Am J Physiol Renal Physiol 311: F469–F486, 2016.
First published May 18, 2016; doi:10.1152/ajprenal.00560.2015.

Review

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

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Submitted 10 December 2015; accepted in final form 31 March 2016

Bankir L, Bouby N, Blondeau B, Crambert G. Glucagon actions on the kidney revisited: possible role in potassium homeostasis. *Am J Physiol Renal Physiol* 311: F469–F486, 2016. First published May 18, 2016; doi:10.1152/ajprenal.00560.2015.—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 α-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.

sodium; calcium; cAMP; membrane receptor; electrolytes; gluconeogenesis

GLUCAGON HAD BEEN A MATTER of great interest in the 1960s-1980s (see reviews in Refs. 37 and 101). Glucagon's metabolic actions on the liver were shown to be mediated by cAMP 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 radioimmuno-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 (CD) 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 below).

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

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receptor. These mice exhibit a reduced blood glucose concentration (74, 173) and fail to become hyperglycemic after streptozotocin administration (61, 100, 134, 198). New nonpeptide 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 GFR and its role in the hepatic synthesis of urea and disposal of nitrogenous end products 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 α-cell and glucagon in the regulation of potassium homeostasis.

Glucagon Receptors and Glucagon Actions Along the Different Nephron Segments and Collecting System

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 the liver and is expressed to a lower extent in the 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 the liver (44, 76, 182). At the protein level, one study suggested that the canine 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 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 (Fig. 1*A*). 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 in 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 with 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. (109) did not observe cAMP production after incubation of rat IMCD with glucagon (in the presence or absence of AVP) whereas Yano et al. (206) obtained an almost 60-fold increase in cAMP production after glucagon treatment of rat IMCD. Their study also showed a PKA-dependent glucagon-mediated increase in water permeability in this segment.

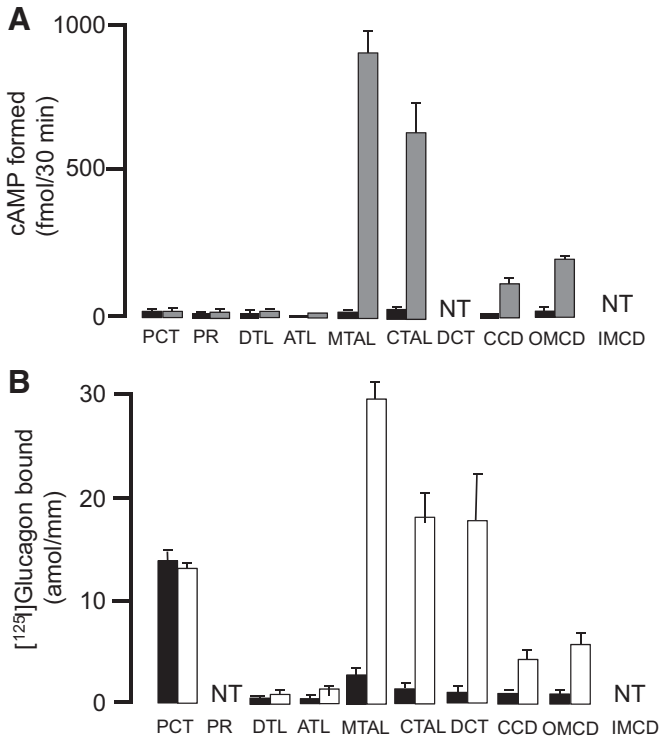

Fig. 1. Adenylate cyclase activity and binding of radiolabeled glucagon in different segments of the rat nephron and collecting duct. *A*: basal (black columns) and stimulated (grey columns) adenylate cyclase activity. *B*: specific binding is the difference between total (white columns) and nonspecific binding (black columns). PCT, proximal convoluted tubule; PR, pars recta; DTL, descending thin limb; ATL, ascending thin limb; MTAL and CTAL, medullary and cortical thick ascending limb, respectively; DCT, distal convoluted tubule; CCD, OMCD and IMCD, cortical, outer medullary, and inner medullary collecting duct, respectively; NT, not tested. Panel *A* is adapted from Ref. 13 and *B* from Ref. 31.

Experiments using a radiolabeled ligand (31) confirmed the functional localization of glucagon receptors along the rat nephron (Fig. 1*B*). 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 nonspecific binding. Of note, Marks et al. (112) 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. 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 CD (34, 35). Interestingly, among the hundreds of GPCR expressed in mouse distal and connecting tubule and CD, the glucagon receptor is one of the most strongly ex-

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, $\mu l \cdot min^{-1} \cdot g$ kidney $weight^{-1}$	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^{2+}	8.12 ± 0.40	0.44 ± 0.08‡	0.05
Mg^{2+}	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
P_i	0.61 ± 0.18	0.89 ± 0.09	1.46
Ca^{2+}	0.61 ± 0.07	0.31 ± 0.02†	0.51
Mg^{2+}	1.66 ± 0.16	0.49 ± 0.08‡	0.30

Values are means ± SE of 5 rats/group. GFR, glomerular filtration rate. Adapted from Tables 2 and 5 in Ref. 161. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ by Student's *t*-test.

pressed (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.

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 were investigated very soon after the discovery of this hormone. In the late 1950s, Staub et al. (50) described that GFR and excretion of different ions, including K^+, Na^+, Cl^-, and P_i, were increased after glucagon infusion in dogs. 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. nontreated kidney. However, the doses of glucagon used in many studies were supraphysiological (0.1–5 μg/min), and a few subsequent works using more physiological doses of glucagon failed to demonstrate an effect on renal Na^+, K^+, Cl^-, and P_i 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. (161) studied the influence of a moderate dose of glucagon (5 $ng \cdot min^{-1} \cdot 100$ g body wt^{-1}) on 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 [(TPTX) to suppress parathyroid hormone (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 three different doses of glucagon (1.20, 12, or 120 $ng \cdot min^{-1} \cdot 100$ g body wt^{-1}). 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 in urinary flow rate were prevented by clamping the level of vasopressin. These well-controlled conditions allowed Ahloulay et al. (4) to reveal two different modes of action of glucagon on the nephron. One was a direct and rapidly reversible effect on glucagon-sensitive nephron segments, influencing the excretion rate of Ca^{2+} and Mg^{2+} (both being reabsorbed) (Fig. 2, *A* and *B*). The other was a slowly reversible, and most likely indirect effect increasing Na^+, Cl^-, and P_i (Fig. 2, *C*, *D*, and *E*), likely secondary to a prior action of glucagon on the liver (release of cAMP in the

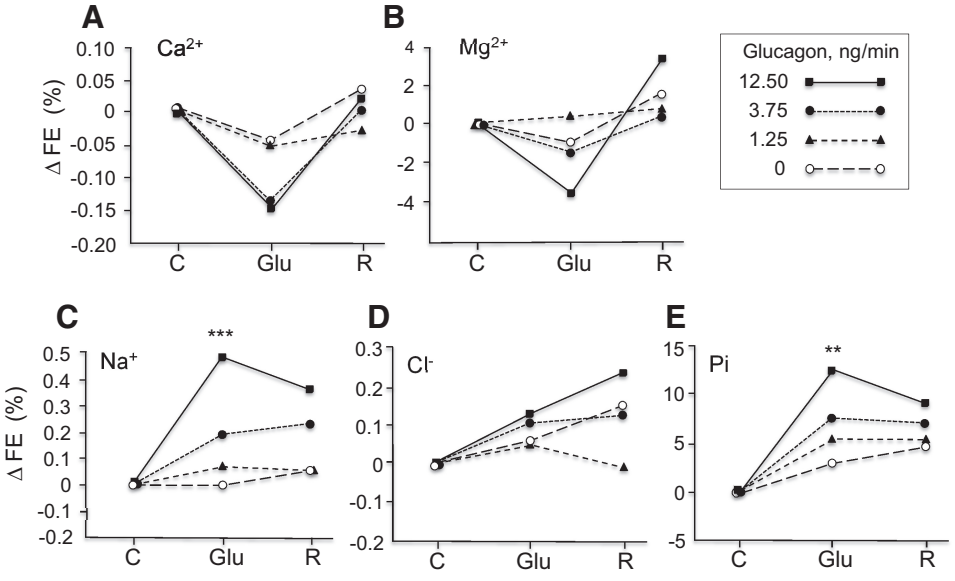

Fig. 2. In vivo clearance experiments in normal anesthetized rats showing changes in the fractional excretion (ΔFE) of several electrolytes induced by glucagon at different infusion rates. C, Glu, and R: control, experimental, and recovery periods, respectively, i.e., before, during, and after glucagon infusion (mean of 3 × 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 $ng \cdot min^{-1} \cdot 100$ g body wt^{-1}, and 0 is time-control. Redrawn and adapted from Ref. 4. **$P < 0.01$. ***$P < 0.001$.

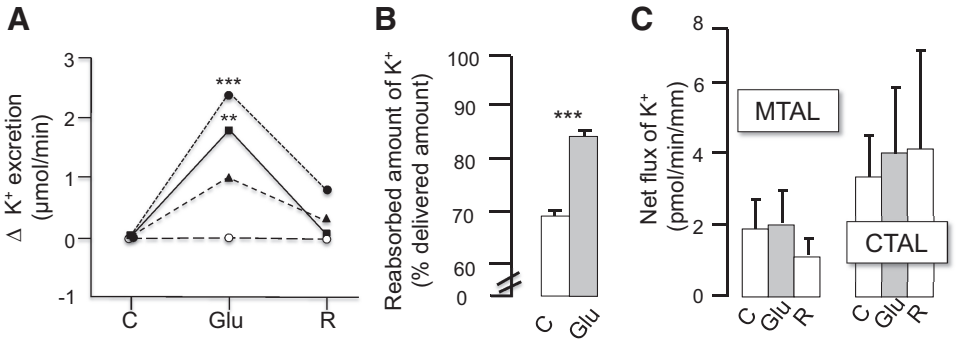

Fig. 3. Influence of glucagon on potassium handling in the rat or mouse kidney. C, Glu, R: control, experimental, and recovery periods, respectively. *A*: during in vivo clearance experiments in normal anesthetized rats. Infusion rates are as in Fig. 2. *B*: results obtained during in vivo micropuncture 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 the 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. Panel *A*, adapted from Ref. 4, *B* and *C* from Ref. 42. $^{**}P < 0.01$. $^{***}P < 0.001$.

blood) as shown in a later study (3, 4) (see review in Ref. 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 (Fig. 3, *A–C*) (see details below). Interestingly, glucagon also increased urea excretion rate (Fig. 4, *A* and *B*) and promoted water reabsorption, inducing an increase in urine osmolality (Fig. 4, *C* and *D*) (2) (see details below).

To our knowledge, only one study investigated the influence of a chronic glucagon administration on urinary electrolyte excretion (18). Glucagon (100 μg/day) infused ip in normal rats by osmotic implantable minipumps for 3 wk raised basal plasma glucagon concentration threefold. Measurements during the last 3 days showed a 14% lower creatinine clearance ($P < 0.05$), a 23% higher urine osmolality, and a 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 vs. control rats (18). On 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 details).

Glucagon is also a significant player in the regulation of the acid-base balance. Paillard and colleagues (40, 116) showed, in 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 a decrease in titratable net acid secretion. As a result, urine alkalinization rose by 0.6 unit of pH (40, 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 plurihormonal control of acid-base balance (131).

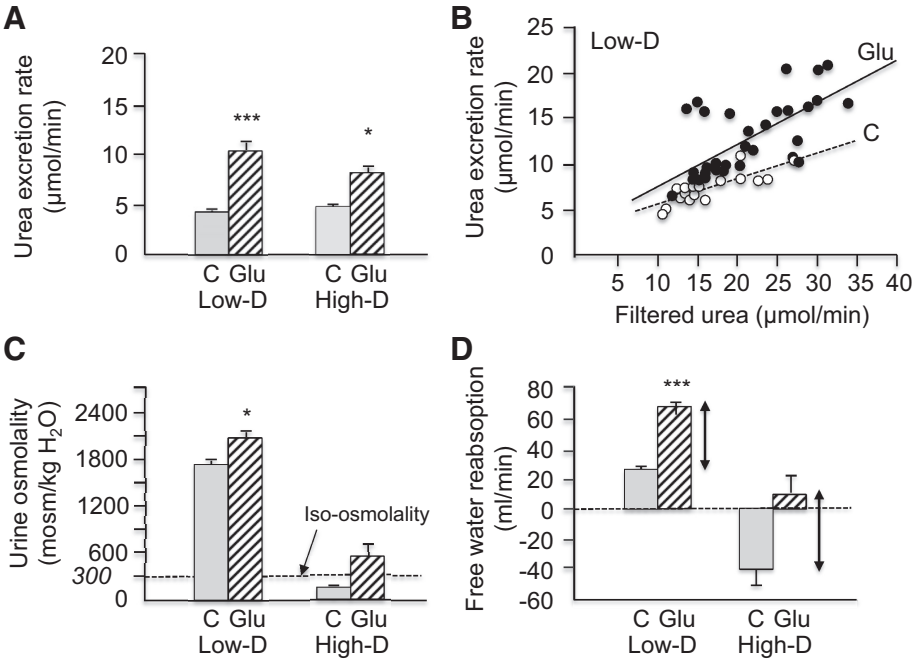

Fig. 4. Influence of glucagon on urinary urea excretion rate (*A* and *B*) and on urine concentration (*C* and *D*) in anesthetized rats during clearance experiments. Measurements were made in separate groups of rats, with either a low diuresis induced by dDAVP infusion (Low-D) or a high diuresis induced by infusion of dilute saline (High-D). C and Glu, control and glucagon infusion periods. Adapted from Ref. 2. Data shown in *B* were 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. $^{*}P < 0.05$. $^{***}P < 0.001$.

The regulatory effects of glucagon on urinary solute excretion have also been observed in humans. Friedlander et al. (59) studied the influence of glucagon, within a physiological range (i.e., a 4-fold increase in its plasma concentration), in eight young healthy volunteers. As shown in Fig. 5, *A* and *B*, 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 unit, 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 subsegments has been obtained by two main techniques: *1*) in vivo micropuncture allowing collection of tubular fluid with micropipettes inserted in different accessible sites along the nephron at the surface of the cortex or papilla; and *2*) in vitro microperfusion of well-identified nephron and CD segments or subsegments isolated by microdissection. The main results of these studies are detailed below and summarized in Fig. 6.

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 threefold increase in the absolute and fractional 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 suggests 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 hypothesized 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 iv infusion of cAMP induced the same effects as did glucagon at a dose susceptible to reproduce the plasma concentration prevailing in the liver (~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, 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

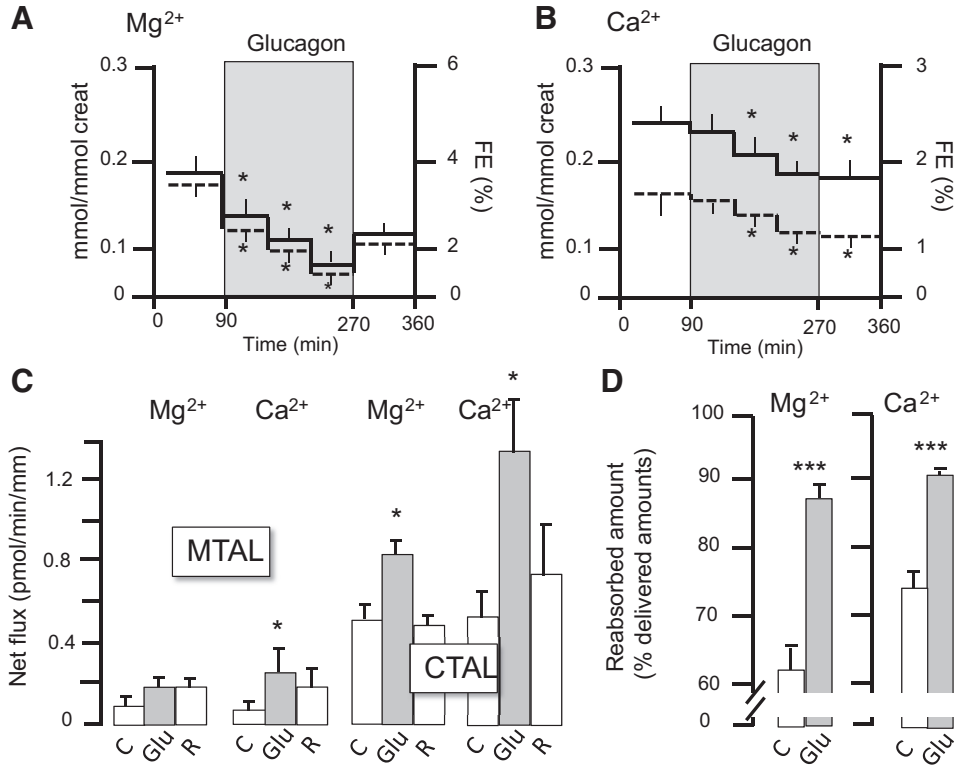

Fig. 5. Influence of glucagon on calcium and magnesium handling in humans and mice. *A* and *B*: clearance study in healthy humans showing the concentration and the fractional excretion of calcium and magnesium. *Significant difference from the basal period. Redrawn and adapted from Ref. 59, $P < 0.05$. *C*: in vitro microperfusion experiment of isolated microdissected mouse mTAL and cTAL during control (C), glucagon infusion (Glu), and recovery (R) periods. *Significant difference from the pre- and posttreatment periods, $P < 0.05$. *D*: in vivo micropuncture experiments of the loop of Henle in "hormone-deprived" rats (see text and Fig. 3) untreated (C) or treated with glucagon (Glu). Redrawn and adapted from Ref. 42. ***Significant difference from the untreated group (unpaired *t*-test, $P < 0.001$).

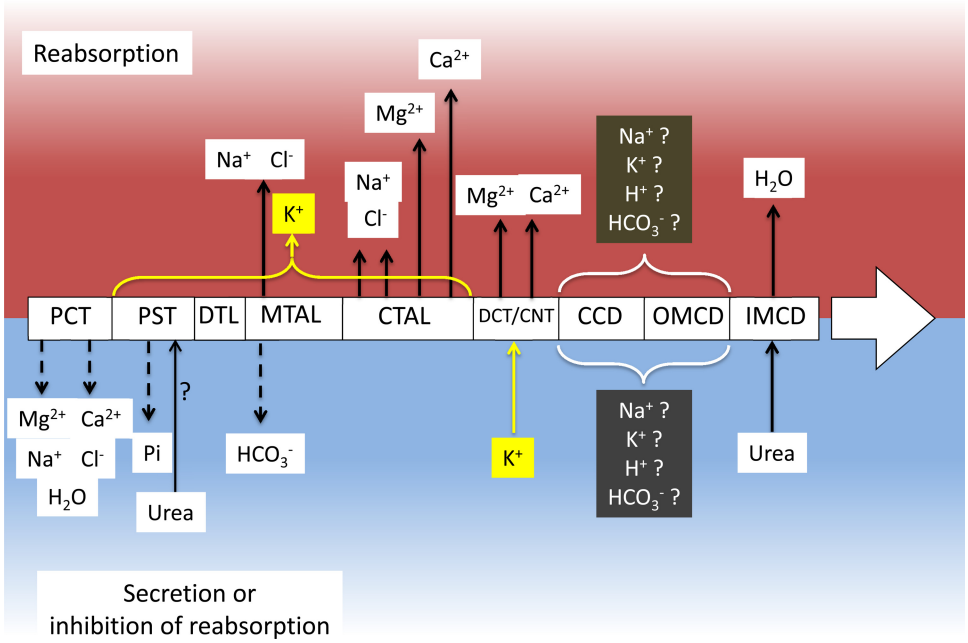

Fig. 6. Diagram showing the effects of glucagon on solute transport along the different nephron segments. *Top*: arrows indicate glucagon-activated reabsorption. *Bottom*: continuous arrows indicate glucagon-activated secretion, whereas dashed arrows indicate glucagon-induced inhibition of reabsorption. Effects on K^+ transport are highlighted in yellow. The lack of information for the CCD is displayed by black boxes. Abbreviations are as in Fig. 1.

in ion transport through a transcellular (Na^+, Cl^-, K^+, HCO_3^-, NH_4^+) or a paracellular (Mg^{2+} and Ca^{2+}) pathway (see recent review in Ref. 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 1 μM 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 flux measurements, Di Stefano et al. (42) reported that glucagon (10 nM) reversibly increased Na^+ and Cl^- reabsorption in both MTAL (by 30–40%) and CTAL (by 10%) (Fig. 5*D*) (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, additional evidence that glucagon stimulates the transcellular pathway. However, in this study, Ando et al. (8) did not observe modifications of the PDte as shown in mouse MTAL. 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. (42) also reported a rather strong stimulation of Mg^{2+} and Ca^{2+} reabsorption (by 60 and 140%, respectively) (Fig. 5*C*). 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 Fig. 3*C*.

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 Figs. 3*B* and 5*C*). 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 (Fig. 3*B*) cannot be attributed to the TAL (Fig. 3*C*). 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. (116) showed that glucagon reduced bicarbonate reabsorption in Henle's loop, leading to a 45% increase in bicarbonate delivery to the early distal tubule. This effect was further confirmed and completed by David Good (65), who showed that incubation of isolated microperfused rat MTAL with 2 nM glucagon lead to a 35% reduction of bicarbonate reabsorption. The CTAL was not studied in these experiments.

DISTAL CONVOLUTED TUBULE. To our knowledge, only one study addressed the effects of glucagon on the DCT. By micropuncture of early and late distal tubules in hormone-deprived rats, Bailly et al. (15) compared the effects of glucagon and PTH on electrolyte transport in hormone-deprived rats. This study showed that glucagon stimulates Ca^{2+} and Mg^{2+} reabsorption in the DCT independently of the loads

delivered to the distal tubules or the plasma concentrations of Ca^{2+} or Mg^{2+}. However, 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 corticomedullary axis and are usually segmented into cortical, outer medullary, and inner medullary CDs (CCD, OMCD, and IMCD, respectively). These subsegments 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 three different cell types, the principal cells (PC), the α-intercalated cells (α-IC) and the β-intercalated cells (β-IC). Here again, each cell type is involved in specific functions. Up until recently, the reabsorption of Na^+ and water was attributed to PC whereas α-IC were involved in acid excretion and β-IC in base excretion. This dogma has been revisited recently with the discovery of paracrine cross talk 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 immunolabeling, or in situ hybridization, etc.) has not been reported for the CD. The opposite responses induced by adrenergic or cholinergic agonists in 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^+ transport in isolated microperfused CCD or OMCD. Laroche-Joubert et al. (98) demonstrated that the incubation of rat OMCD with 1 μM glucagon activated 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 Magaldi group (206, 207) investigated the action of glucagon on IMCD and, more particularly, its role in 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). These modifications are related to changes in aquaporin-2 (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.

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, which 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 Fig. 4, *A* and *B*. 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 yet been clarified. 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 participates in the concentrating activity of the kidney by an effect additive to that of the antidiuretic hormone vasopressin (AVP) (19). Figure 4, *C* and *D*, shows that, in rats with experimentally induced either 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). 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 descending thin limb, 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 significant water economy.

Because some gluconeogenesis occurs in the kidney, it was interesting to evaluate whether glucagon stimulates this metabolic process in the kidney, as it does in the liver. Roobol et al. (159) 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. More recently, Mutel et al. (126) showed that glucagon-stimulated renal gluconeogenesis contributed to maintain plasma glucose concentration in mice in the absence of hepatic gluconeogenesis. However, no such effect had been observed in normal dogs (72). Glucagon's 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 whether glucagon really influences proximal tubule metabolism in addition to its effect on electrolyte and urea transport.

Possible Role of the α-Cell and Glucagon in Potassium Homeostasis

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 a review, see Ref. 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 (38, 67, 71, 115, 127). But although potassium could not be stored in cells permanently, no explanation is provided of how potassium leaves the cells and is excreted after this temporary storage. Yet, potassium excretion is relatively fast compared with 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 before 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 (Fig. 3*A*) (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 (39, 49, 113, 144), and of simultaneous changes in urine flow rate that also influence potassium handling in the CD (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 α-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 humans, or in isolated perfused pancreas (39, 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). However, the most interesting study is that of Santeusanio et al. (167), who showed marked elevations in plasma glucagon and insulin concentrations in conscious dogs during an infusion of KCl, as

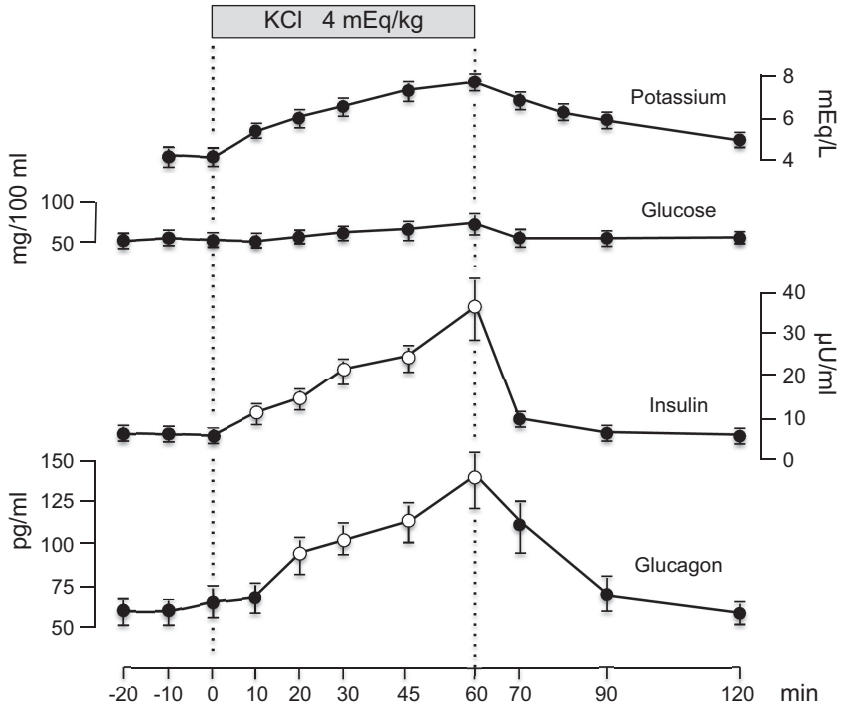

Fig. 7. Influence of KCl infusion on plasma glucagon, insulin, and glucose in normal dogs. Open circles indicate significant differences compared with mean baseline values. Reproduced from Ref. 167.

illustrated in Fig. 7. Glucose concentration did not change. 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 α-cells could "sense" potassium concentration; and *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.

Putative role of the α-cell as a potassium sensor. Could α-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 the 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 iv infusion of KCl in anesthetized dogs induced marked, significant, and parallel increases in plasma insulin and glucagon without affecting glycemia (Fig. 7). When the production of insulin was impeded by treatment with alloxane, the tolerance toward 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 resolve two issues. First, the increase in 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 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 α-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 α-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 debatable (160), it is, however, tempting to assume that an elevation in extracellular K^+, which directly depolarizes the plasma membrane, may stimulate the exocytosis of glucagon. Indeed, Rorsman et al. (160) showed that an experimental depolarization of α-cells activated the P/Q-type Ca^{2+} channel present at the cell surface. 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 α-cells. For a long time, membrane receptors were assumed to bind organic molecules, i.e., hormones or mediators, not minerals. However, because calcemia is tightly regulated, Brown et al. (28) hypothesized that a specific membrane receptor might be sensitive to 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, was identified and shown to play a role in the maintenance of acid-base balance (181). The rationale behind the search for a calcium sensor is also valid for potassium. Because the 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 α-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 β-adrenergic receptors (68). This putative K^+ sensor remains to be identified.

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 vary either 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 the disposal 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. (167) in conscious dogs (Fig. 7).

It is interesting to emphasize the relationships 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 despite a twofold 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 colleagues (73), urea and potassium excretions may be closely interrelated. 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 the 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 α-cells show that glucagon is not required for general health (74). However, mice lacking the glucagon receptor (mimicking human Mahvash disease due to inactivating mutations of the glucagon receptor) became progressively hypoglycemic, lethargic, and cachexic after 12 mo and exhibited a much lower survival rate than heterozygous or wild-type mice (208). Thus glucagon is required for long-term survival.

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) [b]Potassium load or potassium-rich meal	High	High	[a]Increased gluconeogenesis 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,b]Insulin-dependent storage of potassium in cells. Followed by progressive release resulting from glucagon-induced increase in urinary potassium excretion

AAs, amino acids.

Physiological aspects. During a fast, glucagon plays its well-known role of stimulating gluconeogenesis, but this also implies excretion of nitrogen from the endogenous amino acids used as a substrate. After a meal, the end products 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 α-cells could express a potassium sensor protein that would stimulate glucagon secretion and participate, in conjunction with insulin, to potassium homeostasis, as depicted in Fig. 9.

The role of glucagon is obviously related to 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. However, it is important to note that carnivores eat infrequent 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.

Pathophysiological aspects. The biological half-life of glucagon is short (a few minutes) (10, 146). Thus the effects of glucagon occur promptly and are rapidly reversible. It is mostly degraded by the liver and kidneys (102). In chronic kidney disease, 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 DM, glucagon is elevated and its actions are no longer counteracted by insulin. Moreover, glucagon's 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 α-cells in patients with type 2 DM and in diabetic mice (23).

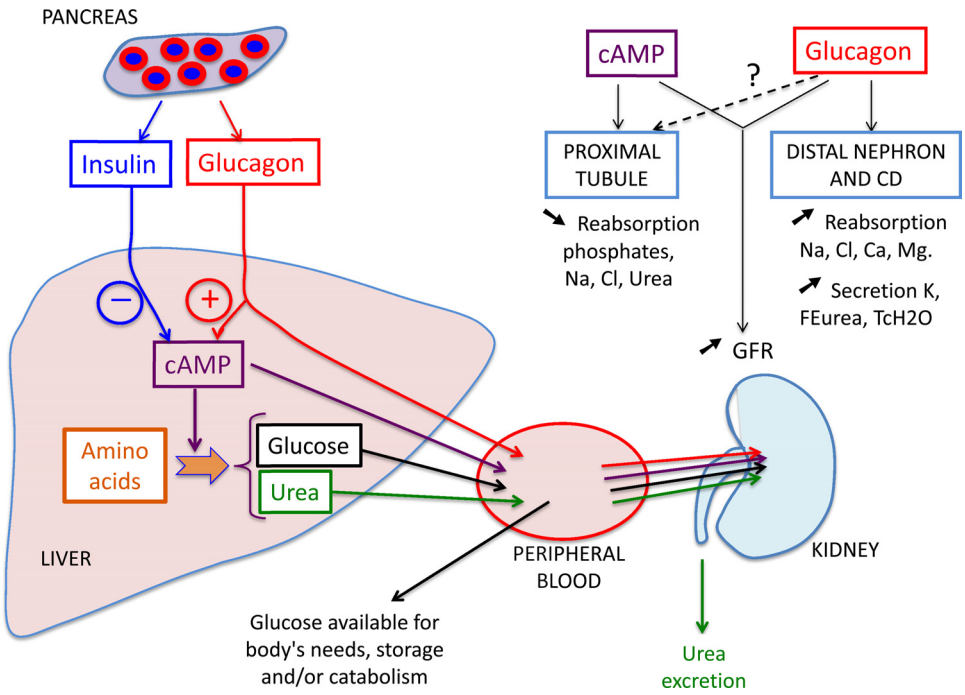

Fig. 8. Proposed view of the direct and indirect effects of glucagon (combined with liver-borne cAMP) on glomerular filtration rate (GFR) and solute handling by the nephron.

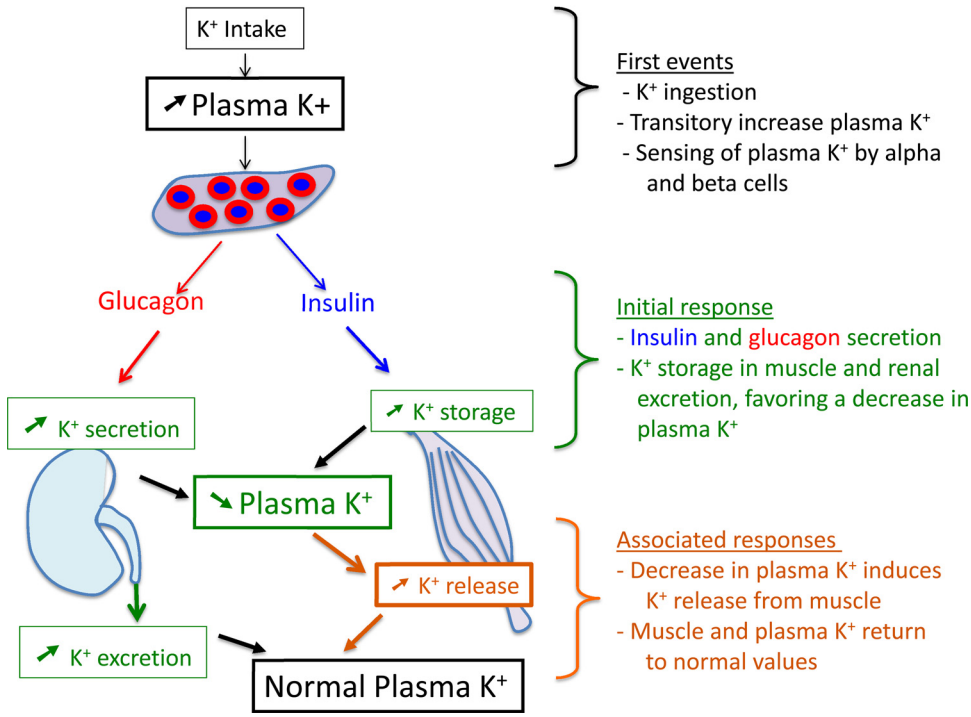

Fig. 9. Proposed view of the combined effects of insulin and glucagon on potassium homeostasis.

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 α-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 the 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 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 α- and β-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 a consideration of 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 Dietary Approaches to Stop Hypertension diet (190). The possible contribution of glucagon to these ethnic differences in potassium handling deserves 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 was done too often for a few decades. Note that glucagon should not be measured only in the morning after a night's fast in humans, or during daytime, the resting period in rodents. It is important to evaluate its elevation above the basal state in the 2–3 h 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 classic metabolic end points.

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 expresses 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 (41)? 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 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 with 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 knockout mice and wild-type mice after an amino acid load or a potassium load, in a setting maintaining stable glycemia and vasopressin concentrations, or during DM, should reveal whether 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 α-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 subsegments of the CD (without or with pretreatment by aldosterone) could confirm whether glucagon indeed stimulates potassium secretion, and investigate the intracellular pathway at the molecular level. Mathematical models may help in an understanding of how glucagon and insulin secretion can combine their respective influences to regulate independently 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.

ACKNOWLEDGMENTS

During her visits to the Mount Desert Island Biological Laboratory in Maine and to Nashville, TN, in 1995, 1997, and 1998, L. Bankir had several discussions with Steve Hebert (who unfortunately passed away in 2008). It is Steve who proposed that there might be a cell membrane potassium "sensor" like the one he had discovered for calcium (78). But, he had no idea in which organ this sensor could be expressed, neither which hormone could be secreted in response to its stimulation. Our observations that physiological concentrations of glucagon stimulate potassium secretion in the rat kidney (4) led us to imagine that glucagon might be this hormone and that, consequently, the α-cell of the pancreatic islets could express a membrane protein that could play the role of a potassium sensor.

GRANTS

The authors acknowledge the financial contribution of the European Foundation for the Study of Diabetes (EFSD/Boehringer Ingelheim Programme 2014).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

L.B. provided conception and design of research; L.B., B.B., and G.C. analyzed data; L.B. and G.C. prepared figures; L.B. and G.C. drafted manuscript; L.B., N.B., B.B., and G.C. edited and revised manuscript; L.B., N.B., B.B., and G.C. approved final version of manuscript; N.B. performed experiments; N.B. interpreted results of experiments.

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