Differences in the hepatic and renal extraction of insulin and glucagon in the dog: evidence for saturability of insulin metabolism

K. Polonsky, J. Jaspan, D. Emmanouel, K. Holmes and A. R. Moossa

The University of Chicago, 950 East 59th Street, Chicago, IL 60637, USA

Abstract. The metabolism of exogenously infused porcine insulin and glucagon was assessed concurrently in normal fasted dogs under anaesthesia. Hepatic and renal extraction of glucagon were 25.6 ± 2.3 and 43.7 ± 3.9%, respectively, and its metabolic clearance 16.5 ± 0.8 ml/kg/min. Hepatic and renal extraction accounted for 28.5 ± 4.2 and 28.7 ± 3.7% of total glucagon clearance, respectively. Insulin MCR was 18.3 ± 1.5 ml/kg/min and its hepatic and renal extraction were 49.6 ± 3.4 and 41.7 ± 4.4% accounting for 51.9 ± 4.4 and 27.3 ± 3.9% of total insulin clearance, respectively. Neither total glucagon metabolic clearance nor its hepatic or renal components saturated even in the face of circulating glucagon levels extending into the pharmacologic range up to 14 ng/ml. In contrast however, with increasing arterial concentrations of insulin, saturability of metabolism was apparent as evidenced by significant reductions in MCR as well as hepatic and renal extraction. This demonstration of saturability of hepatic insulin metabolism occurred at levels encountered in the portal vein after meals and is compatible with the concept that the hepatic capacity for extraction of this hormone may be an important site of control of the proportion of secreted insulin reaching the periphery.

The metabolic handling of each hormone was shown to be independent of the other. Despite similarities in the interaction of insulin and glucagon with the target cell, there are important differences in the mechanisms of metabolism of these peptides at the major degradative sites.

A number of studies have established the importance of the kidney in the metabolism of circulating immunoreactive glucagon (Emmanouel et al. 1978; Sherwin et al. 1976; Lefebvre et al. 1974), this organ contributing approximately 30% to the overall metabolic clearance of the hormone (Emmanouel et al. 1978). It has been suggested that the liver is not an important site of glucagon metabolism (Felig et al. 1974; Fisher et al. 1976) but we and others have shown that the liver is responsible for 30% of its overall metabolic clearance (Jaspan et al. 1981a; Buchanan et al. 1968).

The liver is acknowledged to be the major site of insulin metabolism, extracting 50% of insulin delivered to it on the first pass (Mortimore et al. 1959; Rubenstein et al. 1972; Kaden et al. 1973; Stoll et al. 1970). The kidney is also an important site of insulin extraction and renal arteriovenous differences of 30–40% have been reported (Rubkin & Colwell 1969; Zaharko et al. 1966).

Although the hepatic and renal metabolism of insulin and glucagon have been studied separately in a number of species the simultaneous handling of these hormones by both organs in a single animal has not been addressed. We therefore performed these studies to compare the relative im-
importance of the two organs in the metabolism of insulin and glucagon as well as to highlight any differences in their in vivo handling.

Materials and Methods

Experiments were carried out in 13 healthy dogs using methods previously described (Polonsky et al. 1981). Anaesthesia was induced with sodium pentobarbital (30 mg/kg iv) and maintained by iv boluses (30–60 mg). Bilateral ureteric catheters were inserted to ensure complete and accurate urine collection. End sampling catheters were placed in the portal vein, hepatic vein, left renal vein, and femoral artery. The portal vein catheter was positioned so that its tip lay at the bifurcation of this vessel in the porta hepatis. The peripheral arterial catheter was inserted through a small branch of the left femoral artery into the common femoral artery. The hepatic vein catheter was passed through the right jugular vein into the left common hepatic vein 1–2 cm from the wedged position. The renal vein catheter was advanced through the left femoral vein into the left renal vein so that its tip lay as close to the renal hilum as possible. Two catheters were inserted into the left jugular vein, one for monitoring central venous pressure, and the other for infusion of somatostatin, insulin, glucagon, para-aminohippuric acid (PAH), and inulin. The correct positioning of the catheters was confirmed by a number of independent methods (Jaspan et al. 1981a). Blood flow in the portal vein and hepatic artery was measured by means of electromagnetic flow probes placed around each vessel according to techniques previously outlined (Jaspan et al. 1981a).

Physiological parameters were carefully monitored as described in detail (Jaspan et al. 1981a) and remained normal in each animal for the duration of the experiment.

Preparation of infusates

Porcine glucagon (Novo, Copenhagen) and porcine insulin (Eli Lilly Indianapolis, IN) were added in appropriate concentration to solutions containing 0.9% saline and 1% bovine serum albumen (BSA) just prior to use and were co-infused. Somatostatin (cyclic form Bachem Inc., Torrance, CA) was also prepared in 1% BSA saline just prior to use and infused separately.

Para-aminohippuric acid (PAH) (Sigma Chemical Co., St. Louis, MO) and inulin (Fisher Scientific Co., Fair Lawn, NJ) were dissolves in 0.9% NaCl prior to each experiment.

Experimental protocol

Following surgery, each animal was allowed to stabilize for 30 min. During this period, after priming injections, PAH and inulin were administered at constant rates (constant infusion pump Model 975, Harvard Apparatus Inc., Boston, MA) to achieve arterial plasma concentrations of approximately 1.5 and 50 µg/100 ml, respectively. Simultaneously an iv bolus of somatostatin (100 µg) was injected followed by a constant infusion (800 ng/kg/min) which was continued for the duration of the experiment. Samples were drawn from all 4 sampling sites after 40 min of somatostatin infusion to confirm insulin and glucagon suppression and to measure residual glucagon immunoreactivity.

The same basic experimental protocol was followed for all experiments. Each experiment consisted of from one to four experimental periods, in which, after appropriate priming injections, insulin and glucagon were infused at constant rates. The infusion rate of the hormones was kept constant in some experiments while in others it was increased in successive periods. The rate of glucagon infusion thus varied from 1 to 50 ng/kg/min and the rate of insulin infusion from 0.3 to 4.8 mU/kg/min. Samples for insulin and glucagon were drawn at 35, 40 and 45 min into each period from all 4 vessels. Samples for PAH and inulin were drawn at 35 and 45 min from femoral artery and renal vein and urine was collected over this 10 min time period. The sampling time points were chosen on the basis of preliminary experiments which revealed that this infusion time was necessary to obtain consistent steady state concentrations of insulin and glucagon. An infusate sample was taken from the infusion catheter at the point at which it entered the animal at the end of each infusion period. This value was used for calculation of the hormone infusion rate.

Sample collection

Glucagon samples were collected in chilled tubes at 4°C containing Trasylol (500 KIU/ml) and EDTA (1.2 mg/ml). Plasma was separated immediately at 4°C and stored frozen until assayed. Samples for insulin, PAH and inulin were allowed to clot at room temperature and after centrifugation at 4°C the serum was frozen as for glucagon.

Immunoassay technique

The glucagon radioimmunoassay was performed with 30K antiserum as previously described (Unger et al. 1961). Insulin radioimmunoassay was performed as previously described (Morgan & Lazarow 1963). PAH and insulin concentrations in blood and urine were measured by a technicon Autoanlyser (Technicon, Instrument Corporation, Elmhurst, IL).

Data analysis

Renal and hepatic extraction and clearance and metabolic clearance rates of insulin and glucagon were calculated by standard formulae (Polonsky et al. 1981).
Statistical methods

All results are expressed as mean ± SEM. Statistical significance of differences was assessed by paired or non-paired two-tailed Student's t-tests where applicable. P values < 0.05 being considered significant.

Results

The hepatic and renal metabolism of glucagon (Table 1)
The overall hepatic extraction of glucagon was 25.6 ± 2.3%. The hepatic plasma flow was 20.3 ± 2.3 ml/kg/min and the hepatic clearance was 4.5 ± 0.6 ml/kg/min. The renal glucagon extraction was 43.7 ± 3.9%. Renal plasma flow was 10.3 ± 0.4 ml/kg/min and the renal organ clearance was 4.7 ± 0.5 ml/kg/min. This was significantly greater than the simultaneously measured clearance of insulin which was 3.2 ± 0.5 ml/kg/min (P < 0.01).

The metabolic clearance rate (MCR) of glucagon was 16.5 ± 0.8 ml/kg/min. The hepatic contribution was 28.5 ± 4.2% and was similar to that of the kidney 28.7 ± 3.4%.

The effect of increasing arterial glucagon concentration on the hepatic and renal extraction of glucagon is shown in Fig. 1. In both organs, glucagon extraction was unaffected by its arterial concentration even at concentrations in the pharmacologic range. It can be seen in Fig. 2 that a similar independence of arterial concentration exists for glucagon metabolic clearance rate, a finding that would be expected from the non-saturability of extraction at the two major sites of glucagon clearance.

In order to confirm the non-saturability of glucagon metabolism, and compare this with insulin, measurements obtained at physiological concentrations (<1 ng/ml) were compared with measurements obtained at levels in the pharmacologic range (> 1 ng/ml). Thus hepatic extraction in periods when plasma IRG was < 1 ng/ml (25.5 ± 2.2%) was not different from that in periods when plasma IRG was > 1 ng/ml (25.7 ± 2.4%). Similarly renal extraction was not significantly different over the lower (43.5 ± 3.8%) and higher (40.4 ± 3.0%) concentration ranges.

Hepatic and renal metabolism of insulin (Table 1)
The mean hepatic insulin extraction in all experimental periods was 49.6 ± 3.4% and its hepatic clearance was 9.2 ± 1 ml/kg/min. The renal extraction of insulin was 41.7 ± 4.4% and the corresponding renal clearance was 5.0 ± 0.7 ml/kg/min. This was significantly greater than the simultaneously measured rate of insulin clearance (P < 0.01).

The metabolic clearance rate of insulin was 18.3 ± 1.5 ml/kg/min. The hepatic contribution to MCR was 51.9 ± 4.4% and the renal contribution was 27.3 ± 3.9%.

The effect of increasing the arterial insulin level on its hepatic and renal extraction was studied as for glucagon (Fig. 3). In contrast to glucagon, in the face of increasing arterial insulin concentrations there was a significant reduction in both hepatic and renal extraction. As anticipated, over the same concentration range there was also a reduction in total metabolic clearance rate of insulin (Fig. 2).

To confirm these observations the hepatic extraction in all periods in which insulin levels were below 100 μU/ml was compared to those in which arterial levels were greater than 100 μU/ml. At insulin levels < 100 μU/ml hepatic insulin extraction was 57.3 ± 2.3% and renal extraction 53.0 ± 3.3%. At insulin levels > 100 μU/ml hepatic extraction fell to 43.8 ± 2.2% (P < 0.001) and renal extraction fell to 29.6 ± 3.2% (P < 0.001).

Independence of insulin metabolism on glucagon level and glucagon metabolism on insulin level
The influence of glucagon on insulin metabolism was investigated in 3 dogs by comparing hepatic

Table 1.
The metabolism of insulin and glucagon in 13 dogs.
Results are expressed as mean ± SEM.

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<thead>
<tr>
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<th>Glucagon</th>
<th>Insulin</th>
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<tbody>
<tr>
<td>Hepatic extraction %</td>
<td>25.6 ± 2.3</td>
<td>49.6 ± 3.4</td>
</tr>
<tr>
<td>Hepatic clearance (ml/kg/min)</td>
<td>4.5 ± 0.6</td>
<td>9.2 ± 1</td>
</tr>
<tr>
<td>Renal extraction %</td>
<td>43.7 ± 3.9</td>
<td>41.7 ± 4.4</td>
</tr>
<tr>
<td>Renal clearance (ml/kg/min)</td>
<td>4.7 ± 0.5</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>Metabolic clearance rate (ml/kg/min)</td>
<td>16.5 ± 0.8</td>
<td>18.3 ± 1.5</td>
</tr>
<tr>
<td>Hepatic contribution to MCR %</td>
<td>28.5 ± 4.2</td>
<td>51.9 ± 4.4</td>
</tr>
<tr>
<td>Renal contribution to MCR %</td>
<td>28.7 ± 3.4</td>
<td>27.3 ± 3.9</td>
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The effect of increasing arterial IRG concentration on hepatic (● upper panel) and renal (○ lower panel) glucagon extraction in 34 experimental periods in 13 dogs.

Left hand panel: The effect of increasing arterial IRG concentration on the metabolic clearance rate of glucagon (31 experimental periods in 13 dogs).
Right hand panel: The effect of increasing arterial IRI concentration on the metabolic clearance rate of insulin (23 experimental periods in 13 dogs).
Fig. 3.
The effect of increasing arterial IRI concentration on the hepatic (● upper panel) and renal (○ lower panel) insulin extraction in 31 experimental periods in 15 dogs.

Discussion

The use of this dog model in the study of pancreatic hormone metabolism has previously been validated and the rationale for the use of somatostatin to suppress endogenous insulin and glucagon secretion described (Polonsky et al. 1981; Jaspan et al. 1981a). It should be noted that although somatostatin reduces portal blood flow (Jaspan et al. 1979) it does not appear to affect hepatic insulin or glucagon extraction (Jaspan et al. 1981a).

The hepatic and renal extraction of insulin measured in this study correspond closely with the values obtained by other workers in a variety of experimental models (Mortimore et al. 1959; Rubenstein et al. 1972; Kaden et al. 1973; Rabkin & Colwell 1969; Zaharko et al. 1966; Chamberlain & Stimmmer 1967). The renal extraction of glucagon is also similar to previous data (Lefebvre et al. 1974; Emmanouel et al. 1976). The hepatic extraction of glucagon taken in conjunction with previously published data (Jaspan et al. 1981a,b;
Buchanan et al. 1968) firmly establishes the importance of the liver in the metabolism of glucagon.

Collectively our studies indicate that in the basal state the liver and kidney account for approximately 80% of the metabolic disposal of insulin and approximately 55% of that of glucagon, the difference being accounted for by the greater hepatic extraction of insulin.

We have previously demonstrated that hepatic glucagon metabolism is not saturable in the dog (Jaspan et al. 1981a) and renal glucagon metabolism is not saturable in the rat (Emmanouel et al. 1976). The current data confirms these observations since neither the hepatic nor the renal metabolism of glucagon saturated up to arterial concentrations of 13.733 ng/ml. Accordingly, glucagon MCR was independent of arterial level over this same concentration range.

The question of the saturability of insulin metabolism, however remains open. Thus it has been reported that with increasing hepatic insulin delivery its extraction may increase (Kaden et al. 1973) decrease (Röjmark et al. 1978; Tranberg & Thorell 1979) or stay the same (Harding et al. 1975). Even the studies in which saturation of hepatic insulin extraction was shown suggested that this only occurred at very high insulin concentrations of 500–2000 μU/ml (McCarroll & Buchanan 1973; Mondon et al. 1975).

In the present studies, saturation of hepatic insulin extraction and insulin MCR occurred in the face of increasing serum insulin levels from basal to 300 μU/ml. Hepatic insulin extraction at arterial insulin levels above and below an arbitrarily chosen level of 100 μU/ml was then compared. It was found that hepatic insulin extraction at arterial levels < 100 μU/ml was 57.3 ± 2.3%, which was significantly greater (P < 0.001) than the value at levels > 100 μU/ml (43.8 ± 2.3%). Comparison of insulin MCR at levels > 100 vs < 100 μU/ml revealed a significant difference (20.2 ± 1.7 vs 14.9 ± 11 ml/kg/min; P < 0.01). This data is comparable to that of Sönksen et al. (1973) who in a study in man showed a progressive decline of insulin MCR from 34 to 11.4 ml/kg/min during insulin infusion which increased serum insulin levels from basal to 280 μU/ml.

These data therefore indicate that hepatic insulin metabolism saturates at concentrations encountered in the portal vein after meals, suggesting that saturation of hepatic insulin extraction may be of physiological importance in determining the proportion of secreted insulin which reaches the periphery.

A number of previous studies have concluded that renal insulin extraction is a non-saturable process (Rabkin & Colwell 1969; Chamberlain & Stimmmer 1967; Katz & Rubenstein 1973; Maude et al. 1981). In this light we were surprised to find that renal extraction measured at arterial insulin levels < 100 μU/ml (53.0 ± 3.3%) fell to 29.6 ± 3.2% when the level was raised to between 100 and 400 μU/ml (P < 0.001). The reason for the difference between these and previously published studies is uncertain but probably relates to differences in animal species, experimental model and design. In addition the studies of Rabkin & Colwell (1969), Lefebvre et al. (1974) and Chamberlain & Stimmmer (1967) examined this question at serum insulin levels less than 150 μU/ml, a range within which we were also unable to demonstrate saturability. Katz & Rubenstein (1973), however found that renal insulin extraction in the rat is a linear process even at serum concentrations as high as 375 μU/ml.

The renal clearance of both insulin and glucagon was greater than the simultaneously measured clearance of inulin, indicating that both hormones are cleared from the kidney by peritubular uptake from postglomerular blood in addition to glomerular filtration. Recently specific insulin receptors have been identified in renal tubular membranes (Kurokawa & Lerner 1980; Talor et al. 1982) and saturability of these receptors could explain the reduction in renal insulin extraction which was observed at high serum insulin levels.

The weight of current evidence indicates that hepatic insulin metabolism is a receptor mediated process (Terris & Steiner 1975; Baldwin et al. 1980). The reduction in hepatic insulin extraction observed is consistent with saturation of hepatic insulin receptors. The linear relationship between hepatic glucagon extraction and arterial glucagon level suggests that glucagon extraction is either not receptor mediated or occurs by receptor mediated and other mechanisms, since a purely receptor mediated mechanism would be expected to have a finite capacity.

In view of the close interaction between insulin and glucagon in the regulation of hepatic glucose production and similarities in the interaction of these two hormones with the cell including cell surface binding, internalization and intracellular disposal, the possibility of interdependent regula-
tion of their metabolism is of considerable importance. It is therefore of interest that consistent with the in vitro finding of Terris & Steiner (1976) and contrary to those in vivo in the dog by Röjdmark et al. (1978) the present studies demonstrated a lack of effect of glucagon on insulin metabolism. Hepatic and renal glucagon extraction and glucagon MCR were likewise shown to be independent of insulin level.

In conclusion, these studies indicate that there are major differences in the mechanisms whereby insulin and glucagon are metabolized in vivo. Of particular significance is our demonstration that at liver and kidney, the two major sites of degradation, insulin metabolism saturates at physiological serum levels. Glucagon metabolism, in contrast, is non-saturable at either hepatic or renal metabolic sites. In addition the lack of effect of insulin on glucagon metabolism and glucagon on insulin metabolism indicates that the cellular processing of the two hormones is independently regulated.

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