The Site of Insulin Resistance in Acute Uremia

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SUMMARY

In order to define the mechanism of glucose intolerance in acutely uremic rats, various studies were carried out 24 hours after bilateral nephrectomy. Glucose removal following intravenous glucose was *significantly* ($p < 0.001$) decreased in uremic rats compared with sham-operated rats ($k = 2.1 \pm 0.03$ per cent vs. 5.1 ± 0.2 per cent). This deterioration in glucose tolerance was associated with higher insulin levels in uremic rats from one to 40 minutes after glucose administration, suggesting that insulin resistance accounted for the decrease in glucose removal by uremic rats. To identify the site of the insulin resistance, we compared the ability of insulin to enhance net glucose uptake by isolated perfused liver and muscle (hindlimb) preparations obtained from uremic and sham-operated rats. Insulin suppressed glucose outflow from perfused livers of uremic rats at least as well as it did from livers of sham-operated rats, and suppression occurred at both maximal (> 600 μU./ml.) and threshold (75 μU./ml.) perfusate insulin levels. In contrast, there was a significant decrease in the ability of insulin (mean perfusate level = 225 μU./ml.) to enhance glucose uptake of perfused hindlimbs of uremic as compared with sham-operated rats. These results suggest that the insulin resistance of acute uremia may be due primarily to decreased insulin-mediated uptake of glucose by skeletal muscle without any decrease in sensitivity of the liver to insulin. DIABETES 27:571-76, May, 1978.

Considerable evidence has accumulated to suggest that the glucose intolerance of the uremic syndrome is secondary to a loss of normal insulin sensitivity.[1,2] However, there is less consensus regarding the organ systems operative in the development of insulin resistance.[3-6] In an effort aimed at defining the cause of the insulin resistance of uremia, it seemed reasonable to determine which anatomic sites demonstrate resistance to insulin action as uremia develops. In an attempt to provide such information, we have used the technique of isolated organ perfusion. Both perfused liver and skeletal muscle have been shown to readily respond to added insulin,[7,8] and these tissues are also recognized to be the ones primarily responsible for the disposal of a glucose load. Therefore, in this study we have attempted to define the locus of insulin resistance in uremia by assessing the sensitivity to insulin of isolated perfused liver and hindlimb preparations from acutely uremic rats.

METHODS

Intravenous glucose tolerance tests (IVGTT) in intact animals and perfusion studies on livers and hindlimb preparations were performed on male rats of the Sprague-Dawley strain weighing between 120 and 200 gm. Rats were anesthetized with light ether two to three hours after removal of food and either nephrectomized by bilateral dorsal excision of the kidneys or sham-operated. All animals were fasted for the 24 hours following surgery, and uremic animals were deprived of water to prevent water loading.

IVGTT. All animals were unanesthetized and wrapped in a terry-cloth towel to minimize struggling and physical activity during the test period. The initial blood sample was taken from the cut tip of the tail into four microhematocrit capillary tubes rinsed with 5 per cent EDTA solution. Glucose was then injected into the tail vein (0.25 ml. of a 50 per cent dextrose solution per 100 gm. body weight). Subsequent blood samples were taken at 10, 20, 30, and 40 minutes postinjection for determination of plasma glucose and

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insulin concentrations by methods previously described.[8] Glucose removal rate (K) for each interval of time was calculated from the formula $K = 2.3 (\log GT_1 - \log GT_2)/t_2 - t_1$. Additional animals were killed by decapitation one minute after glucose administration to note the maximal rise in plasma glucose and insulin levels after dextrose injection.

Liver perfusion. The apparatus and technique for simultaneous in situ perfusion of four livers in a multiple perfusion box maintained at 37° C. has been previously described.[8,9] The perfusing medium consisted of 45 per cent blood prepared from four parts defibrinated blood obtained on the day of the experiment from large normal male rats and five parts Krebs-Ringer bicarbonate (KRB) buffer. The KRB solution contained 3 gm. bovine serum albumin per deciliter, which was filtered through a 0.45-μ Millipore filter to remove bacterial contaminants before mixing with the blood. The perfusate volume averaged 33 ml. at the start of recirculation, and livers from sham and uremic animals, weighing approximately 5 gm., were perfused at flow rates averaging 1 mg./gm. per minute for a period of 120 minutes. Initial perfusate glucose concentration averaged 3 mM. Livers from sham-operated and acutely uremic rats were separated into three groups, and varying amounts of insulin were added at rates to maintain perfusate insulin concentrations at three different sustained levels.[8] The first group did not get added insulin; receiving only KRB buffer with 0.5 gm. bovine serum albumin at flow rates of 0.435 ml. per hour from zero to 120 minutes. The second group received 8 to 10 mU. porcine insulin per hour to sustain perfusate insulin concentration at physiologic levels (75 μU./ml.), while the third group received 75 to 90 mU. insulin per hour to sustain perfusate levels at maximally effective concentrations (> 600 μU./ml.).

Hindlimb perfusion. The technique and methodology of perfusion of the bisected hindlimb preparation has been recently described.[10] The perfusing medium consisted of 75 ml. of 60 per cent blood (6:4 mixture of defibrinated rat blood and KRB buffer) prepared in a manner similar to the liver perfusion studies. Both sham-operated and uremic animals were circulated with blood perfusate prepared from normal fed rats. After cannulation of the inferior vena cava (IVC), the arterial perfusate flow into the abdominal aorta was started and maintained at constant rates averaging 8.6 ml. per minute (range 7.5 to 10). The initial 20 ml. of perfusate passing through the preparation was discarded, and during this period the carcass was bisected below the left renal artery and the hindlimb placed into a polypropylene funnel. After the initial 20 ml. were flushed out, recycling of the residual perfusate volume was initiated by connecting the IVC cannula and the tip of the polypropylene funnel to the perfusate reservoir flask. The drops flowing into the flask from the funnel per unit time provided a measure of blood flow through the vertebral plexus and transected dorsal lateral muscle that averaged 0.325 ml. per minute, or 5 per cent of the arterial inflow (range 0 to 13 per cent) in the 30 hindlimb preparations used in this study. Glucose was added to the perfusing medium to elevate initial levels to 80 mg./dl., and it was infused at constant rates averaging 93 mg. per hour from zero to 120 minutes. Porcine insulin was added to half of control and uremic preparations to elevate initial levels to 170 μU./ml. as well as being infused continuously for 120 minutes. Since uremic muscle removes insulin at one-third the efficiency of normal muscle,[10] the rate of insulin infusion to sham-operated hindlimbs was set at 21 mU. per hour and to 7.5 mU. per hour to uremic hindlimbs. With this approach, perfusate insulin concentration was maintained at comparable levels, averaging 225 μU./ml. between 20 and 120 minutes.

Estimation of insulin responsiveness. The response of the liver to insulin was assessed by its demonstrated effect on suppressing the net efflux of glucose. The amount of substrate released to the perfusing medium at physiologic and maximal insulin levels was compared with the outflow by control livers by multiplying the net change in concentration of perfusate constituents between zero and 120 minutes by the perfusate serum volume after correcting for sampling losses. The results were expressed per unit weight liver, and the significance of differences between means was established by Student's *t*-test. The responsiveness of perfused hindlimb muscle to insulin was assessed by the difference in elevation of perfusate glucose concentration between control and insulin-treated preparations during a continuous infusion of dextrose (93 mg. per hour). The quantitative uptake of glucose by the hindlimb between 20 and 120 minutes was calculated from the quantity of dextrose infused minus sampling losses and the net accumulation of glucose in the perfusing medium during this period. The results were expressed as nmoles glucose taken up per unit skeletal muscle mass per minute, and the significance of difference between control and insulin-treated muscle was established by Student's *t*-test.

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RESULTS

Glucose and insulin responses. Mean (± S.E.) fasting plasma glucose concentrations before the intravenous glucose challenge were 83 ± 4 mg./dl. in sham-operated as against 99 ± 5 mg./dl. in acutely uremic rats. The results in figure 1 (left) reveal that the rate of disappearance of the intravenous glucose load between 10 and 40 minutes was significantly slower ($p < 0.001$) in uremic (k = 2.13 ± 2.9) than in sham-operated rats (k = 5.05 ± 2.4). The data in figure 1 (right panel) display the mean (± S.E.) plasma insulin responses of the two groups of animals and demonstrate that plasma insulin levels were significantly elevated initially ($p < 0.01$) as well as at every time point following glucose administration.

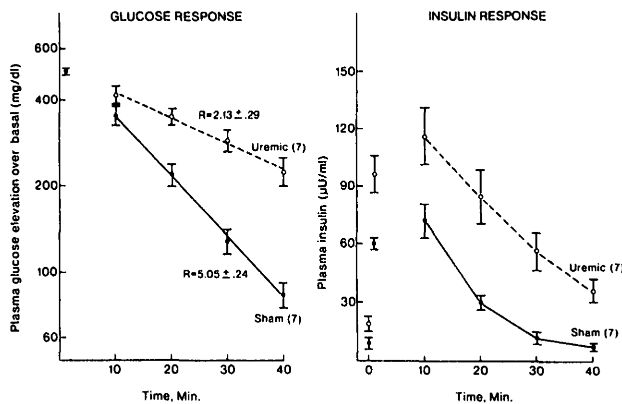

FIG. 1. (Left) Elevation of serum glucose concentration over basal levels after intravenous administration of 125 mg. glucose per 100 gm. body weight to seven uremic and sham-operated rats. (Right) Elevation of plasma insulin concentration following intravenous administration of glucose to uremic and sham-operated rats. Values at one minute represent Mean ± S.E. of five to six animals at each point.

The effect of insulin on perfused liver. The ability of insulin to suppress glucose outflow is illustrated in figure 2. The data indicate that in the absence of insulin (zero concentration), glucose outflow from sham livers (17.1 ± 1.5 μmol/gm.) was significantly greater ($p < 0.02$) than from livers of uremic rats (11.3 ± 1.3). The ability of insulin to suppress net glucose outflow from livers of sham and uremic rats was dose-related, with perfusate insulin levels greater than 600 μU./ml. eliciting a greater effect than insulin levels of 75 μU./ml. Of particular note was the observation that uremic livers were at least as responsive to insulin action as sham livers. If anything, at physiologic perfusate insulin levels (75 μU./ml.) the

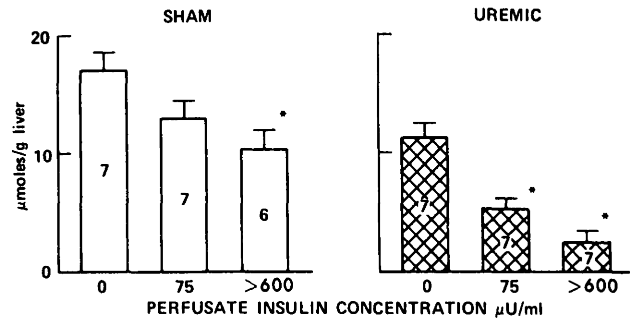

FIG. 2. Effect of physiologic (< 75 μU./ml.) and maximal (> 600 μU./ml.) perfusate insulin concentration on suppression of glucose outflow by sham-operated and uremic rats after 120 minutes' perfusion. Number of experiments in each group are listed in the bars, and significance of differences from control perfusions are indicated by *$p < 0.02$ sham and $p < 0.005$ uremic.

absolute decrease of net glucose outflow in uremic livers was slightly larger (6.0 μmol) than in sham livers (4.2 μmol). Relative to the total outflow, this effect of insulin amounted to a 53 per cent ([11.3 − 5.3]/11.3 × 100) reduction of glucose outflow by uremic livers and a 25 per cent decrease ([17.1 − 12.9]/17.1) in sham livers at this insulin concentration. In a similar fashion, insulin suppressed hepatic glucose release from uremic livers by 78 per cent at levels > 600 μU./ml., in contrast to a 40 per cent reduction when sham livers were perfused at the same insulin level.

The effect of insulin on perfused muscle. Studies of the ability of insulin to promote glucose uptake by hindlimb muscle from uremic and sham-operated rats were conducted under conditions in which a continuous infusion of glucose was provided to ensure adequate substrate for insulin action during the course of perfusion. In addition, insulin was given to half of each group at infusion rates calculated to provide identical steady-state perfusate insulin levels. These results are seen in figure 3 and permit several conclusions. In the first place, these data show that a comparable rise in perfusate glucose occurs when hindlimbs from sham and uremic rats are perfused in the absence of insulin. Second, they demonstrate that comparable mean perfusate insulin levels were achieved that significantly ($p < 0.005$) inhibited the rise in perfusate glucose levels when hindlimbs from both sham-operated or acutely uremic rats were perfused. Finally, they suggest that the magnitude of this effect of insulin was greater in hindlimbs of sham-operated than in acutely uremic rats.

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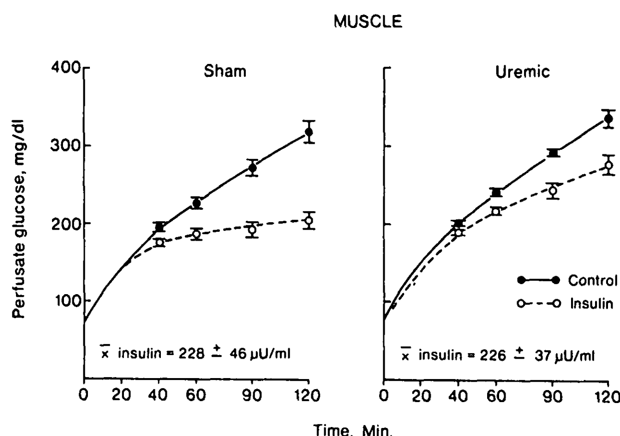

FIG. 3. Effect of insulin on disappearance of glucose from perfusing medium during perfusion of hindlimbs from sham-operated and uremic rats. Mean (x̄) insulin was calculated from average perfusate concentration between 20 and 120 minutes. The effect of insulin on lowering perfusate glucose concentration was significant to $p < 0.005$ at 60, 90, and 120 minutes in both sham-operated and uremic perfusions.

In an effort to more precisely quantify this difference, we calculated glucose uptake by the perfused hindlimbs by subtracting the net accumulation of glucose in the perfusate between 20 and 120 minutes from the amount of glucose infused during this period. In order to relate this to the mass of skeletal muscle used in this process, we used data from previous studies[10] that indicated that skeletal bone and cartilage in the hindlimb portion of fasted rats weighing 170 ± 10 gm. average 13.4 per cent of "stripped" hindlimb weight, i.e., that portion remaining after removal of the skin and gross subcutaneous, abdominal, perianal, and intermuscular fat as well as the nonperfused ligated tail, genitalia, and bladder. Therefore, an approximation of maximal skeletal muscle mass was made by multiplying the stripped-carcass weight after perfusion by 0.863 (1 − 0.134), and the glucose uptake by control and insulin-treated hindlimb for uremic and sham-operated rats was expressed as nmoles glucose uptake per gram muscle per minute. The results of these calculations are seen in table 1 and indicate that hindlimbs from uremic and sham-operated rats exhibit comparable uptake of glucose by skeletal muscle when perfused in the absence of insulin. Furthermore, these data demonstrate that glucose uptake was significantly increased by about 40 per cent (62/160 × 100) when hindlimbs from sham-operated rats were perfused at insulin levels of 222 μU./ml. On the other hand, the increase in glucose uptake effected by comparable insulin levels in hindlimbs from acutely uremic rats was considerably smaller, averaging only 15 per cent (23/156 × 100), than in control perfusions.

DISCUSSION

The data we have presented indicate that the glucose intolerance of acute uremia in the whole rat is not due to a defect in insulin secretion and is most likely secondary to the development of insulin resistance. Furthermore, the results of the studies of isolated perfused liver and muscle suggest that insulin resistance develops only in the muscle. It is tempting to relate the results of studies of the isolated perfused organs back to the intact organism, and in so doing we would have to conclude that the development of glucose intolerance in the acutely uremic rat is due in part to the loss of normal insulin responsiveness by skeletal muscle. This conclusion is consistent with the studies by Westervelt,[4] who noted that basal glucose uptake by forearms of uremic subjects was comparable to a control group but that glucose uptake of uremics was only 25 per cent that of controls following intra-arterial infusion of insulin. These results suggest that uremic forearm muscle was not resistant to glucose entry per se but was unable to respond normally to insulin stimulation of glucose uptake. Other studies have demonstrated that glucose utilization by rat diaphragm was decreased following incubation in uremic serum in the absence of insulin,[11-13] suggesting that uremia may also lead to an alteration of metabolic activity of muscle independent of insulin antagonism. However, these findings do not exclude the possibility that inhibitors in uremic serum that decrease utilization directly may also antagonize insulin activity. Thus, the prevailing evidence to date is consistent with the view that glucose intolerance of acute uremia is associated with an abnormality of glucose uptake by

TABLE 1

Effect of insulin on glucose uptake in perfused hindlimbs of sham-operated and uremic rats

	Glucose uptake (nmol/gm. muscle/min.)	
	Sham (7)	Uremic (8)
Control	160 ± 9	156 ± 13
Insulin	222 ± 12	189 ± 7
ΔI-C	62 ± 15	23 ± 15
p	< 0.005	N.S.

Numbers of control and insulin-treated experiments are listed in parentheses. Values are expressed as Means ± S.E.

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muscle and that this defect arises from the accumulation of uremic toxins and a developing resistance to insulin action.

With regard to the role of the liver in the glucose intolerance of uremia, earlier studies by Cohen[4] and others on uremic patients and experimental animals concluded that the liver had a part in this process, either by virtue of a primary defect in hepatic glycogenesis or one arising secondarily to the accumulation of uremic toxins.[14-16] Although others have reported that the deposition and breakdown of liver glycogen is not impaired in uremia,[17-20] it would appear that in some cases the degree and length of uremia may modify the nutritional status of the animal resulting in decreased or altered glycogen stores.[21] However, in well-maintained uremic subjects or acutely uremic fasting rats, it seems unlikely that altered hepatic glycogen stores can account for the abnormalities in glucose tolerance. On the other hand, increased gluconeogenesis may contribute to the glucose intolerance of uremia. Enhanced glucose output, uptake of glycogenic amino acids, and transaminase activity have been noted in livers from rats 44 hours after nephrectomy.[22-25] Furthermore, recent studies have documented both hyperglucagonemia[26] and increased glucagon sensitivity[27] in subjects with chronic renal failure. Thus, a number of factors may lead to increased hepatic glucose production in uremia and in so doing contribute to the glucose intolerance. However, our results suggest that this is not likely to be the case. In the first place, hepatic glucose release from perfused livers of acutely uremic rats was actually less than that from livers of normal rats in the absence of insulin. Furthermore, the ability of insulin to suppress hepatic glucose outflow was greater at both physiologic (75 μU./ml.) and maximally effective perfusate insulin levels in acutely uremic than in sham-operated rats (figure 2). The results of these studies do not permit us to define the cause of this decreased glucose outflow from livers of acutely uremic rats either in the basal state or in response to insulin, and a variety of changes in glycogenolysis and/or gluconeogenesis could account for the experimental observations. On the other hand, and regardless of the mechanism, it is clear that uremic livers are not resistant to insulin action in suppressing glucose outflow. Since patients with uremia as well as experimental animals have increases in both fasting and postglucose insulin levels[1,2,28] (figure 1), we would suggest that increased hepatic glucose production is not a major cause of glucose intolerance as long as insulin secretion is maintained.

Finally, our suggestion as to the anatomic site responsible for the glucose intolerance of uremia does not provide any insight as to the cause of the defect in insulin-induced glucose uptake by skeletal muscle. It is obvious that it could be due to a primary change in the binding or responsiveness to insulin in the tissue itself and/or be secondary to the accumulation of toxic factors in the blood of uremic subjects. Current efforts in our laboratory are aimed at an attempt to provide additional information in this regard.

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