Glucagon Metabolism in the Rat

CONTRIBUTION OF THE KIDNEY TO THE METABOLIC CLEARANCE RATE OF THE HORMONE

D. S. EMMANOUEL, J. B. JASPAN, A. H. RUBENSTEIN, A. H-J. HUEN, E. FINK, and A. I. KATZ, *Department of Medicine, Division of Biological Sciences of the University of Chicago, Pritzker School of Medicine, Chicago, Illinois 60637*

ABSTRACT The renal handling of the biologically active glucagon component (the 3,500-mol wt fraction of immunoreactive glucagon [IRG]) and the contribution of the kidney to its overall peripheral metabolism were studied in normal and uremic rats. The metabolic clearance rate of glucagon was 31.8 ± 1.2 ml/min per kg in normal animals and was diminished by approximately one-third in each of three groups of rats with compromized renal function: 22.3 ± 1.6 ml/min per kg in partially (70%) nephrectomized; 22.9 ± 3.3 ml/min per kg in bilaterally ureteral ligated; and 23.2 ± 1.2 ml/min per kg in bilaterally nephrectomized animals. In normal rats the kidney contributed 30% to the overall metabolic clearance of the hormone and the renal extraction of endogenous and exogenous glucagon was similar, averaging $22.9 \pm 1.6\%$ and was independent of plasma IRG levels over a wide range of arterial concentrations. The remnant kidney of partially (70%) nephrectomized animals continued to extract substantial amounts ($16.6 \pm 4.2\%$) of the hormone, but accounted for only 8% of the total peripheral catabolism of IRG. In the two groups of animals with filtering kidneys, renal glucagon uptake was linearly related to its filtered load and could be accounted for by glomerular filtration and tubular reabsorption. However, the kidneys of animals with both ureters ligated (renal extraction of inulin = $3.2 \pm 1.8\%$) and hence virtual absence of glomerular filtration, continued to extract $11.5 \pm 1.9\%$ of the renal arterial glucagon, contributing by 9% to its overall metabolic clearance, indicating that IRG uptake occurs also from the post glomerular capillaries.

INTRODUCTION

Alterations in the endocrine milieu, which result in part from the inability of the diseased kidney to degrade polypeptide hormones, are prominent features of the uremic syndrome (1). Glucagon is such a hormone, and in uremic man and animals the substantial elevations of its circulating levels and alterations in its immunoreactive composition have been attributed to the impaired renal degradation rather than the biochemical environment of uremia (2–9). In fact, the uremic state per se fails to cause hyperglucagonemia or changes in immunoreactive glucagon (IRG)[1] composition in animals with normal kidneys rendered azotemic by urine autoinfusion. On the other hand, cessation of glomerular filtration rate (after ligation of both ureters) or bilateral nephrectomy leads to marked and quantitatively similar increments in total plasma IRG (9).

Compositional alterations of the circulating IRG patterns are observed only in anephric animals, in which a proglucagon-like material with a molecular weight of 9,000 constitutes more than a third of the hormonal immunoreactivity. In contrast, the hyperglucagonemia observed after ureteral ligation is almost entirely a result of accumulation of the 3,500-mol wt glucagon component, which is the biologically active fraction of the circulating hormone (10). These findings confirm the importance of the renal parenchyma in the peripheral metabolism of the hormone, and suggest that the various immunoreactive glucagon components may be handled by different renal mechanisms, that have not yet

been clarified (9). The present study was therefore undertaken in order to elucidate the disposition of the 3,500-mol wt glucagon component by the normal rat kidney and to define the renal contribution to the overall peripheral metabolism of the hormone. In addition, the renal handling and the metabolic clearance rate of this component of IRG was studied in animals with nonfiltering kidneys and in rats in which rates of glomerular filtration, renal plasma flow, and renal mass were markedly reduced by partial (70%) nephrectomy (70% Nx).

METHODS

Animals. All experiments were performed on male albino rats (ARS/Sprague-Dawley Division, The Mogul Corp., Madison, Wis.) weighing 250–350 g. 24 h before the study bilateral nephrectomy, bilateral ureteral ligation (BUL), or a sham operation was performed through a midline incision under light pentobarbital anesthesia (50 mg/kg body wt i.p.). An additional group of rats, 3 wk before the experiment, underwent a partial nephrectomy (11) that resulted in ablation of 70±1% of their renal mass.[2] After surgery all animals received chloramphenicol (100 mg/kg body wt i.m.) and were fasted during the 24-h preceding the study, while water was withheld only from anuric rats.

On the morning of the experiment animals were anesthetized with Inactin (Promonta, Hamburg, West Germany) 100 mg/kg body wt i.p., and the trachea, the bladder, one jugular vein, and carotid artery were cannulated. In non-nephrectomized animals the left renal vein was entered with a hooked 27-gauge needle segment connected to silastic tubing, which was kept patent throughout the experiment by a constant infusion (10 μl/min) of heparinized saline (25 U/ml). This approach allowed the withdrawal of renal venous blood up to 3 h after catheter placement, without manipulation of the retroperitoneum, a maneuver that in preliminary experiments was shown to cause acute increments in IRG levels, making the interpretation of renal extraction data difficult. After surgery, isotonic saline equal to 1.5–2.0% of the body weight was infused to replace estimated fluid losses. Rats were placed on heated boards and their rectal temperature, monitored by a thermistor probe (Yellow Springs Instrument Co., Inc., Yellow Spring, Ohio), was maintained between 36° and 38°C.

Infusion studies. Evaluation of the renal handling and metabolic clearance rates of glucagon necessitated the suppression of endogenous IRG to a defined level before administration of exogenous hormone. Preliminary experiments showed that this was achieved in all four experimental groups 45 min after the initiation of a constant infusion of somatostatin and glucose: a priming glucose injection (100 mg/kg body wt) was followed by a sustaining infusion of somatostatin (100 μg/h per kg body wt) and glucose (50 mg/min per kg body wt) delivered at the rate of 40 μl/min in anuric rats and 100 μl/min in control and 70% Nx animals. In subsequent experiments, a blood sample for IRG determination was collected 45 min after the start of the somatostatin/glucose infusion and its value subtracted as background from the steady-state hormonal level attained during the constant infusion of exogenous glucagon. Priming doses of glucagon (20–100 ng), [*methoxy*-³H]inulin (100 μCi/kg body wt), and [¹⁴C]*p*-aminohippurate (PAH) (20 μCi/kg body wt) (both from New England Nuclear, Boston, Mass.) were followed by a sustaining infu-

sion (20 μl/min) delivering 2–10 ng/min of the peptide and appropriate amounts of inulin and PAH. After an additional equilibration period of 45 min, three urine specimens and carotid blood samples (at the midpoint of urine collections) were obtained for clearance measurements. Renal arteriovenous differences were estimated from renal venous blood collected simultaneously with the last arterial sample. All blood samples were withdrawn in chilled heparinized plastic syringes and immediately transferred to iced glass tubes containing Trasylol 500 U (Mobay Chemical Co., Pittsburgh, Pa.) and EDTA 1.5 mg/ml of blood. Urine was collected in chilled plastic tubes; blood samples were centrifuged at 4°C and all samples were stored at −20°C until assayed.

Evaluation of the renal handling of low arterial levels of endogenous hormone was carried out by omitting the infusion of exogenous glucagon and the renal metabolism of high endogenous IRG concentrations was investigated in normal rats during the infusion (40 μl/min) of a 25% solution of arginine hydrochloride. In these experiments, samples of urine and blood were collected 30 min after the start of the arginine infusion when maximal alpha cell stimulation and stable peripheral glucagon levels were achieved (12).

To estimate the renal clearance of glucagon (RC_{IRG}) in BUL rats renal blood flow and hormonal extraction rates (E)$_{IRG}$ were determined during glucagon infusion in seven animals which underwent 24 h previously BUL. Renal blood flow in this group was determined by a modification of the tracer microsphere technique of Hsu et al. (13). The surgical preparation was identical to the one described above with the exception that, in addition, one femoral artery was cannulated with polyethylene-50 tubing. After blood collection for measurement of renal glucagon extraction and replacement of blood losses with normal saline over 20 min, a suspension of ≅60,000 ¹⁴¹Ce-labeled microspheres 15 μm in diameter (3M Co., Nuclear Division, St. Paul, Minn.) in 0.1 ml of 10% dextran was injected rapidly into the carotid artery. 10 s before the injection, withdrawal of blood was commenced at a rate of 0.68 ml/min and continued for 60 s with a continuous automatic infusion-withdrawal pump (model 600-950, Harvard Apparatus Co., Inc., Millis, Mass.). Whole kidney and blood radioactivity were determined in a gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.) and blood flow (expressed in milliliters per minute) calculated from their ratios ×0.68. Renal plasma flow (RPF) was estimated from the product of renal blood flow × (1-hematocrit).

Materials, and assay procedures. The somatostatin (from Dr. K. H. Gabbay, Harvard Medical School, Boston, Mass.) infusate was prepared in hypertonic dextrose (15 or 42%) from a stock solution containing 1 mg of the peptide per milliliter of isotonic saline (pH 7.4 with Tris-HCl buffer) and 0.2% bovine serum albumin. The glucagon infusate (crystalline porcine glucagon, Eli Lilly & Co., Indianapolis, Ind.) was prepared in isotonic saline from a stock solution of the polypeptide (70 μg/ml) dissolved in glycine buffer (pH 8.8) containing 1% bovine serum albumin to prevent loss by absorption onto the glassware and polyethylene tubing.

IRG was measured by a double-antibody radioimmunoassay and gel filtration of plasma samples was carried out as described (6). A specific alpha cell glucagon antibody (30K) obtained from Dr. Roger Unger, University of Texas Southwestern Medical School, Dallas, Tex. was used. The intraassay coefficient of variation for samples above 80 pg/ml in this system averaged 3.7±0.5% and 11.9±2.4% for samples containing <80 pg/ml. Radioactive inulin and PAH concentrations in urine and blood were determined using a liquid scintillation counter (Packard Instrument Co., Inc.). Urea was determined by a modification of the Berthelot method (14) and glucose was measured by the glucose oxidase method (15).

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[2] In this calculation it was assumed that the weight of the right kidney, which was totally excised, was approximately equal to that of the left, which was only partially removed.

Calculations. The urinary clearance (UC) of glucagon was calculated from its urine (U): plasma concentration ratio and the urine flow: $UC_{IRG} = U_{IRG} \times$ urinary volume/arterial$_{IRG}$. Extration (E) rates of inulin, PAH, and glucagon were calculated from their arterial (A) and renal venous (RV) concentrations: $E = (A-RV)/A \times 100$. RPF was derived from the ratio of the clearance (C) of PAH to its renal extraction: $RPF = C_{PAH}/E_{PAH}$. The renal clearance (RC) of glucagon was estimated from the product of its extraction rate and the RPF: $RC_{IRG} = E_{IRG} \times RPF$. Renal glucagon uptake was calculated from the product of its RC and arterial hormonal concentration, while the product of the arterial IRG concentration and the glomerular filtration rate (GFR) was taken to represent the filtered load of the hormone, assuming that glucagon has a glomerular sieving coefficient of one: renal glucagon uptake = $RC_{IRG} \times A_{IRG}$; filtered glucagon load = $GRF \times A_{IRG}$.[3]

Metabolic clearance rates (MCR) were measured by the constant infusion technique (17) and calculated from the ratio of the infusion rate to the steady-state plasma concentration minus the postsuppression IRG level: $MCR_{IRG} =$ infusion rate/A_{IRG}—nonsuppressible IRG. In view of the considerable adherence of IRG to glass and plastic tubing, infusion rates of the hormone were estimated at the point of entry into the animal and calculated from the IRG concentrations of timed infusate aliquots, collected after completion of the experiment from the cut end of the jugular catheter. These samples were measured in the same assay together with urine and arterial and renal venous blood specimens from each individual experiment.

Statistical methods. Results are presented as mean±SEM. Regression lines were calculated by the method of least squares and the similarity of the slopes evaluated according to a standard formula (18). The statistical significance of differences between means of each experimental group and that of controls was assessed by the Student's *t* test, *P* values <0.05 being considered significant.

RESULTS

Preliminary experiments. In preliminary experiments it was shown that the infusion of somatostatin and glucose resulted in maximal and sustained suppression of endogenous IRG levels in all four experimental groups after 45 min. Fasting IRG levels of 232 ±17 pg/ml in controls (*n* = 36), 226±30 pg/ml in 70% Nx (*n* = 10), 623±64 pg/ml in BUL (*n* = 22), and 517 ±46 pg/ml in bilateral nephrectomy (Nx) (*n* = 17) were suppressed to 67±8, 62±8, 237±34, and 267±24 pg/ml respectively. Interestingly, the degree of suppression was approximately proportional to the previously described contribution of the 3,500 mol wt component to the total plasma IRG concentration (9). Thus, suppression was least complete (48±2% of basal IRG) in nephrectomized animals, in which the 3,500-mol wt

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[3] The validity of this assumption is suggested by the absence of protein binding or formation of aggregates by the 3,500-mol wt glucagon component in the circulation and by its almost neutral isoelectric point ($\cong$7.0). Thus, this molecule although slightly anionic at physiologic pH, carries less negative charges than dextran sulfate molecules, the sieving coefficient of which approaches or reaches unity as long as their molecular radius does not exceed 18 Å (as compared to 14 Å for inulin) (16).

material comprises only 57% of the total IRG, whereas basal hormonal levels were suppressed most in control and 70% Nx rats (71±2% and 72±2% respectively), in reasonable agreement with the 88% contribution of this component to total IRG. Suppressibility was of intermediate degree in BUL rats (63±3%) in which plasma IRG comprises 83% of the 3,500-mol wt component. These results are in agreement with reports by others showing that somatostatin suppression of IRG in a variety of species was never complete (19–22).

Evaluation of IRG composition in selected post suppression plasma samples revealed that the 9,000-mol wt proglucagon-like material was largely responsible (70%) for this finding in anephric animals, while the 3,500-mol wt component persisted in measurable, albeit markedly decreased, amounts in all four experimental groups during maximal somatostatin and glucose suppression. Blood glucose levels, measured 90 min after beginning of its infusion together with somatostatin, were elevated in all groups, but were slightly higher in uremic rats: (636±6 vs. 599±14 mg/100 ml, *P* < 0.05). Although it is appreciated that the glucagon infusion experiments were performed in hyperglycemic animals, the need to suppress endogenous glucagon release necessitated this approach.

Renal handling of endogenous and exogenous glucagon in normal rats. The renal metabolism of endogenous glucagon was studied in sham-operated animals during constant infusions of somatostatin and glucose, or arginine (*n* = 13). The disposition of exogenous hormone by the kidney was investigated in identically handled rats in which administration of porcine glucagon was superimposed on a somatostatin and glucose infusion (*n* = 21). This approach allowed the evaluation of renal extraction and clearance rates of IRG of both endogenous and exogenous origin over a wide range of arterial levels (101–2170 pg/ml), glucose concentrations, and filtered loads (834–22164 pg/min per kg body wt). In experiments in which glucagon suppression was maximal (arterial IRG: 47±5 pg/ml, range 27–63 pg/ml), the sensitivity of the assay did not allow the accurate measurement of renal arterio-venous differences and these data were therefore excluded. The mean renal extraction (E) of IRG was 22.9±1.6% and its average clearance was 7.5±0.6 ml/min per kg body wt. These values remained constant despite >20-fold changes in IRG arterial levels and no differences in the renal handling of glucagon of either endogenous (E_{IRG} = 21.0 ±2.7% and RC_{IRG} = 6.7±0.9 ml/min per kg body wt) or exogenous origin (E_{IRG} = 24.1±1.9% and RC_{IRG} = 7.9 ±0.7 ml/min per kg body wt) were observed (Fig. 1). Furthermore, in agreement with a previous report (23) the degree of glycemia did not appear to affect the renal metabolism of the hormone (Fig. 1). Data from all normal animals therefore were pooled for further analysis. Urinary clearance rates of glucagon measured
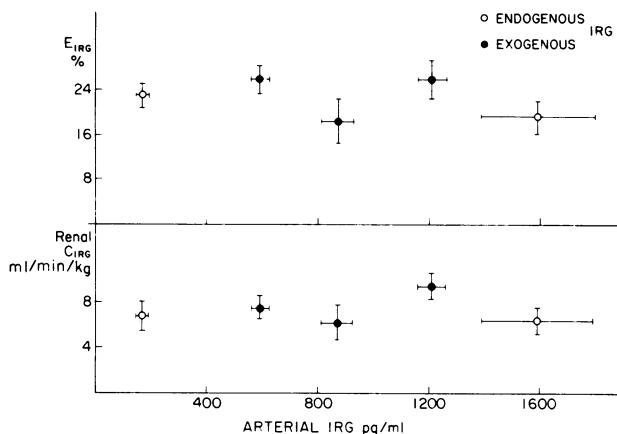
FIGURE 1 The renal handling of endogenous and exogenous IRG and its relation to arterial hormonal levels in normal rats. Renal metabolism of endogenous hormone was studied during infusions of somatostatin and glucose (low IRG levels), or arginine hydrochloride (high IRG levels). Exogenous glucagon handling was investigated during the infusion of porcine hormone, superimposed on the administration of somatostatin and glucose. Renal extractions and clearances of endogenous and exogenous hormone are quantitatively similar, independent of its arterial concentration and are not affected by the levels of plasma glucose, as evidenced by comparing the four glucose-infused groups to the animals infused with arginine alone.

in 17 rats infused with exogenous hormone were negligible, averaging 10.0 ± 1 μl/min per kg body wt, or <0.2% of the corresponding renal IRG clearance rates.

In normal animals renal glucagon extraction (22.9 ± 1.6%) did not differ significantly from that of inulin (24.2 ± 1.2%) (Fig. 2), and the RC rate of the hormone (7.5 ± 0.6 ml/min per kg body wt) was only slightly lower
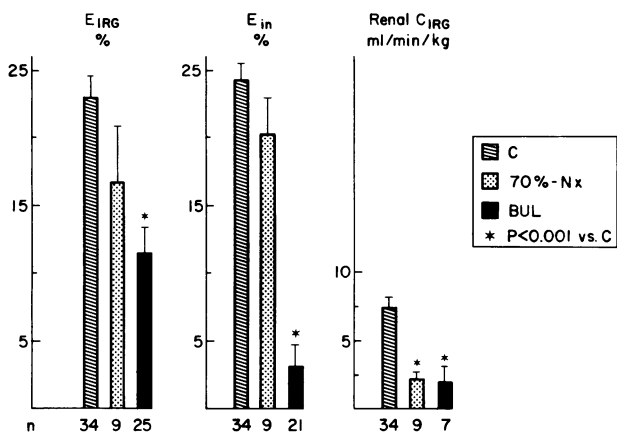

FIGURE 2 The renal handling of glucagon in normal, partially nephrectomized and ureter-ligated rats. Although in animals with filtering kidneys the RC of the hormone can be accounted for by glomerular filtration and tubular reabsorption, peritubular entry of glucagon from postglomerular blood can also occur and is in fact the predominant mode of renal uptake by the nonfiltering kidney.

than the corresponding GFR (8.9 ± 0.4 ml/min per kg body wt).

Effects of partial nephrectomy and ureteral ligation on renal glucagon handling (Fig. 2). Partial nephrectomy, accomplished by ablation of 70 ± 1% of the renal mass, resulted 3 wk later in moderate azotemia blood urea nitrogen: 67 ± 11 vs. 20 ± 2 mg/100 ml in controls, $P < 0.001$) and marked reduction in GFR and RPF rates (GFR: 2.2 ± 0.2 vs. 8.9 ± 0.4 ml/min per kg body wt; and 0.5 ± 0.07 vs. 1.3 ± 0.05 ml/min per g kidney wt, both $P < 0.001$; and RPF: 14.6 ± 1.5 vs. 32.9 ± 1.2 ml/min per kg body wt; and 3.0 ± 0.6 vs. 4.8 ± 0.2 ml/min per g kidney wt, both $P < 0.001$). Partially nephrectomized rats gained only 11% of body weight over the 3 wk after the renal ablation, as compared to a normal growth rate of $\cong$33% in animals of the same strain and age. 70% Nx animals had similar fasting glucagon levels to controls (239 ± 20 pg/ml), despite their significantly impaired renal IRG clearance rates (2.2 ± 0.5 vs. 7.5 ± 0.6 ml/min per kg body wt, $P < 0.001$), and a decrement in renal glucagon extraction, which was not statistically significant (16.6 ± 4.2 vs. 22.9 ± 1.6%, $0.1 > P > 0.05$). Urinary IRG clearance in these animals was also negligible (6.7 ± 2.8 μl/min per kg body wt), accounting for only 0.3% of the organ clearance of the hormone. The observed decrement in the RC of IRG was proportional to the reduction in glomerular filtration and, analogous to the finding in normal rats, neither the extractions (E) nor the RC of glucagon and inulin (in) were significantly different ($E_{IRG} = 16.6 \pm 4.2$%, $E_{in} = 20.2 \pm 2.6$%; $RC_{IRG} = 2.2 \pm 0.5$, and $C_{in} = 2.2 \pm 0.2$ ml/min per kg body wt).

BUL (Fig. 2) led 24 h later to marked azotemia (blood urea nitrogen: 235 ± 13 mg/100 ml) and fasting hyperglucagonemia (arterial$_{IRG}$: 609 ± 57 pg/ml). Despite virtual absence of glomerular filtration (E_{in}: 3.2 ± 1.8%), renal glucagon extraction continued at significant, albeit decreased rates (E_{IRG}: 11.5 ± 1.9 vs. 22.9 ± 1.6% in controls, $P < 0.001$). In seven BUL rats in which renal blood flow was also measured, by the microsphere method, shortly after blood collections for determination of IRG renal extractions, RPF, and glucagon clearance averaged 16.1 ± 2.4 and 2.0 ± 0.9 ml/min per kg body wt respectively.[4]

Elution profiles of plasma IRG in sham operated, partially nephrectomized and BUL rats confirmed that the immunoreactive material measured in both arterial and renal venous samples was exclusively the 3,500-mol wt component (Fig. 3).

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[4] Since with this method in the rat it is not technically possible to measure renal extraction (E) ratios and blood flow simultaneously, E_{IRG} was determined first, followed by measurement of renal blood flow. Although RPF and RC_{IRG} determined by this technique probably represent only minimum values, these results strengthen the conclusion that peritubular uptake contributes to the renal handling of IRG.
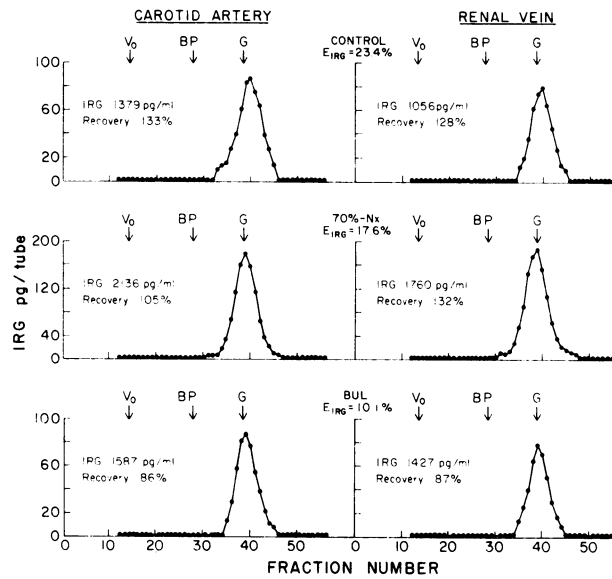
FIGURE 3 Elution profiles of immunoreactive glucagon from simultaneously drawn arterial and renal venous plasma from normal, partially nephrectomized and ureter-ligated rats during the infusion of arginine hydrochloride. In each set of experiments equal volumes of arterial and renal venous sample were applied on the columns, that were calibrated with ^{125}I-gamma globulin, ^{131}I-proinsulin, and ^{125}I-glucagon. Vo = Void volume (mol wt > 40,000); BP = bovine proinsulin (mol wt 9,000); G = glucagon (mol wt 3,500).

Metabolic clearance. Fasting IRG levels and the metabolic clearance rates of glucagon measured in all four groups of animals are summarized in Fig. 4. In control animals the MCR of glucagon averaged 31.8 ±1.2 ml/min per kg body wt (n = 11), and the simultaneously measured RC (9.2±0.9 ml/min per kg body wt) accounted for 29.7±3.1% of the overall peripheral metabolism of the hormone. In partially nephrectomized rats the kidney contributed only 7.6±1.1% to the total MCR of glucagon (MCR:22.3±1.6 and the simultaneously measured RC: 1.7±0.2 ml/min per kg per body wt).

In animals with both ureters-ligated MCR was 22.9 ±3.3 ml/min per kg body wt, whereas the RC of seven BUL rats studied separately averaged 2.0±0.9 ml/min per kg body wt, suggesting that the nonfiltering kidney contributes ≅9% to the overall metabolic disposition of this hormone.

Each of the three renal ablative maneuvers depressed the metabolic clearance rate of IRG by approximately one-third: (70% Nx = 22.3±1.6 ml/min per kg body wt, (n = 8); BUL: 22.9±3.3 ml/min per kg body wt, (n = 9); Nx: 23.2±1.2 ml/min per kg body wt, (n = 12). Partially nephrectomized rats were not hyperglucagonemic despite the decrement in their MCR (IRG: 238±36 pg/ml vs. 191±27 pg/ml in controls), whereas bilaterally nephrectomized and ureteral-ligated rats exhibited substantial and quantitatively similar elevations of their fasting IRG levels (Nx: 440±36 pg/ml, BUL 405±47 pg/ml; both P < 0.001 vs. controls).

DISCUSSION

Since the autoradiographic demonstration of luminal uptake of labeled glucagon by proximal tubular cells (24), a number of investigators have stressed the importance of the renal parenchyma in the metabolism
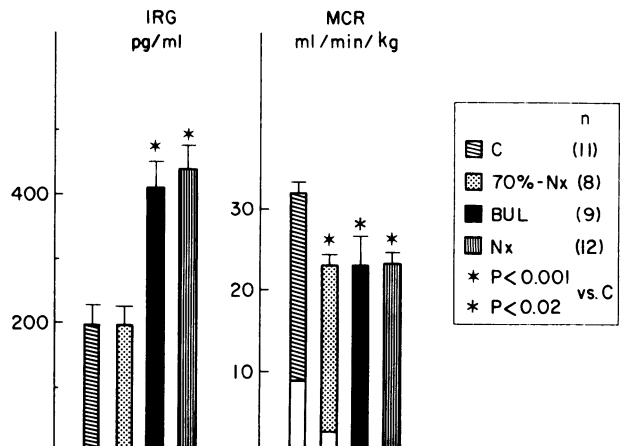

FIGURE 4 Base-line IRG levels and the MCR of the hormone in normal, partially nephrectomized, BUL, and nephrectomized animals. The simultaneously measured renal contribution to the overall MCR rate of the hormone in control and 70% Nx rats is indicated by the clear bars. In seven BUL animals studied separately renal glucagon clearance (not shown) averaged 2.0±0.9 ml/min per kg body wt and thus contributed ≅ 9% to the total metabolic disposition of the peptide.

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of this hormone. Kidney tissue contains specific glucagon-degrading enzymes in both the proximal tubular cytosol and brush border (25, 26). Glucagon is taken up avidly by the autotransplanted as well as by the in vitro perfused kidney of the dog (23, 27) and by the rat kidney in situ (28). In animals, bilateral nephrectomy, ureteral ligation, or renal pedicle-clamping lead promptly to hyperglucagonemia (3, 8, 9, 22, 29, 30). In patients with renal failure basal glucagon levels are substantially elevated (2–8) and the MCR of exogenously administered hormone is markedly decreased (5).

Most of the above mentioned studies did not take into account the heterogeneity of circulating IRG and the alterations in its composition which occur in renal insufficiency (6, 8, 9). Because the various immunoreactive components of the hormone are probably handled by different renal mechanisms (9), the present investigation was focused on the renal handling and the contribution of the kidney to the overall peripheral metabolism of the biologically active component of glucagon (the 3,500-mol wt fraction) (10). The renal handling and the MCR of this material was evaluated during a constant infusion of crystalline porcine glucagon (3,500 mol wt) superimposed on the administration of somatostatin and glucose, which in preliminary studies was shown to produce a sustained suppression of endogenous hormonal levels in all experimental groups of animals. Our results thus describe the renal and the overall peripheral disposition of 3,500 mol wt glucagon, that was studied in normal, as well as in partially nephrectomized, ureteral ligated, and bilaterally nephrectomized rats. Furthermore, studies conducted in normal rats infused with maximally effective stimulating doses of arginine demonstrate that the renal handling of the endogenous 3,500 mol wt component of the rat is not different from that of the porcine hormone.

The renal handling of the 3,500-mol wt glucagon was studied over a wide range of arterial concentrations thus simulating filtered hormonal loads encountered in a variety of physiologic and pathologic states. In normal rats the arteriovenous IRG concentration difference averaged $22.9\pm1.6\%$, a value quite similar to that found in the dog (25%) by Lefebvre et al. (23, 27), but lower than the one described by Bastl et al. (28) in rats (39%). In the latter study though, endogenous IRG release was not suppressed and renal vein cannulation immediately preceded blood collection for measurement of arterio-venous differences, this approach in our experience, causes substantial increments in plasma glucagon levels and acute changes in renal hemodynamics, making extraction data difficult to interpret. Despite marked reduction in the GFR and RPF, the remnant kidney of partially nephrectomized rats continued to extract substantial amounts ($16.6\pm4.2\%$)

of glucagon from the circulation, a finding, which is also at variance with the results of Bastl et al. (28). In our study the decrement in the RC of the peptide in these animals could be attributed to the reduced filtration rate and the resulting decrease in the filtered load of the hormone. The renal parenchyma of both control and 70% Nx rats catabolized virtually all of the extracted peptide, since in both groups urinary glucagon excretion was negligible, representing <0.3% of the overall renal hormonal clearance,[5] an estimate comparable to that reported for insulin (0.2%) in the rat (31). In both groups the renal uptake of the hormone was linearly related to its filtered load (controls: $y = 0.70x + 0.87$, $r = 0.82$, $P < 0.001$; 70% nephrectomized: $y = 1.15x - 0.25$, $r = 0.72$, $P < 0.05$) and the slopes of these linear regressions did not differ, suggesting that at equivalent IRG-filtered loads both groups extracted and catabolized similar amounts of the hormone. Furthermore, evaluation by column fractionation of the IRG measured in arterial and renal venous samples showed similar immunological patterns in the two groups, indicating that the 3,500-mol wt glucagon component is handled similarly by the normal and by the remnant kidney (Fig. 3).

In these two groups of animals glucagon extraction was not significantly different from that of inulin. In 70% Nx rats inulin and glucagon clearances were comparable, while in sham-operated rats the clearance of the hormone was only slightly lower than the GFR. Although these results could be interpreted as indicating that renal glucagon extraction, in animals with filtering kidneys, can be accounted for by glomerular filtration and tubular reabsorption, it is likely that uptake from postglomerular capillaries, as postulated for insulin (31), also occurs. In fact, animals with both ureters ligated, maintained renal perfusion and barely measurable extraction of inulin continued to extract 11.5% of the renal arterial glucagon, indicating that peritubular glucagon uptake occurs, at least in the nonfiltering kidney. This might also be the case for the 9,000-mol wt proglucagon-like material, which accumulates only after bilateral nephrectomy but not after BUL (9).

In agreement with reports stressing the prominent role of nonrenal degrading sites, such as the liver, in the overall peripheral disposition of the 3,500-mol wt glucagon component (32) the kidney contributed in normal animals only 30% to its MCR. All three renal ablative maneuvers used in the present study depressed the metabolic clearance of IRG also by $\cong30\%$ and in BUL and 70% Nx rats this decrement could

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[5] Urinary clearances of both insulin and glucagon are substantially higher in the dog (4.7 and 4.1% of their respective RC) suggesting that the renal tubular handling of these peptides is different in this species (23).

be accounted for almost entirely by the impaired renal clearance of the hormone. Whereas, bilaterally nephrectomized and ureteral-ligated rats exhibited substantial and quantitatively similar elevations of their basal IRG levels, partially nephrectomized rats were not hyperglucagonemic despite their comparably decreased MCR. Because chronic starvation markedly depresses both the secretory and MCR of IRG (33), this factor might account at least in part for our results in the partially nephrectomized rats.

In summary, this study has demonstrated that the normal rat kidney extracts and catabolizes 23% of the 3,500-mol wt glucagon component, independent of its arterial concentration, and that this organ contributes 30% to the overall metabolic disposition of glucagon. The remnant renal parenchyma of 70% Nx rats, while contributing <8% to the total peripheral metabolism of this component of glucagon, continues to extract and catabolize substantial amounts of the hormone in a manner similar to that of the normal kidney. Although glucagon uptake by the filtering kidney could be accounted for by glomerular filtration and tubular reabsorption, this mechanism is not solely responsible for renal IRG handling. In fact, animals in which GFR has virtually ceased continue to extract substantial amounts of the hormone, indicating that peritubular entry from postglomerular capillaries also occurs and actually is the predominant mode of IRG uptake by the nonfiltering kidney.

ACKNOWLEDGMENTS

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