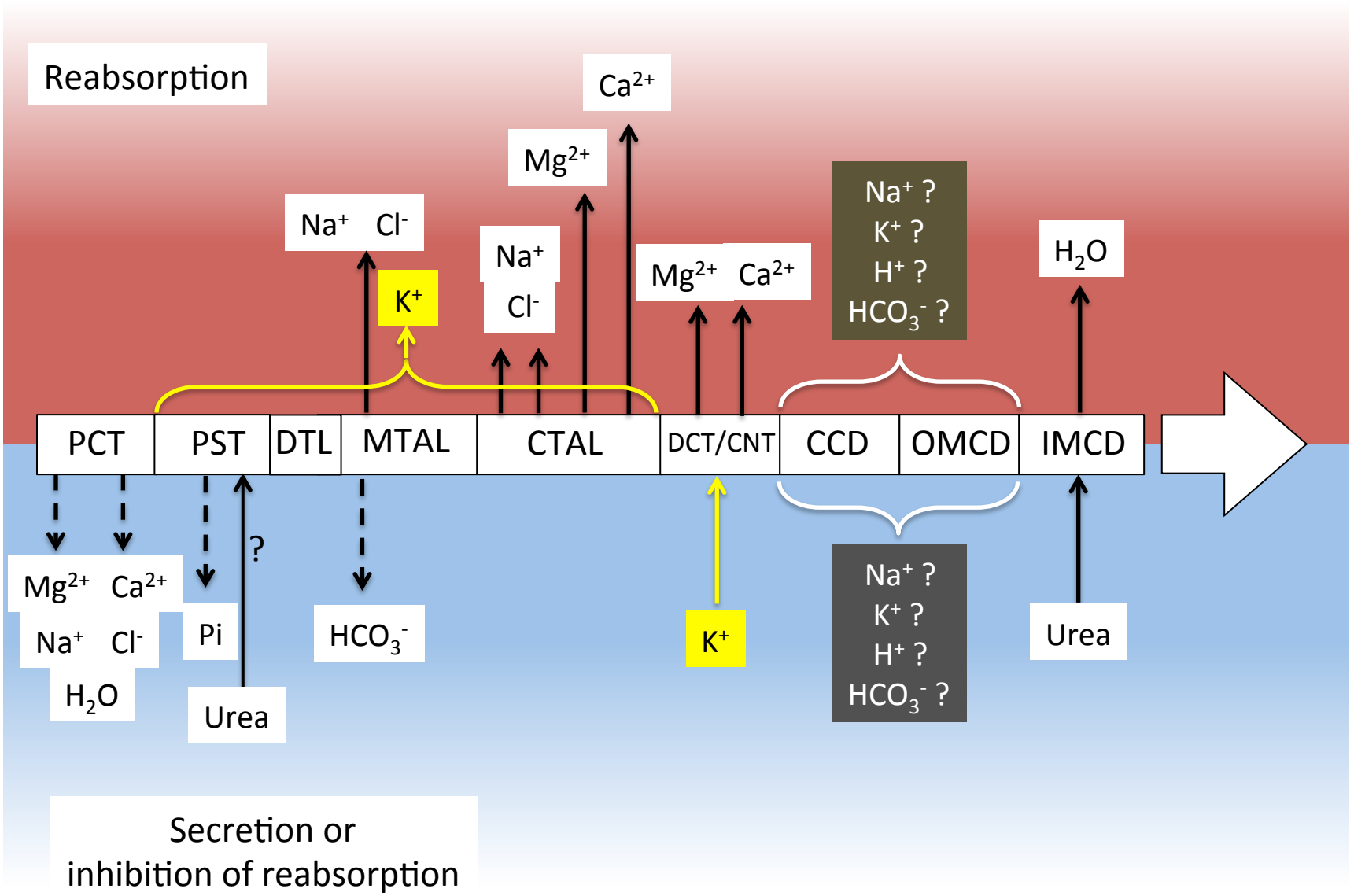
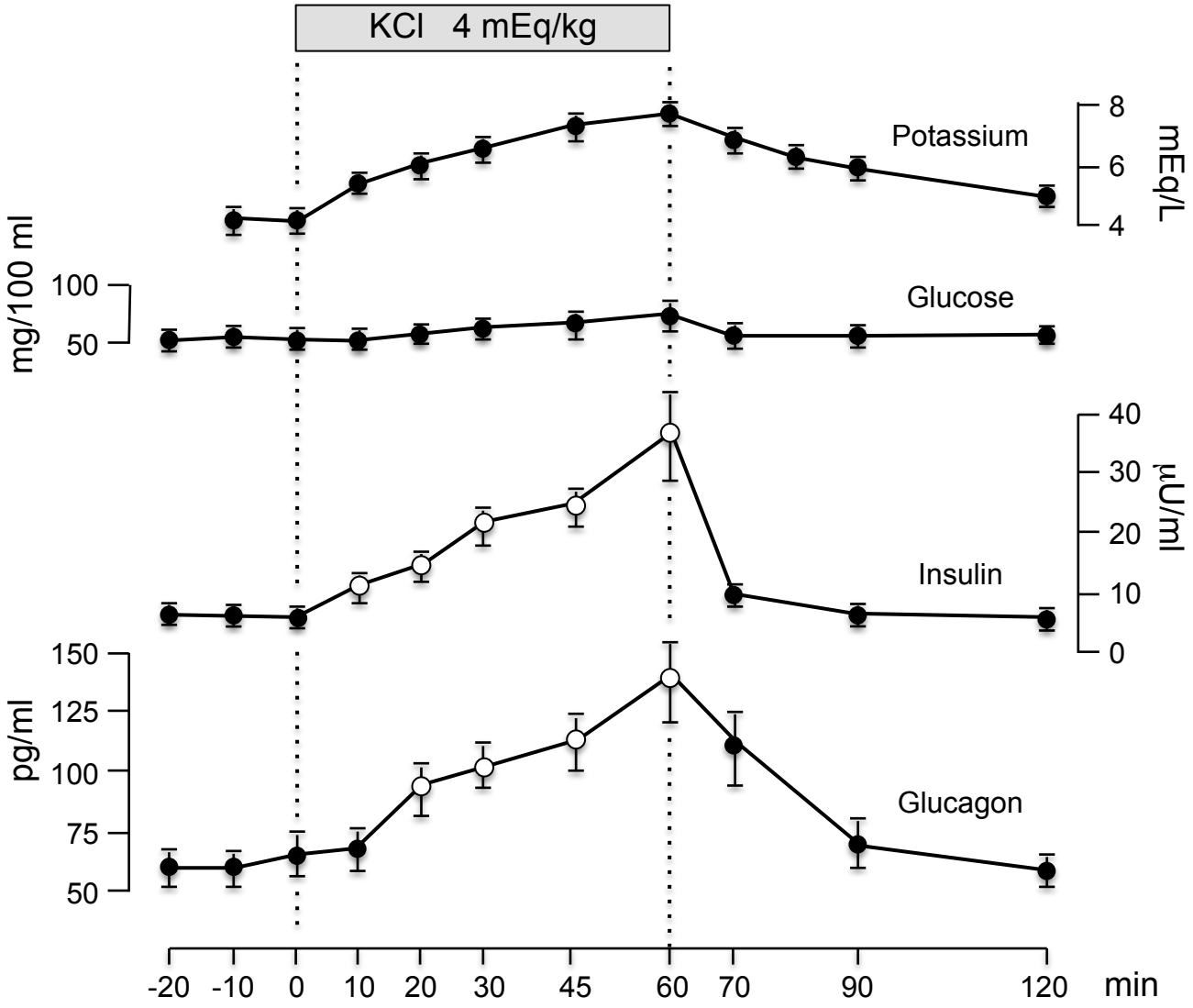
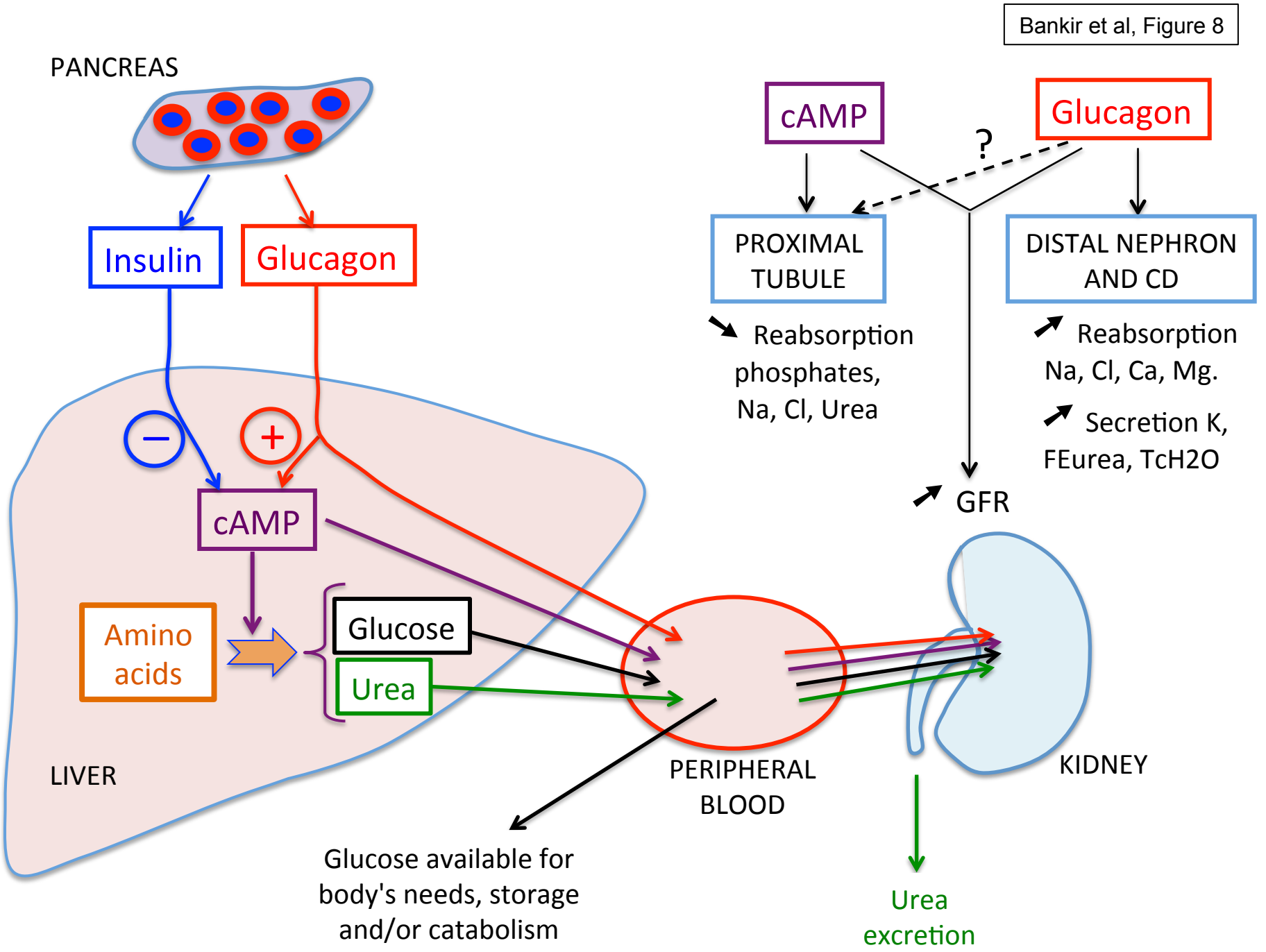


Bankir et al, Figure 6

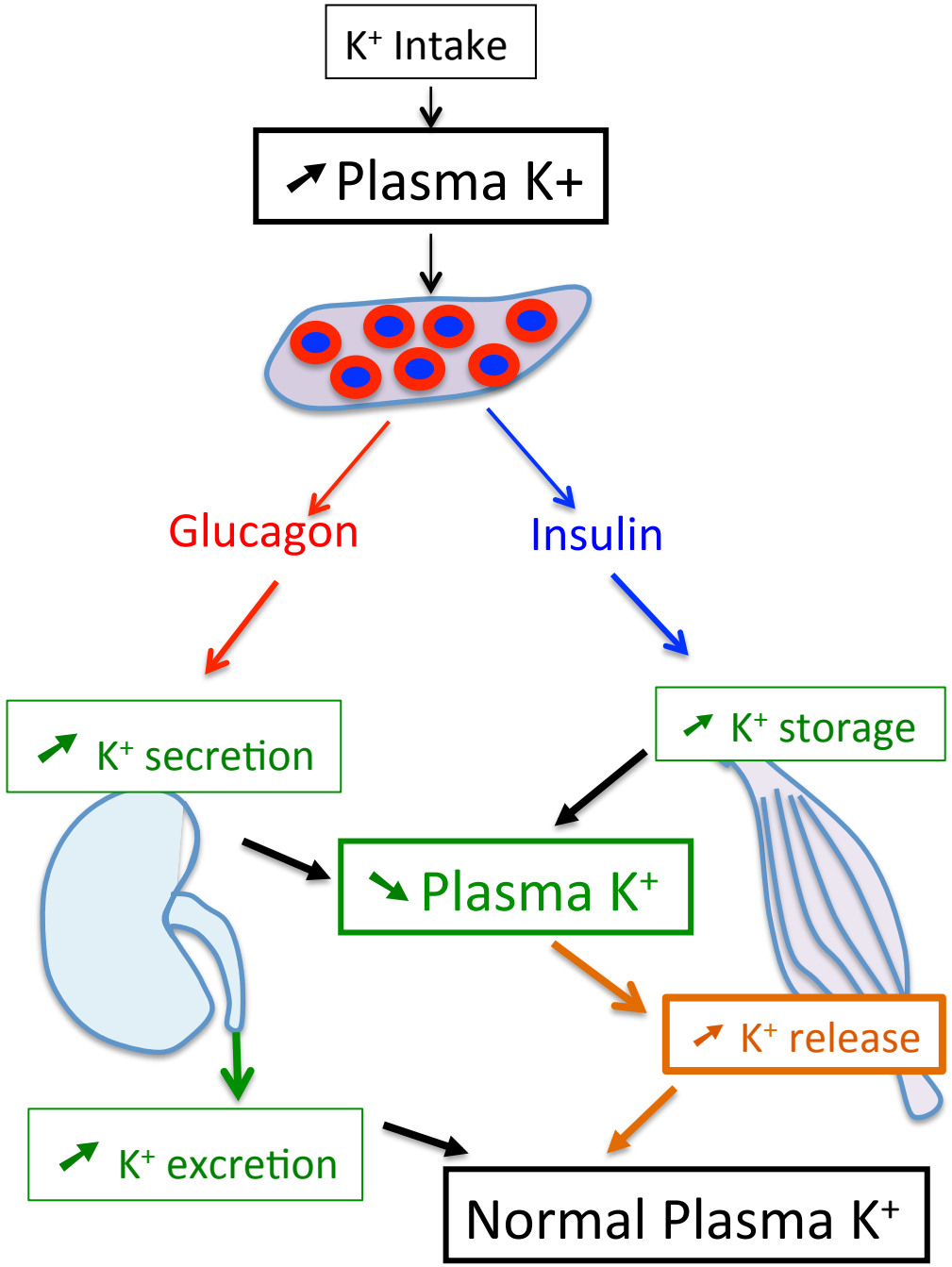


Bankir et al, Figure 7




Bankir et al, Figure 8
PANCREAS
Insulin
Glucagon
cAMP
Glucagon
?
PROXIMAL TUBULE
DISTAL NEPHRON AND CD
Reabsorption phosphates, Na, Cl, Urea
Reabsorption Na, Cl, Ca, Mg.
Secretion K, FEurea, TcH2O
GFR
cAMP
Amino acids
Glucose
Urea
LIVER
PERIPHERAL BLOOD
KIDNEY
Glucose available for body's needs, storage and/or catabolism
Urea excretion

Bankir et al, Figure 9



First events
- K^+ ingestion
- Transitory increase plasma K^+
- Sensing of plasma K^+ by alpha and beta cells

Initial response
- Insulin and glucagon secretion
- K^+ storage in muscle and renal excretion, favoring a decrease in plasma K^+

Associated responses
- Decrease in plasma K^+ induces K^+ release from muscle
- Muscle and plasma K^+ return to normal values