Dissociation of potassium and glucose efflux in isolated perfused rat liver

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The dissociation of potassium and glucose movement during glucagon-induced glycogenolysis was studied in the isolated rat liver utilizing a small volume perfusion apparatus. When glucagon was added to a preparation assumed to be insulin deficient and depleted of a fraction of liver K⁺ after 60-min perfusion, a marked outflow of glucose occurred in the absence of an early efflux of liver K⁺. However, when glucagon was administered to a liver pretreated with insulin to restore intracellular K⁺, a large efflux of potassium occurred during the onset of glycogenolysis. The administration of insulin after glucagon effected a recovery of potassium previously lost from the liver and reduced the outflow of glucose. These findings indicate that a fraction of potassium dependent upon insulin for retention within the liver moves independently of glucose and suggests that the early release of potassium during glucagon-induced glycogenolysis involves an inhibition of the effect of insulin on maintaining potassium within the cell.

Methods

Liver donor animals, male Sprague-Dawley rats weighing 220–260 g, were fed ad lib. until the time of the experiment. The surgical removal of the liver and the technique of liver perfusion employed in these studies have been described previously (3).

Previous studies have shown that the addition of insulin to the isolated perfused liver caused a prompt uptake of potassium which was not accompanied by an immediate increase in the uptake of glucose (3, 11). These findings suggested that the movement of potassium into the liver following the administration of insulin was independent of a concomitant transfer of glucose. In the intact animal, the release of potassium prior to the outflow of glucose following the administration of glucagon indicates the dissociation of the time sequence of potassium and glucose release from the liver during glycogenolysis (9, 16). Although numerous studies have been made of the glycogenolytic effect of glucagon on the isolated perfused rat liver (12, 13, 17), the time sequence of potassium and glucose release has not been reported.

The present experiments were designed to test whether or not glucagon evokes an early release of potassium during the initial outflow of glucose when administered after 60-min perfusion to livers isolated from fed rats. The effect of glucagon on the efflux of potassium and glucose under these conditions was compared to experiments in which the liver was treated with insulin before and after administration of glucagon.

The results indicated that the continuous infusion of insulin after glucagon administration effected a recovery of potassium by the liver and decreased the outflow of glucose and suggest that the early efflux of potassium from the liver effected by glucagon during the onset of glycogenolysis involves the release of a fraction of intracellular K⁺ dependent upon insulin for retention within the cell.

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Modifications of the perfusion apparatus, described in detail in the following section, were introduced to reduce hemolysis of the red blood cells, and permit simultaneous arterial and venous sampling and measurement of blood flow through the liver.

The perfusing medium consisted of nine parts heparinized blood, obtained from large, nonfasted male rats of the same strain and one part NaCl solution (0.9 g/100 ml). The initial circulating volume of perfusate was comparable to the extracellular fluid space of the intact animal and totaled 40 ml in control and insulin-treated perfusions. The initial volume was increased to 45 ml in liver perfusions to which glucagon was added to permit more frequent sampling of the perfusing medium for determination of plasma potassium concentration. Surgical preparation of both liver and blood donor animals was performed under ether anesthesia.

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Addition of insulin and glucagon. Crystalline Zn insulin, glucagon-free, (lot No. 466368, Lilly Research Laboratories, Indianapolis, Ind.) was dissolved in weak acid or base and diluted to a concentration of 1 U/ml with a salt solution containing 145 mEq NaCl and 5.6 mEq KCl/liter. Crystalline glucagon (Eli Lilly Co.) was dissolved in weak base and diluted with the above salt solution to a concentration of 2 pg/ml. These hormone solutions or the hormone diluent were added directly to the liver through the arterial catheter at the time intervals indicated in the figures and text.

Analysis of perfusing medium. Perfusate samples for determination of blood glucose were taken from the arterial overflow tube by inserting capillary tubes through openings in the reservoir flask. Glucose was determined by a commercial glucose oxidase procedure (Blood sugar—Boehringer and Sons, Mannheim, Germany) on perchloric acid filtrates of the perfusing medium. Perfusate samples of 1.0 ml, obtained from the reservoir at selected time intervals were analyzed for plasma potassium concentration by flame photometry. Calculation of the net changes of perfusate glucose and plasma K⁺ during the perfusion were corrected for sampling losses and addition of hormone or hormone diluent.

Modification of perfusion apparatus (4). The roller pump previously used for circulating blood through the apparatus was replaced with a modified Dale-Schuster pump to reduce trauma to the red blood cells (6). As depicted in Fig. 1, the pump assembly (A), consisting of two aortic-type valves connected by a rubber sleeve, is activated by an alternating positive and negative pressure arising from an air piston pump (B) cycling 60 times/min. The air pressure exerted against the rubber sleeve inside the Lucite cylinder (A) is regulated by adjusting the aperture of the bleed-off tube attached to the air manometer gauge (C). The blood passes from the pump through a nylon filter (D) (Don Baxter, Inc., Glendale, Calif.) to the aerator (E). The tubing through which the blood flows enters the aerator through an opening at the top and terminates above a convoluted glass column. With each stroke of the pump, the volume of blood ejected completely covers the convoluted column, eliminating the need for the multiple-channeled glass joint formerly used at the top of the aerator to assure adequate coverage of the column with blood when a roller pump was used to circulate the perfusing medium.

The former blood reservoir was replaced with an inverted 50-ml Erlenmeyer flask (F). A ground glass ball and socket joint provides an attachment for the receptacle containing the liver. Two small openings on the side of the flask permit addition of infusate or withdrawal of blood from either the reservoir, the venous effluent cannula, or the overflow tube containing arterial blood. A paper clip coated with polyethylene is suspended within the reservoir from a stainless steel wire passed through one of the two sampling holes. A magnetic mixer (G) positioned on the outside of the reservoir creates a gentle waving motion of the paper clip which facilitates mixing of the venous effluent and arterial overflow with the perfusate in the reservoir.

Since visual counting of drops of liver effluent per unit time as an index of volume flow through the liver becomes unreliable at flow rates greater than 10 ml/min (approx. 4 drops/sec), a drop counter and recording device was added to the perfusion apparatus. The unit consisted of a photovoltaic cell (H) with a neutral density filter (G.E. model 8PV) placed behind the blood reservoir and a small light (I). Interruption of the light beam by drops of blood flowing from the outflow cannula were recorded as deflections of an ECG stylus (Cardiotron, model PCIA) connected to the photo cell. Volume flow through the liver was obtained by recording the number of drops passing from the effluent cannula and reading the corresponding volume flow per minute from a pre-calibrated graph relating drops per second to milliliters per minute. At a constant hydrostatic pressure of 14 cm H₂O, the rate of blood flow through the 22 perfused livers reported in this study averaged 1.10 ml/min per g liver 5 min after the start of the perfusion. The flow rate increased to 1.54 ml/min by 30 min and plateaued at 1.95 ml/min after 75-min perfusion. The rate of bile flow during 2-hr perfusion was comparable to that found in the intact rat (5) and averaged 1.22 ± SE 0.04 ml/min per g liver.
**RESULTS**

The changes in plasma $K^+$ and perfusate glucose concentration during 2 hr perfusion of three control livers (group A), three livers receiving insulin from 60 to 120 min (group B), and three experiments in which the perfusing medium was circulated in the absence of the liver are illustrated in Fig. 2.

**Perfusate recirculation.** Recirculation of the perfusing medium in the absence of the liver resulted in a progressive decrease in perfusate glucose concentration due to red blood cell (RBC) glycolysis and a linear increase in plasma $K^+$ concentration arising from trauma to the circulating RBC's. In one experiment, insulin was infused at a rate of 1 unit in 1 ml salt solution at 60 min and an infusion of 1.5 ml from 60 to 120 min, 3 livers receiving 1 unit insulin in 1 ml salt solution at 60 min and an infusion of 1.5 units in 1.5 ml from 60 to 120 min, and 3 perfusate recirculations in the absence of the liver receiving the same dose of insulin or volume of hormone diluent. Values are expressed as the mean ± se change in $K^+$ and glucose concentration after 60-min perfusion.

**Control liver perfusion (group A).** The prompt increase in the concentration of perfusate glucose during the early phase of extracorporeal liver perfusion has been previously described (3, 12, 13). This response, due in part to glycogenolysis arising from the initial 4 min interruption of hepatic blood flow, subsided within 15 min and was followed by a partial recovery of glucose by the liver. Between 60 and 120 min, the net accumulation of perfusate glucose amounted to $80 ± 11 \mu$moles (Table 1). As shown in Fig. 2, a marked increase in plasma $K^+$ concentration, amounting to a net accumulation of $74 \mu$moles $K^+$, occurred during the first 90-min perfusion. After correcting for the increase in plasma $K^+$ due to RBC trauma ($21 \mu$moles/30 min), it was calculated that 53 $\mu$moles of $K^+$ were released from the liver during this period. Over the remaining 90-min perfusion, the further rise in plasma $K^+$ approximated the rate of increase due to RBC trauma, indicating that the major portion of $K^+$ release from the liver occurred during the first 90-min perfusion.

**Effect of insulin (group B).** The administration of a single dose of 1 unit insulin directly into the arterial cannula at 60 min followed by an infusion of 1.5 U/hr from 60 to 120 min effected an uptake of $90 ± 10 \mu$moles $K^+$ which was not accompanied by a significant increase in the net uptake of perfusate glucose (Table 1, $\Delta B - A$). These results confirm previous studies which demonstrated that the addition of insulin after 60-min perfusion effected a prompt recovery of potassium previously lost from the liver in the absence of any increase in the uptake of glucose from the perfusing medium (3).

The changes in plasma $K^+$ and perfusate glucose concentration resulting from the addition of 1 $\mu$g glucagon directly into the liver after 61-min perfusion and the addition of 0.125 unit insulin 1 min before glucagon are illustrated in Fig. 3.

**Effect of glucagon on potassium and glucose efflux (group C).** The data indicate that the initial outflow of glucose evoked by glucagon was not accompanied by an early release of $K^+$ from the liver. It was calculated that within the first 9 min after hormone administration, $-1 ± 4 \mu$moles of $K^+$ accumulated in the perfusing medium and over the same period the accumulation of perfusate glucose averaged $186 ± 18 \mu$moles. However, a slight increase in the outflow of $K^+$ from the liver was observed between 75 and 120 min. When compared with control liver perfusions, the administration of glucagon increased the total outflow of potassium and glucose between 60 and 120 min by 25 ± 7 and 761 ± 30 $\mu$moles, respectively (Table 1, $\Delta C - A$).

**Effect of insulin administered 1 min before glucagon (group D).** As noted in Fig. 3, a prompt outflow of glucose occurred in the absence of an early egress of liver $K^+$ during the first 9 min after the administration of both hormones. However, the late rise in plasma $K^+$ effected by glucagon was decreased by the prior administration of insulin. It was calculated that insulin administered 1 min before glucagon decreased the net accumulation of plasma $K^+$...
TABLE 1. Net accumulation of plasma K+ and perfusate glucose between 60- and 120-min perfusion; effect of insulin, glucagon and insulin administered 1 min prior to glucagon

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose, µmoles</th>
<th>Diff.</th>
<th>P</th>
<th>Potassium, µmoles</th>
<th>Diff.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td>89±11</td>
<td></td>
<td></td>
<td>53±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Insulin</td>
<td>28±33</td>
<td>D - A</td>
<td>-61±33</td>
<td>NS</td>
<td>B - A</td>
<td>-90±10</td>
</tr>
<tr>
<td>C. Glucagon</td>
<td>850±33</td>
<td>C - A</td>
<td>76±30</td>
<td>&lt;.001</td>
<td>C - A</td>
<td>95±7</td>
</tr>
<tr>
<td>D. Insulin + glucagon</td>
<td>390±106</td>
<td>D - C</td>
<td>-260±106</td>
<td>NS</td>
<td>D - C</td>
<td>-44±8</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of 3 perfusions in each group. Group A received 1 ml salt solution (145 mM NaCl and 5.6 mM KCl/liter) at 60 min and an infusion of 1.5 ml from 60 to 120 min. Group B received 1 unit insulin in 1 ml of salt solution at 60 min and an infusion of 1.5 units of 1.5 ml salt solution from 60 to 120 min. Group C received 1 µg glucagon in 0.5 ml salt solution at 61 min, and Group D 0.125 unit insulin in 1.0 ml salt solution at 60 min and 1 µg glucagon in 0.5 ml at 61 min.

The changes in plasma K+ and perfusate glucose concentration after 60-min perfusion were not significantly different in Group A and B. Group C showed a greater recovery of potassium and reduction of glucose outflow than the other groups. Group D showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group E showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group F showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group G showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group H showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group I showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group J showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group K showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group L showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group M showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group N showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group O showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group P showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group Q showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group R showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group S showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group T showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group U showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group V showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group W showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group X showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group Y showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion. Group Z showed a decrease in glucose concentration and a prompt efflux of potassium during the initial 10 min of perfusion.

Effect of glucagon administered after 75-min perfusion (group E). As previously shown, glucagon administered to livers depleted of K+ lost during the first 75-min perfusion did not affect an increase in plasma K+ during the initial outflow of glucose. However, a late increase in plasma K+, amounting to a net accumulation of 55 ± 4 µmoles was noted between 90- and 120-min perfusion (Table 2). After correcting for the increase in plasma K+ due to RBC trauma (21 µmoles/30 min), it was estimated that 34 µmoles of K+ were released from the liver during this period. The net accumulation of glucose in the perfusing medium totaled 723 µmoles, one-half accumulating between 75 and 90 min and the remainder between 90- and 120 min perfusion.

Effect of insulin administered 15 min before the addition of glucagon (group F). The addition of 0.125 unit insulin at 60 min followed by an infusion of 1.5 U/hr from 60 to 74 min effected a prompt recovery of 65 ± 4 µmoles of K+ by the liver during this period in the absence of a significant uptake of perfusate glucose (62 ± 33 µmoles, Table 2). The administration of glucagon under these conditions effected a prompt efflux of potassium during the onset of glycogenolysis. It was calculated that 46 ± 13 µmoles of K+ and 50 ± 10 µmoles of glucose were released from the liver 3 min after the administration of glucagon. The net changes in plasma K+ listed in Table 2 indicate that between 75 and 90 min perfusion, 67 ± 6 µmoles K+ accumulated in the perfusing medium. When compared with perfusions receiving glucagon alone (group E), the increased efflux of 58 µmoles of K+ (67 - 9) was associated with a decreased outflow of 84 µmoles glucose (279 - 363) between 75 and 90 min.
Between 90 and 120 min, livers pretreated with insulin released the same amount of glucose as those treated with glucagon alone (350 ± 9, 360 ± 18), although the total release of potassium during this period was decreased 23 ± 8 μmoles (Table 2, ΔF – E).

Effect of continuous infusion of insulin between 60 and 120 min and a single dose of glucagon at 75 min (group G). The administration of 0.125 unit insulin at 60 min followed by an infusion of 0.125 unit insulin/ml salt solution at a rate of 1.5 ml/min from 60–120 min effected a prompt recovery of potassium by the liver prior to the addition of glucagon similar to that noted in group F. A prompt efflux of potassium was again observed during the first 15 min of glucose administration. It was calculated that insulin reduced the outflow of glucose 163 ± 38 μmoles and effected a net recovery of 56 ± 6 μmoles of potassium by the liver (Table 2, G – E).

DISCUSSION

The results of the present study indicate that the release of glucose from the liver following the administration of glucagon can be clearly separated from the efflux of potassium. When glucagon was administered after 60-min perfusion to a liver which had previously lost potassium to the perfusing medium, the initial outflow of glucose occurred in the absence of an early release of potassium. By contrast, when glucagon was administered to a liver pretreated with insulin to recover the initial potassium loss, a prompt efflux of potassium occurred during the onset of glycogenolysis without increasing the early outflow of glucose. These findings suggest that the prompt efflux of potassium effected by glucagon in the isolated perfused rat liver involves the release of a fraction of liver K+ responsive to insulin whose movement out of the cell is not accompanied by an increased outflow of glucose.

Previous perfusion studies have shown that the movement of an insulin-dependent fraction of liver K+ is dissociated from the transfer of glucose. Mortimore (14) demonstrated that retention of potassium effected by the addition of insulin to livers isolated from fasted rats was not accompanied by a demonstrable effect of the hormone on perfusate glucose. Subsequently, Keestens et al. (11) and Burton and Ishida (3) noted that the addition of insulin after 60-min perfusion to the isolated dog and rat liver, respectively, effected a prompt recovery of potassium by the liver in the absence of an increased uptake of perfusate glucose. Thus, it appears from the above studies and the current findings that insulin has an im-
portant role in retaining a fraction of potassium within the liver or effecting its recovery when previously lost. Since the hyperkalemic effect of glucagon in the perfused liver occurs only when the insulin-dependent fraction of liver K⁺ is present within the cell, the data suggest that glucagon opposes the effect of insulin on retaining potassium.

It has been inferred from studies in the intact animal that the early release of potassium from the liver following the administration of epinephrine may be incidental to the action of the hormone on the glycogenolytic enzyme system (7). Finder et al. (16) and Tsujimoto et al. noted that the efflux of potassium from the intact dog liver following administration of glucagon and epinephrine, respectively, preceded activation of liver phosphorylase. Finder and co-workers suggested that the initial release of potassium by glucagon may represent a primary action of the hormone on activating glycogenolysis. Our studies on the isolated rat liver indicate that glucagon activated glycogenolysis without an early efflux of potassium when a fraction of liver K⁺ responsive to insulin was absent from the cell. However, when this fraction was present, glucagon evoked its prompt discharge concurrently with the onset of glucose outflow. These findings imply that the loss of potassium from the isolated liver is not a primary event in the activation of glycogenolysis. However, release of an insulin-dependent fraction of liver K⁺ coincides with the onset of glucagon-induced glycogenolysis when it is present within the cell. Accordingly, the question may be raised whether or not the recovery of liver K⁺ by insulin is associated with an effect of this hormone on suppressing glycogenolysis. Studies on the intact dog have demonstrated that insulin reduces glucose outflow from the liver, due in part to inhibition of glycogen breakdown (1, 8). Our studies on the isolated liver, in contrast to those of others (19, 19), show that continuous administration of insulin significantly decreased the glucagon-induced outflow of glucose from the liver and this event coincided with recovery of plasma K⁺ (Fig. 4, Table 2). Although the effect of insulin on reducing glucose outflow was not of sufficient magnitude to be correlated with detectable changes in liver glycogen, a greater inhibitory effect of insulin on glucose outflow and glycogen breakdown might be attained by employing more physiological doses of the two hormones for a longer period. Sokal et al. (17) reported that the minimal glycogenolytic concentration of glucagon effective in the isolated perfused rat liver was 0.4 μg/ml plasma, a concentration comparable to levels observed in vivo, and suggested that glucagon may have a physiological role as an hepatic glycogenolytic agent. Of note is the observation that the molar concentration of glucagon (1.2 × 10⁻⁸ M) found by Sokal and co-workers to be physiologically active, is of the same magnitude as the minimal concentration of insulin found to be effective in decreasing potassium and glucose outflow from the isolated liver (14, 15).

Further studies are required to evaluate whether or not the competitive effect of insulin and glucagon on the transfer of potassium is incidental to or associated with opposing effects of these hormones on the maintenance and breakdown of liver glycogen.

REFERENCES