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Independance of Glucagon and Insulin Handling by the Isolated Perfused Dog Kidney

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Summary. The effect of raising arterial plasma glucagon concentrations on kidney glucagon uptake was investigated using an isolated dog kidney perfused with whole blood. In addition, the effect of insulin on the magnitude of glucagon uptake by the kidney was studied at various glucagon concentrations. Renal vein plasma glucagon (V) has been found to be proportional to renal artery plasma glucagon (A). V and A were highly significantly correlated. In the absence of exogenous insulin infusion, V equalled 0.733 ± 0.034 A, while in the presence of insulin V equalled 0.747 ± 0.015 A. When kidney glucagon uptake was measured directly it increased as a function of arterial plasma glucagon. The calculated regression lines were similar in the presence and in the absence of insulin. The mean clearance rate of glucagon by the kidney was similar at low, medium or high concentrations of glucagon and was not affected by the presence of insulin at a mean concentration of 335.7 ± 15.7 μU/ml. At this concentration of insulin, kidney insulin uptake was not affected by glucagon at concentrations ranging from 32 to 1600 pg/ml. Comparison of kidney glucagon uptake at similar arterial plasma glucagon concentrations, but with different renal plasma flows, indicated that kidney glucagon uptake is more dependant on arterial plasma glucagon concentration than on the quantity of glucagon entering the kidney per minute. *It is concluded that:* 1) kidney glucagon uptake increases as a function of arterial plasma glucagon concentration; 2) the clearance rate of glucagon is similar at low, medium or high arterial concentrations of glucagon; 3) at concentration of 300–350 μU/ml, insulin does not affect kidney glucagon uptake, and 4) at concentrations of glucagon up to 1600 pg/ml, renal insulin uptake is not affected by glucagon. These studies indicate that insulin and glucagon are handled independantly by the kidney of the dog.

Key words: Glomerular filtration rate, glucagon, insulin, kidney, metabolism, renal plasma flow.

Previous studies from our Laboratories, using dog kidneys transplanted acutely to the neck vessels of a perfusing anaesthetized dog, have indicated that under basal conditions, the glucagon uptake by the kidney averaged 89 ± 14 pg/min/g of kidney, a value which was significantly reduced to 42 ± 5 pg/min/g after massive intravenous glucose load [1]. Contrary to insulin, no correlation was found between arterial glucagon concentration and kidney glucagon uptake; it was suggested that this might be due to the fact that the glucagon concentrations were too low and that the range of arterial glucagon concentrations reached was relatively narrow. In order to overcome these limitations, we performed the present experiments using isolated dog kidneys perfused with whole blood. In the first part of this work, we investigated the effect on renal glucagon uptake of raising arterial plasma glucagon concentrations by exogenous infusion of this hormone. Recent investigations have demonstrated the presence in the kidney and muscle of a glucagon degrading enzyme and it was suggested that the same enzyme might be involved in the degradation of both insulin and glucagon [2–4]. After isolation and purification of this enzyme from rat skeletal muscle, it was demonstrated that insulin served as a competitive inhibitor of glucagon degradation and that glucagon itself was a competitive inhibitor of insulin degradation [2]. In view of the potential physiologic significance of this finding, we investigated, at various glucagon concentrations, the effect of insulin on the magnitude of glucagon uptake by the kidney. This constitutes the second part of the present investigation.

Material and Methods

1. Perfusion of Isolated Dog Kidneys

Perfusion equipment and basic experimental conditions have been described previously [5, 6]. Two identical machines were used for simultaneous separate perfusion of both kidneys of the same animal. After an overnight fast, kidney donor dogs, of both sexes, weighing 10.1 ± 0.7 kg (range 8–12) and blood donor dogs, weighing 27.1 ± 1.2 kg (range 22–34) were anaesthetized using pentobarbital (30 mg/kg i. v.) and were given 25 000 I. U. heparin intravenously. Blood was obtained from the carotid arteries of the blood donor; approximately 900 ml were collected in about 2 minutes. The blood was mixed by gentle shaking and divided into two equal portions, one for each perfusing machine. Ten per cent Trasylol® (5000 KI per ml) was added to the blood prior to its introduction in the machines. In preliminary experiments, the effect of Trasylol® on glucagon conservation during perfusion as well as the possible influence of Trasylol® on kidney function was investigated (see section on Results). After careful surgical preparation, both kidneys of the kidney donor dog were isolated and simultaneously transfered to the perfusion machines. The duration of renal ischaemia never exceeded 90 seconds. The experiments were conducted at a temperature of 37° C; the blood flow of the pump was continually adjusted throughout the experiments in order to hold perfusion pressure at a permanent level of 140 mm Hg. Transient initial vasoconstriction was minimized by the addition of 25 mg Promethazine (Phenergan Specia®) to the blood immediately before introducing it into the perfusion equipment.

2. Infusions

In all instances, exogenous creatinine was infused in order to measure glomerular filtration rate (GFR): a priming dose of 4.5 ml of a 2 per cent solution of creatinine in Ringer's fluid was added at the beginning and was followed by a continuous infusion at 0.09 ml/min of the same solution. In the *control experiments,* saline supplemented with crystallized bovine albumin (1 g/100 ml) (Calbiochem, San Diego, Calif.) was infused during 3 consecutive periods of 30 min each at a rate of 0.01, 0.02 and 0.05 ml/min respectively. In the *glucagon infusion experiments,* 60 ng of glucagon (Pork glucagon, lot MC6770, Novo, Copenhagen) in a volume of 1 ml saline supplemented with albumin (1 g/100 ml) was injected at the beginning of the infusion; this was followed by 3 consecutive periods of 30 min during which a 600 ng/ml solution of glucagon in saline enriched with albumin (1 g/100 ml) was infused at a rate of 0.01, 0.02 and 0.05 ml/min, or 6, 12 and 30 ng glucagon/min, respectively. In two additional series, saline infusions or glucagon infusions were associated with *insulin infusions:* insulin (Actrapid®, Novo Copenhagen) was injected at a loading dose of 300 mU in a volume of 1 ml of saline enriched with albumin (1 g/100 ml) followed by a continuous infusion of insulin at a dose of 4 mU/min in a volume of 0.05 ml/min of the same diluent. In some of these experiments, a fourth glucagon infusion period at a rate of 0.1 ml/min (i. e. 60 ng/min) was performed. All infusions were performed in the renal vein, in order to insure proper mixing prior to return of blood to the kidney.

3. Collection of Samples

Urine samples were collected during the last 5 min of each infusion period and their volume was measured with graduated cylinders. At the beginning, midpoint and end of each urinary collection period, blood was taken simultaneously from the artery and the vein of the perfused kidney by free flow into heparinized tubes, using sidebranched cannulas. Blood flow through the kidney was determined immediately after each sampling by means of a side-branched graduated 25-ml pipette and temporary occlusion of the venous return. Preliminary experiments indicated that satisfactory steady state levels of plasma creatinine, glucagon, and insulin were reached during the determinations of renal and urinary clearances.

4. Analytical Procedures

The plasma glucose concentration was determined by an enzymatic procedure [7] and plasma and urinary creatinine levels were measured by the Auto-Analyzer® method. For hormone assays, 0.4 ml of a solution containing Trasylol® 5000 U/ml and Na_2EDTA 12 mg/ml were added to 3.6 ml of blood. The latter was immediately centrifuged at +4° C and the separated plasma was stored at −20° C. Plasma insulin was assayed by radioimmunoassay [8] using an antiporcine insulin antiserum. Human insulin was used as a standard, and all samples from a single experiment were assayed in the same series using the same standard curve. The identity of reactivity of dog and human insulin with regard to the anti-insulin antiserum used was validated by demonstrating the parallelism of the two dilution curves. Plasma insulin was determined in duplicate. Plasma glucagon was determined in duplicate by a classical radioimmunoassay procedure, using ^{131}I-pork glucagon as tracer, 30K antiserum (kindly provided by Dr. Roger H. Unger, Dallas, Texas), which is considered to be specific for

glucagon, and dextran-charcoal separation of free from antibody-bound hormone [9]. The haematocrit was determined for each arterial sample.

5. Calculations

At the end of each experiment, the kidneys were carefully dissected, decapsulated, washed and weighed. GFR was determined on the basis of creatinine clearances. The glucagon concentrations of the three samples obtained during each collection period were averaged and the mean was used to indicate the „mean arterial plasma glucagon concentration" of the period under consideration; the "mean venous plasma glucagon concentration" was calculated in the same manner. The mean renal plasma flow was calculated from the measured blood flow and the haematocrit.

The arteriovenous difference in plasma glucagon concentrations multiplied by the renal plasma flow gave the kidney glucagon uptake. The arterial plasma glucagon concentration multiplied by the renal plasma flow gave the quantity of glucagon entering the kidney per minute. The "clearance rate of glucagon by the kidney" was defined as the number of mililiters of plasma cleared of the hormone per minute. It was calculated by dividing the kidney glucagon uptake by the arterial glucagon concentration. All calculations were expressed per gram of kidney. Similar calculations were performed for insulin. The results were analyzed statistically using the Student's *t* test for paired or non-paired data. Correlation coefficients were calculated according to classical procedures [10].

Results

1. Presence of Trasylol® in the Perfusion System

a) *Does Trasylol® Affect Kidney Function?* In 3 preliminary experiments, one kidney was perfused with blood supplemented with Trasylol® (500 U per ml of blood), while the other kidney of the same dog was perfused with blood lacking Trasylol® (the latter was replaced by an appropriate volume of the Trasylol® diluent). Eleven parallel collections were obtained. The results, given in Table 1, show that the mean values of urinary output and renal blood flow were not affected by the presence of Trasylol® while mean GFR was slightly increased. However, paired comparison of the results showed urinary output and GFR to be slightly but significantly increased in the presence of Trasylol®, while renal blood flow was not affected.

b) *Does Trasylol® Protect Glucagon against Destruction in the Perfusion System?* In a preliminary experiment, one kidney of the same donor was perfused with and the other without Trasylol®, as described above. Plasma glucagon concentration in the perfusing blood before its addition to the machines was 118 pg/ml. After 30 min perfusion, glucagon concentration was 111 ± 14 pg/ml in the blood containing Trasylol® and 70 ± 8 pg/ml in the blood lacking Trasylol®. After 90 min perfusion, the respective glucagon plasma concentrations were 124 ± 3 pg/ml and 56 ± 2.5 pg/ml. At these plasma glucagon levels, no significant kidney glucagon uptake, as calculated from the arterio-venous differences in glucagon was recorded.

It was concluded that 1), Trasylol® has only slight effects on kidney function and that 2) Trasylol® effectively protects glucagon from degradation in the blood during perfusion. It was therefore decided to use Trasylol® in all subsequent experiments.

2. Renal Vein Versus Renal Artery Plasma Glucagon Concentration

Glucagon infusions resulted in arterial plasma glucagon concentrations ranging from 26 to 1184 pg/ml and in venous plasma glucagon concentrations ranging from 6 to 800 pg/ml. Renal vein plasma glucagon concentration (V) has been found to be proportional to renal artery plasma glucagon level (A). V and A were highly significantly correlated ($r = 0.987$; $n = 31$; $p < 0.001$) with V being equal to 0.733 ± 0.034 A. When exogenous insulin was infused, reasonably stable plasma insulin levels were obtained: mean plasma insulin averaged 336 ± 16 μU/ml ($n = 41$). *In these experiments,* when glucagon was perfused with insulin, glucagon infusions resulted in arterial plasma glucagon concentrations ranging from 32 to 1600 pg/ml and in venous plasma glucagon concentrations ranging from 26 to 1275 pg/ml. Here again, V was proportional to A, V and A were highly significantly correlated ($r = 0.980$; $n = 39$; $p < 0.001$) and V equalled 0.747 ± 0.015 A. No systematic change in renal plasma flow was observed in both experimental conditions Thus, the above-mentioned calculations indicate that, at any glucagon concentration investigated, an average of 25% of glucagon in the arterial blood is taken up by the kidney. This value was not affected by the presence of insulin.

3. Measured Kidney Glucagon Uptake as a Function of Arterial Plasma Glucagon. Effect of Insulin

As shown in Figure 1, kidney glucagon uptake increased as a function of arterial plasma glucagon ($r = 0.940$; $p < 0.01$). Since blood flow was not systematically modified, a similar correlation existed between kidney glucagon uptake and the quantity of glucagon

Table 1. Effect of Trasylol® on the function of the isolated perfused dog kidney

	Saline controls	Trasylol® 500 U/ml of blood	Paired comparison
Urine output[a] (ml/min/g of kidney)	0.045 ± 0.005 (n = 11)	0.058 ± 0.07 (n = 11)	0.013 ± 0.002 ($p < 0.01$)
	N. S.[a]		
Glomerular filtration rate (ml/min/g of kidney)	0.40 ± 0.02 (n = 11)	0.47 ± 0.02 (n = 10)	+ 0.07 ± 0.02 ($p < 0.01$)
	$p < 0.05$		
Renal blood flow (ml/min/g of kidney)	4.45 ± 0.50 (n = 11)	4.69 ± 0.38 (n = 11)	+ 0.24 ± 0.19 (N. S.)
	N. S.		

[a] All results are expressed as mean ± standard error of the mean N. S. = not statistically significant.

Table 2. Clearance rate of glucagon by the kidney at various glucagon or insulin concentrations

A. Effect of glucagon concentrations (at all insulin levels)			
Arterial plasma glucagon (pg/ml)	Low (0–200)	Medium (201–500)	High (> 500)
Glucagon clearance[a] (ml/min/g of kidney)	0.53 ± 0.16 (n = 7)	0.52 ± 0.09 (n = 12)	0.57 ± 0.05 (n = 17)
	N. S.	N. S.	
		N. S.	
B. Effect of Insulin concentrations (at all glucagon levels)			
Arterial plasma insulin (μ U/ml)	Low (0–10)	Medium (150–300)	High (400–500)
Glucagon clearance[a] (ml/min/g of kidney)	0.45 ± 0.06 (n = 26)	0.50 ± 0.06 (n = 14)	0.43 ± 0.12 (n = 8)
	N. S.	N. S.	
		N. S.	

[a] Results are expressed as mean ± SEM N. S. = not statistically significant

Table 3. Insulin uptake by the kidney at various glucagon concentrations[a]

Arterial plasma glucagon (pg/ml)	0–200	201–800	801–1600
Insulin uptake[b] (μU/min/g of kidney)	122.3 ± 17.7 (n = 9)	138.1 ± 7.5 (n = 17)	119.5 ± 15.9 (n = 12)
	N. S.	N. S.	
		N. S.	

[a] Results are expressed as mean ± standard error of the mean
[b] Mean arterial plasma insulin averaged 335.7 ± 15.7 U/ml; plasma insulin concentrations were similar in the 3 subgroups

entering the kidneys per minute ($r = 0.952$; $p < 0.01$). The mean renal clearance rate of glucagon, calculated on the basis of all the kidney glucagon uptake data, averaged 0.45 ± 0.06 ml/min/g of kidney (n = 26). The intercept of the regression line with the X axis was found at about 100 pg/ml: statistical analysis, however, revealed that this point was not statistically different from zero. When kidney glucagon uptake was studied in the presence of insulin, insulin infusion resulted in reasonably stable arterial plasma insulin concentrations. Under these conditions, kidney glucagon uptake was again significantly correlated with both arterial plasma glucagon ($r = 0.913$; $p < 0.01$) and the quantity of glucagon entering the kidneys per minute ($r = 0.866$; $p < 0.01$). As indicated in Figure 1, the calculated regression lines were similar in the presence and in the absence of insulin. Statistical analysis revealed no significant dif-

ference. The mean clearance rate of glucagon by the kidney during insulin infusion averaged 0.55 ± 0.05 ml/min/g (n = 36), a value which is not statistically different from the one obtained in the absence of insulin infusion. As indicated in Table 2, the clearance rate of glucagon by the kidney was similar at low, medium or high arterial plasma glucagon or insulin concentrations.

4. Insulin Uptake by the Kidney at Various Arterial Concentrations of Glucagon

As depicted in Table 3, for a mean arterial plasma insulin of 335.7 ± 15.7 μU/ml (n = 41), kidney insulin uptake was of the same order of magnitude in the presence of varying arterial glucagon concentrations. None of the differences was statistically significant.

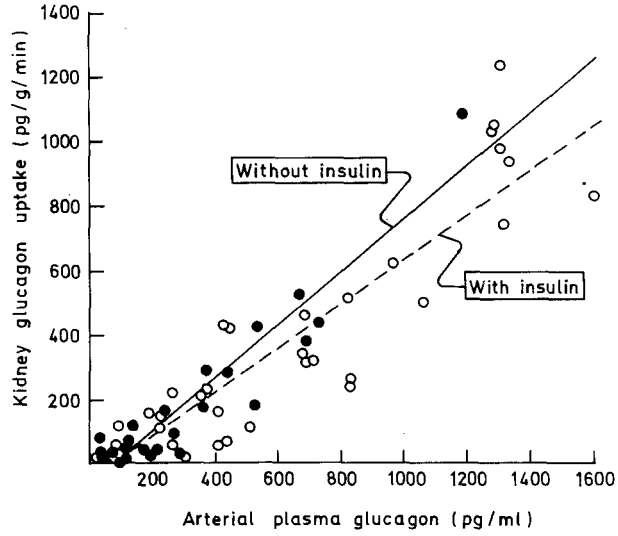

Fig. 1. Correlation between arterial plasma glucagon (A) and kidney glucagon uptake (U) in the absence (•) and in the presence (○) of insulin. U equalled 0.686 ± 0.046 A in the absence, and 0.622 ± 0.032 A in the presence of insulin. At a 95% threshold, the confidence limits were 0.592–0.780 in the absence, and 0.557–0.687 in the presence of insulin

Discussion

As recently reviewed by one of us [6], the isolated dog kidney perfused with whole blood has been shown to be a physiologically valid model of the normal kidney. Since glucagon preservation in blood or plasma requires the presence of an anti-proteolytic agent [11] such as Trasylol®, we investigated the possible effects this substance may have on kidney function. We demonstrated that the main parameters of kidney function were not (renal blood flow) or only slightly (urinary output, glomerular filtration rate) affected by Trasylol® included in the perfusion fluid. In previous experiments, renal handling of glucagon was investigated in dog kidneys acutely transplanted to the neck vessels of an anaesthetized dog. In this system [1], under basal conditions the clearance rate of glucagon averaged 0.482 ± 0.063 ml/min/g of kidney, a value very similar to the one found in the present experiments (0.450 ± 0.060 ml/min/g of kidney). Kidney glucagon uptake averaged 89 ± 14 pg/min/g of kidney when the mean arterial plasma glucagon concentration averaged 193 ± 14 pg/ml. In the present investigation, kidney glucagon uptake averaged 90 ± 20 pg/min/g when arterial plasma glucagon values were selected to average 192 ± 28 pg/ml (n = 8); thus in both systems, renal glucagon uptake was practically identical in the presence of similar arterial plasma glucagon concentrations. Renal blood flow in the isolated perfused dog kidney is slightly higher than that measured in situ or in the kidney transplanted to the neck [6]. This was also true in the present series: renal plasma flow was 2.30 ± 0.22 ml/min/g of kidney (n = 9) versus 1.53 ± 0.07 ml/min/g (n = 15) in our neck. The difference is statistically significant ($p < 0.01$). Since almost identical values of kidney glucagon uptake were obtained in the two systems, at similar arterial plasma glucagon concentrations but with different renal plasma flows (and, therefore, different quantities of glucagon entering the kidney per minute), one can conclude that kidney glucagon uptake is more dependant on arterial plasma glucagon concentration than on the quantity of glucagon entering the kidney per minute. In the kidneys transplanted to the neck system, urinary glucagon excretion accounted for less than 5 per cent of the total glucagon uptake [1]; this parameter was therefore not investigated in the present study. A surprising finding in our previous study was the apparent lack of correlation between arterial plasma glucagon concentration and kidney glucagon uptake [1]. However, in these experiments, glucagon concentrations were basal or were lowered by glucose infusion so that the range of arterial plasma glucagon concentrations studied was relatively narrow. Despite the lack of correlation, it was found that kidney glucagon uptake was, on the average, reduced by 50% when arterial plasma glucagon decreased from 193 to 136 pg/ml. In the present study, measurements made when glucagon was at basal levels as well as when it was raised by exogenous glucagon infusion, allowed kidney glucagon uptake to be determined at arterial plasma glucagon concentrations ranging from 0 to 1600 pg/ml (a range covering most concentrations encountered in physiological or pathophysiological situations as well as those resulting experiments using the kidney transplanted to the

from pharmacological stimulation of glucagon secretion [12]). Under these conditions, kidney glucagon uptake was significantly correlated with both arterial plasma glucagon or the quantity of glucagon entering the kidney per minute (Q). On average, kidney glucagon uptake was 21.1 ± 2.7 per cent of Q. At low glucagon concentrations, i. e. below 125 pg/ml, no glucagon uptake was usually detectable. This might suggest the existence of a threshold for glucagon clearance but, as indicated in the section on results, statistical analysis of our data do not permit us to draw any firm conclusion in this respect. As previously demonstrated [1], the urinary clearance of glucagon represents only a small percentage of the clearance of glucagon by the kidney. This observation is in agreement with earlier data which demonstrated that glucagon may be filtered through the glomerulus and reabsorbed at the tubular level [13]. This does not however exclude the possibility of a direct uptake from renal capillaries without previous filtration and reabsorption, as has been demonstrated for insulin [14–15]. The existence of such mechanism is supported by our finding that renal clearance rate of glucagon may be higher than the glomerular filtration rate. The intimate mechanism by which the kidney degrades glucagon remains unknown. In rat skeletal muscle, it has been demonstrated that insulin and glucagon can be degraded by the same enzyme; moreover it was shown that insulin was a competitive inhibitor of glucagon degradation ($Ki = 1.5 \times 10^{-8}$ M) and that glucagon was a competitive inhibitor of insulin degradation ($Ki = 5.3 \times 10^{-6}$ M). Since a similar enzyme was extracted from the kidney [16], we were interested in a possible interaction between glucagon and insulin uptake by the whole kidney. Our data clearly indicate that, over a wide range of arterial concentrations, glucagon uptake by the kidney was not significantly affected by the presence of insulin. This finding suggests that insulin and glucagon are handled independently by the kidney. This is consistent with the finding that, in rat kidney homogenates and at physiological hormonal concentrations, insulin degrading activity is located primarily in the cytosol, while glucagon degrading activity is found primarily in the $10^5 \times$ g pellet and is apparently associated with the brush border [4, 17]. The cytosolic enzyme would correspond with the previously described "insulin specific protease" from muscle and could also degrade glucagon. The enzyme apparently associated with the brush border would be more specific for glucagon and would not metabolize insulin. Our data suggest that, under physiological conditions, the bulk of the renal glucagon degrading activity is carried out by mechanisms not affected by insulin. Further studies are needed to identify these mechanisms with the brush border associated neutral protease previously described by others [4, 17–19].

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