Delayed Insulin Transport Across Endothelium in Insulin-Resistant JCR:LA-cp Rats

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Capillary endothelial cells are thought to limit the transport of insulin across the endothelium, resulting in attenuated insulin action at target sites. Whether endothelial insulin transport is altered in dysglycemic insulin-resistant states is not clear and was therefore investigated in the JCR:LA-cp corpulent male rat, which exhibits the metabolic syndrome of obesity, insulin resistance, hyperlipidemia, and hyperinsulinemia. Lean littersmates that did not develop these alterations served as controls. Animals of both groups were normotensive (mean arterial pressure 136 ± 2 mmHg). Hearts from obese and lean rats aged 7 (n = 6) or 18 (n = 8) weeks were perfused in vitro at 10 ml/min per gram wet wt over 51 min with Krebs-Henseleit buffer containing 0.1 or 0.5 U human insulin/l (equivalent to 0.6 and 3 nmol/l). Interstitial fluid was collected using a validated method, and interstitial insulin was determined with a radioimmunoassay. At 0.1 U/l, insulin transfer velocity was similar in both experimental groups (half-times of transfer: 11 ± 0.2 min in obese and 18 ± 4 min in lean rats; NS), but at 0.5 U/l, the respective half-times were 7 ± 1 min in lean and 13 ± 2 min in obese rats (P < 0.05). The steady-state level of insulin in the interstitium was 34 ± 1% of the vascular level at 0.1 U/l and reached the vascular level (102 ± 2%) at 0.5 U/l in both lean and obese rats. In rats aged 18 weeks, the half-times of insulin transfer were 31 ± 2 and 14 ± 1 min in obese rats and 10 ± 0.3 and 7 ± 0.3 min in lean rats (P < 0.05). Again, interstitial steady-state levels were similar in both groups. Finally, postprandial insulin dynamics were simulated over a period of 120 min with a peak concentration of 0.8 U/l in rats aged 27 weeks (n = 4). The maximal interstitial level was 0.38 ± 0.02 U/l in lean rats and 0.24 ± 0.02 U/l in obese rats (P < 0.05), and a similar difference was noted throughout insulin infusion (areas under the transudate concentration–time curves: 17 and 11 U/min per l, respectively). These data show, for the first time in a genetic animal model of insulin resistance, that transfer of insulin across the endothelium is substantially delayed in obese insulin-resistant rats and that it likely contributes to the postprandial alterations of glucose metabolism observed in the metabolic syndrome.

The dynamics of glucose uptake in peripheral tissue closely correspond to changes in lymphatic insulin concentrations, suggesting that glucose uptake rates are a function of lymphatic (interstitial) insulin levels (1). Insulin concentrations measured in lymph (2–4) and interstitial fluid (5) are substantially lower than corresponding plasma concentrations. In addition, the kinetics of interstitial insulin are delayed in comparison with those of plasma insulin, so that peripheral glucose uptake lags behind changes of plasma insulin levels (6). These findings led to the hypothesis that the capillary endothelium constitutes a barrier for insulin transfer and that transendothelial insulin transport is receptor mediated, saturable, unidirectional (7), and therefore rate limiting for insulin action in muscle and liver (8). Recently, in a model of isolated perfused rat hearts, we were able to provide evidence that insulin is transported across the capillary wall via a bidirectional convective transport and that capillary endothelial cells affect kinetics of insulin transport without being crucial for the generation of a gradient (9). In line with our findings, Steil et al. (10), in a clamp-study using physiological and pharmacological insulin concentrations, found no evidence for a receptor-operated insulin transport in the hind-limb of dogs and suggested diffusion processes to be involved. In addition to insulin resistance, the onset of insulin action is delayed in human obesity and type 2 diabetes (10–12). Recently, the kinetics of interstitial insulin were found to be slower in a model of artificially hyperinsulinemic dogs (13), and reduced capillary permeability was reported for the insulin-resistant fructose-induced hypertensive rat (14).

By regulating the dynamics of insulin transport, the capillary endothelium is likely to be involved in the control of glucose tolerance after acute carbohydrate loads and might contribute to the metabolic alterations seen in insulin resistance. However, in dysglycemic insulin-resistant states, it remains to be determined whether transendothelial transport of insulin is altered. This hypothesis was investigated in the JCR:LA-cp rat. In this model, animals homozygous for the autosomal recessive cp gene (cp/cp) become obese and insulin-resistant, resulting in a syndrome very close to the human metabolic syndrome with hyperinsulinemia, dysglycemia, hyperlipidemia, and spontaneously accelerated vascular disease. In contrast, homozygous normal (+/+ or heterozygous (+/cp) animals are lean and do not develop metabolic alterations (15). Our study was designed to compare transendothelial transport...
of insulin in isolated hearts of obese and lean rats of the JCR:LA-cp strain. Intestinal and vascular insulin levels were measured simultaneously using a sensitive and validated method of interstitial fluid collection (16,17).

**RESEARCH DESIGN AND METHODS**

Human insulin (Actrapid HM) was obtained from Novo Nordisk A/S ( Bagsvaerd, Denmark). The anti-human insulin antibody was from Phoenix Pharmaceuticals (Belmont, CA). All other chemicals were obtained from Merck (Darmstadt, Germany).

**Animals and heart perfusion.** Male rats of the JCR:LA-cp strain were bred in our own breeding colony using standard husbandry techniques and a formal system of outbreeding (18). The young rats were identified either as obese and homozygous for the cp gene (cp/cp) or as lean and homozygous/heterozygous (+/?). The animals were housed in a pathogen-free environment and were weaned at 3 weeks of age. Animals were anesthetized with diethyl ether, and their hearts were removed and perfused retrogradely (Langendorff mode) at a rate of 9.0 ml/min per gram heart wet wt with a modified Krebs-Henseleit bicarbonate buffer (composition in mmol/l: NaCl 118, NaHCO3 25, KH2PO4 1.2, KCl 4.8, MgSO4 1.2, CaCl2 1.25, glucose 11) using the ISOCOR-1 perfusion system (Hugo Sachs Elektronik, March-Hugstetten, Germany). The perfusion solution was continuously gassed with carbogen (95% O2 and 5% CO2) in an oxygen chamber and had a pH of 7.4 ± 0.02 and an oxygen partial pressure of >450 mmHg. Heart temperature was measured with a Physitemp probe (Physitemp Instruments, Clifton, NJ) located directly above the heart for 51 min to give a final concentration of 0.1 U/l by using a roller pump (Ismatec, Glattbrugs-Zürich, Switzerland). Then the insulin concentration was measured using an RIA as previously described (15). The method is fairly reproducible in rats that have been trained to the procedure (range of coefficients of variation of 10 consecutive measurements in 9 cp/cp animals 3.2-10.4%, mean 6.2).

**Data analysis and calculations.** Intestinal insulin concentration-time curves were fitted to a monoequational equation using nonlinear regression analysis. This procedure yielded the apparent first-order rate constants for the influx of insulin into the interstitium (k1), the clearance of insulin after cessation of perfusion (k2), and the steady-state level of insulin reached. The corresponding half-times (t1/2 and t2/2) were calculated from 0.693/k1 and 0.693/k2, respectively. All statistical comparisons were performed using Student’s unpaired t test. Significance was assumed at P < 0.05. Data are reported as means ± SE.

**Perfusate sample collection**

Protocol 1. Intestinal transudate was collected quantitatively at intervals of 3–6 min (Figs. 1–6) during insulin perfusion and every 3 min during the clearance period. The insulin concentration in the perfusion buffer was checked by collecting coronary effluent for 5 s at the end of every insulin infusion.

Protocol 2. Intestinal transudates were collected quantitatively in intervals of 5 min. At the end of every transudate-sampling interval, coronary effluent was collected for 5 s. All samples were collected in polypropylene tubes and were immediately frozen at −20°C until analysis.

**Measurement of insulin.** Insulin levels were determined by RIA using a polyclonal rabbit antibody specific for human insulin but lacking cross-reactivity with human proinsulin and human C-peptide (Phoenix Pharmaceuticals, Mountain View, CA). Either 100 µl insulin standard (25–800 µU/ml perfusion buffer) or 100 µl effluent or transudate sample was incubated with 100 µl antibody solution (prepared in perfusion buffer) overnight (~16 h) at room temperature, which was followed by addition of ~10,000 cpm [3H]-labeled insulin at tyrosine A-14 (Amersham, Amersham, U.K.) for 5 h. To facilitate separation of bound from free insulin, 100 µl γ-globulin (10 mg/ml) and 750 µl polyethylene glycol 6000 (20%) were added; the tubes were vortexed, allowed to stand for 45 min, and centrifuged; and the pellet was counted in a gamma-counter (Packard, Canberra, Vienna). The concentration of insulin inhibiting 50% of binding of [3H]insulin (IC50) of the standard curve was 165 ± 21 µU/ml, and the detection limit was 3.5 ± 0.3 µU/ml (n = 5). The intra- and interassay coefficients of variation were determined with 100 µU/ml insulin assayed 4 times in 1 run and in 4 different runs and were 5.6 and 9.6%, respectively. In rats aged 7 weeks, the time-matched transudates from 2 hearts were pooled and subjected to RIA, whereas enough transudate was produced in hearts from rats aged 18 weeks for individual transudate concentration-time curves. Rat-specific insulin was measured using an RIA as previously described (15).

**Measurement of blood pressure.** Systolic blood pressure was measured in conscious animals using the tail-cuff system (series 9000; Technical & Scientific Equipment, Bad Homburg, Germany). Animals were immobilized in a heated restraining box, the cuff was placed at the root of the tail, and the infrared sensor was placed in the middle portion of the tail. The cuff is automatically inflated and deflated, and the pressure reading coincides with the restoration of the caudal arterial pulse. The method is fairly reproducible in rats aged 18 weeks for individual transudate concentration-time curves. Rat-specific insulin was measured using an RIA as previously described (15).

**Experimental protocols**

Protocol 1. Hearts were perfused for 30 min to establish stable baseline conditions. Human insulin was then added to the perfusion buffer via a sidetrack located directly above the heart for 51 min to give a final concentration of 0.1 U/l and was followed by a perfusion for 24 min without insulin (clearance period). A second perfusion period ensued with a concentration of human insulin of 0.5 U/l for 51 min and a clearance period of 24 min. The total duration of the experiment was 180 min. The functional performance of the hearts was stable through the clearance period. The corresponding half-times (t1/2 and t2/2) were calculated from 0.693/k1 and 0.693/k2, respectively. All statistical comparisons were performed using Student’s unpaired t test. Significance was assumed at P < 0.05. Data are reported as means ± SE.

![FIG. 1. Intestinal transudate flow rates in isolated hearts of J CR:LA-cp rats aged 7 weeks. The animals were either obese (cp/cp) or lean (+/?). Transudates were collected as described in RESEARCH DESIGN AND METHODS. Data are means ± SE from 6 hearts.](image)
RESULTS

Experimental groups. The body weight of cp/cp rats was significantly higher than that of lean controls in all of the age-groups: 246 ± 2 vs. 183 ± 3 g (7 weeks, n = 6); 574 ± 5 vs. 357 ± 7 g (18 weeks, n = 8); and 685 ± 13 vs. 418 ± 12 g (27 weeks, n = 4) (P < 0.05 in each case). However, the corresponding heart weights were not different between groups: 0.70 ± 0.018 vs. 0.68 ± 0.025 g (7 weeks, n = 6); 1.23 ± 0.02 vs. 1.18 ± 0.02 g (18 weeks, n = 8); and 1.26 ± 0.02 vs. 1.23 ± 0.02 g (27 weeks, n = 4). Systolic arterial blood pressure was measured in animals aged 14–16 weeks and was not different between groups (134 ± 1.5 vs. 138 ± 1.6 mmHg, n = 9). All of the animals showed similar heart rates, LVDevP measurements, and CPP measurements, regardless of age (Table 1).

Transudate flow. As described previously for another rat strain, transudate flow rate was ~45 µl/min per gram, and it approximately doubled within several hours. Throughout the protocol (2.5 h), transudate flow rates were similar in lean and obese rats aged 7 weeks (Fig. 1) or 18 weeks (data not shown). The continuous increase in flow was probably due to washout of albumin, as discussed previously (19).

Transcapillary insulin transfer in hearts from animals aged 7 and 18 weeks. The transudate insulin concentrations during and after perfusion of insulin are shown in Figs. 2 and 3. At both 0.1 U/l (Fig. 2) and 0.5 U/l (Fig. 3), insulin appearance in the transudate was slower in cp/cp rats than in +/? rats. A detailed kinetic analysis of individual curves yielded the parameters listed in Table 2. At 0.5 U/l, transfer velocity to the interstitium was 1.8-fold slower in hearts from cp/cp rats than in +/? rats (P < 0.05), but, at 0.1 U/l, the difference (1.6-fold) was not significant. The efflux rate constants were not different between groups for either concentration. As reported previously for these concentrations (9), the steady-state transudate levels of insulin were lower in cp/cp rats than in +/? rats.

### Table 1

<table>
<thead>
<tr>
<th>Insulin concentration (U/l)</th>
<th>Hearts (n)</th>
<th>Heart rate (beats/min)</th>
<th>LVDevP (mmHg)</th>
<th>CPP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Infusion</td>
<td>End of Infusion</td>
<td>Before Infusion</td>
</tr>
<tr>
<td>+/? Rats (7 weeks)</td>
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<td></td>
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</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>325 ± 8</td>
<td>323 ± 6</td>
<td>86 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>317 ± 6</td>
<td>316 ± 6</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>cp/cp Rats (7 weeks)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>329 ± 5</td>
<td>321 ± 5</td>
<td>84 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>310 ± 6</td>
<td>308 ± 6</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>+/? Rats (18 weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>317 ± 5</td>
<td>303 ± 6</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>310 ± 5</td>
<td>296 ± 6</td>
<td>82 ± 1</td>
</tr>
<tr>
<td>cp/cp Rats (18 weeks)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>308 ± 7</td>
<td>303 ± 6</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>301 ± 6</td>
<td>296 ± 6</td>
<td>82 ± 1</td>
</tr>
</tbody>
</table>

Data are n and means ± SE.
were ~30% (0.1 U/l) and ~100% (0.5 U/l) of vascular levels, and they were not different between groups. The differences between experimental groups were even more pronounced in rats aged 18 months. At 0.1 U/l (Fig. 4) and 0.5 U/l (Fig. 5), respectively, insulin transfer was 3.3- and 2.0-fold slower in obese cp/cp-derived hearts than in those from lean littermates (P < 0.05 in both cases). The corresponding rate constants are detailed in Table 2. Again, maximal interstitial insulin concentrations were not affected.

**Simulation of postprandial insulin passage to interstitium.** In an additional protocol using hearts from rats aged 27 weeks, a biphasic postprandial insulin profile was simulated, and the resulting interstitial insulin levels were determined (Fig. 6). In hearts from +/? rats, interstitial insulin reached a maximum of 49% of the luminal level, and, in hearts from cp/cp rats, the corresponding value was 31%. In both cases, the maximal interstitial insulin level temporally coincided with the maximal luminal level. Thus, for the major part of the simulation, insulin transfer was considerably slower in obese cp/cp rats than in controls. This is also reflected in the areas under the transudate concentration-time curves, which were 11.3 and 16.6 U/min per liter in cp/cp and +/? hearts, respectively (P < 0.05).

**Insulin resistance and delay of insulin transfer.** Insulin resistance in the JCR:LA-cp rat was assessed previously (20,21). In the present study, plasma insulin levels were determined in rats aged 7 weeks and were found to be significantly higher in the cp/cp animals than in the +/? rats (306 ± 49 vs. 135 ± 37 µU/ml, P < 0.05). When used as a surrogate for insulin resistance, a negative correlation between plasma insulin level and k_in for transcapillary insulin transport in individual rats was found (r = –0.46, n = 6) (data not shown).

**DISCUSSION**

Our present study provides for the first time evidence of a slower transendothelial transport of insulin in experimental

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**TABLE 2** Parameters for appearance of insulin in interstitial transudate (k_in, t_1/2in) and disappearance from interstitial transudate (k_off, t_1/2off)

<table>
<thead>
<tr>
<th>Insulin concentration (U/l)</th>
<th>Hearts (n)</th>
<th>k_in (min⁻¹)</th>
<th>t_1/2in (min)</th>
<th>k_off (min⁻¹)</th>
<th>t_1/2off (min)</th>
<th>Steady-state levels (% of maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/? Rats (7 weeks)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0.1</td>
<td>6</td>
<td>0.06 ± 0.001</td>
<td>10.84 ± 0.17</td>
<td>0.49 ± 0.01</td>
<td>1.42 ± 0.02</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>0.10 ± 0.01</td>
<td>7.40 ± 0.72</td>
<td>0.17 ± 0.01</td>
<td>4.04 ± 0.15</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>cp/cp Rats (7 weeks)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>0.04 ± 0.01</td>
<td>17.75 ± 3.73</td>
<td>0.49 ± 0.02</td>
<td>1.41 ± 0.06</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>0.06 ± 0.01</td>
<td>13.37 ± 2.21*</td>
<td>0.18 ± 0.01</td>
<td>3.89 ± 0.16</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>+/? Rats (18 weeks)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>0.07 ± 0.002</td>
<td>9.53 ± 0.27</td>
<td>0.45 ± 0.03</td>
<td>1.56 ± 0.08</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>0.10 ± 0.01</td>
<td>7.25 ± 0.30</td>
<td>0.18 ± 0.01</td>
<td>3.87 ± 0.09</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>cp/cp Rats (18 weeks)</td>
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<td></td>
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</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>0.02 ± 0.001</td>
<td>31.19 ± 2.08*</td>
<td>0.47 ± 0.01</td>
<td>1.49 ± 0.04</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>0.05 ± 0.002</td>
<td>14.31 ± 0.83*</td>
<td>0.18 ± 0.01</td>
<td>3.79 ± 0.04</td>
<td>104 ± 3</td>
</tr>
</tbody>
</table>

Data are n and means ± SE. *P < 0.05 vs. the respective value in +/? rats.
animals exhibiting the characteristics of the insulin resistance syndrome. In previous studies of insulin action (22–24) and transport (25), the rat heart has served as a useful model for the following reasons: 1) rat capillary endothelial cells possess functional insulin receptors (26); 2) the capillaries in the heart are of a tight unfenestrated type similar to those in skeletal muscle (27); and 3) rat cardiac muscle shows insulin-mediated metabolic actions, such as glucose uptake and release of lactate (22).

The modified Langendorff preparation used in this study was initially developed for metabolic studies (28,29). By use of this model, we recently demonstrated that transcapillary transport of insulin is governed by a bidirectional convective mechanism and that the capillary endothelium is crucial for regulating the velocity of this transport (9) without affecting the well-established (1,4,5,10) gradient between arterial (perfusate) and interstitial (transudate) insulin concentrations. Consequently, rapid changes in serum insulin, such as those that occur after carbohydrate ingestion, may entail inadequate hormone action despite adequate timing of insulin release due to a retarded transport of insulin across the endothelium. A slowed transport would more likely affect glucose tolerance after acute carbohydrate loads more than insulin resistance per se. This question was addressed in the present work in the JCR:LA-cp rat. In this strain, animals (18 weeks). Steady-state levels, on the other hand, were not different between cp/cp and +/? animals. In this model, transudate flow increased similarly in both experimental groups so that the interstitial insulin concentrations shown on the ordinate of Figs. 2–5 were similarly affected. Because the kinetic parameters were derived from these concentration–time curves, increased fluid flow was of no relevance and did not need to be corrected.

To further investigate the sequelae of a slower transendothelial transport of insulin under dynamic non–steady-state conditions, a biphasic postglucose-challenge insulin response was simulated after a previous equilibration period. Not surprisingly, hearts from cp/cp animals showed a marked reduction of peak transudate insulin concentration as well as a considerably lower area under the insulin concentration–time curve for the entire insulin profile. Because insulin clearance is found to be substantially reduced in the cp/cp rat (20), an increased clearance of insulin can be excluded. Thus, even though reduced insulin transfer velocity does not affect steady-state interstitial insulin levels, it resulted in lower dynamic insulin levels in the interstitial compartment in insulin-resistant cp/cp animals. Because interstitial insulin levels determine glucose uptake rates (1), and because the kinetics of glucose disposal is related to interstitial insulin rather than arterial insulin (30), such a defect is likely to contribute to the glucose intolerance observed in these animals. The impaired insulin dynamics may also contribute to compensatory hyperinsulinemia and large postprandial increases in plasma insulin concentrations that can exceed 1,000 mU/l in cp/cp rats (21).

In our experiments, peak insulin values in the vascular and the interstitial compartment coincided, whereas Getty et al. (30) found peak interstitial insulin levels to lag ~20 min behind peak plasma levels. Generally, the microvasculature of isolated perfused organs is more permeable to macromolecules (31) than that observed in intact animals. A higher permeability in our experimental model would mean that the contribution of transcapillary insulin transport to dynamic interstitial insulin levels would probably be underestimated.

Because the insulin resistance of the JCR:LA-cp rat has been recently characterized and quantified in detail (20,21), we have not repeated such investigations in the present study. However, when plasma insulin concentration is used as a surrogate marker for insulin resistance, a negative correlation between the supposed insulin resistance and k_i for transcapillary insulin transport in individual rats is calculated \( r = -0.46, n = 6 \). These findings support those of Ader and Bergman (32) and suggest a contribution of transcapillary insulin transport to insulin’s overall effect across the spectrum of insulin sensitivity.

Our experiments were not designed to clarify the mechanisms involved in the delay of transendothelial insulin transport observed in the cp/cp animals. However, several conclusions can be drawn from our results. Although cp/cp rats grow obese, heart weight was not different between genotypes, and hemodynamic performance of the isolated hearts was comparable between cp/cp and +/? animals, excluding...
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cardiac hypertrophy as a possible confounding factor. Also, transudate production per unit heart weight was similar for the 2 experimental groups. Therefore, altered capillary solute exchange, possibly due to a modified ratio of pre- to post-capillary resistance or a change in insulin transit time, seems to not be involved in the observed delay in transendothelial insulin transport. Finally, we found no difference in capillary density, a variable suggested to contribute to insulin resistance (33), in the present study between the 2 groups of animals (T.C.W., G.W., J.C.R., F.B., unpublished data). Other mechanisms that possibly contribute to insulin resistance include alterations of the receptor-mediated part of transendothelial insulin transport (7) as well as alterations in nutritive to non-nutritive capillary blood flow (34).

We have not investigated the effect of the delay in insulin transport on glucose metabolism in these hearts because, unlike other strains, neither the cp/cp nor the +/- genotype shows insulin-dependent glucose uptake into the heart, as shown previously (35). Clearly, such additional information is important to judge the pathological importance of the observed kinetic phenomenon. The supposed effect of delayed insulin transport with respect to total-body metabolism could be studied in terms of glucose uptake or glycogen formation in other insulin-dependent tissues, in particular skeletal muscle. In view of our primary aim (i.e., to investigate the transcapillary transport of insulin in a capillary bed with tight nonfenestrated capillaries, as present in both skeletal and heart muscle), we have not extended our present studies to other tissues. In addition, the lack of an appropriate experimental preparation involving an isolated rat skeletal muscle model currently prevents us from pursuing this question further. However, the pathophysiologic relevance of the slowed insulin transport kinetics is strongly suggested by several investigations in the dog. Two articles by the Olefsky group suggested that 1) in quadriceps muscle, the delay in the insulin-stimulated glucose disposal rate is due to the time required for plasma insulin to gain access to the interstitial compartment (36), and that 2) in animals with experimentally induced hyperinsulinemia, as compared with control animals, a retardation of insulin transport into lymph accounted for 30% of the delay in glucose disappearance rate (13). By use of the dynamic intravenous glucose tolerance test in normal dogs, Ader and Bergman (32) found a 21% contribution of transendothelial insulin transport to minimal model-derived estimations of insulin sensitivity.

In summary, we have shown that transendothelial transport of insulin is delayed in hearts from obese insulin-resistant JCR:LA-cp rats. Because of this delay, markedly lower myocardial interstitial insulin levels were found in these animals under dynamic conditions. The exact mechanism underlying this defect and the possible contribution(s) to the metabolic alterations seen in insulin resistance and diabetes need to be addressed in further investigations.

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