Effect of Prolonged Glucose Infusion on Insulin Sensitivity in the Conscious Normal Rat

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Introduction

It has been proposed that hyperglycemia contributes to the insulin resistance normally seen in non-insulin-dependent diabetes mellitus (*DeFronzo, Simonson* and *Ferrannini* 1982; *Kolterman, Gray, Griffen, Burstein, Insel, Scarlett* and *Olefsky* 1981). Fasting hyperglycemia is correlated to the severity of insulin resistance in human type II diabetes (*Kolterman, Gray, Griffen, Burstein, Insel, Scarlett* and *Olefsky* 1981) and reductions of hyperglycemia produced by insulin therapy have been shown to reduce the degree of insulin resistance (*Garvey, Olefsky, Griffen, Hamman* and *Kolterman* 1985). Insulin resistance has been reversed in insulinopenic states such as in insulin-dependent diabetic subjects (*Foss, Vlachoksta, Cunningham* and *Aoki* 1982) or in experimental diabetes induced in the rat (*Rossetti, Smith, Shulman, Papachristou* and *DeFronzo* 1987). The hyperglycemia, hyperinsulinemia and glucosuria developed in intact rats receiving a 50% glucose intravenous infusion (60–66 mg/kg/min) for 72 h have been shown to produce insulin resistance (*Hager, Jochen* and *Kalkhoff* 1991). However, conditions of prolonged hyperinsulinemia in the presence of different degrees of hypoglycemia have been shown to either decrease (*Whittaker, Alberti, York* and *Singh* 1979) or increase (*Trimble, Weir, Gjinovci, Assimacopoulos-Jeannet, Benzi* and *Renold* 1984; *Wardzala, Hirshman, Pofcher, Horton, Mead, Cushman* and *Horton* 1985) insulin sensitivity. The present study was addressed both to testing a method for developing continuous hyperinsulinemia in the conscious normal rat and to studying further the role of hyperinsulinemia in the potential changes of insulin sensitivity in this species.

Material and Methods

Animals

Adult female Wistar rats weighing 190–210 g, bred and raised in our own colony, having free access to food and water and subjected to controlled lighting (lights from 08:00 to 20:00 h) and temperature (22–24 °C), were used.

Surgical procedure

A Silastic catheter (Dow Corning, 0.02 inch ID, 0.037 inch OD) was inserted 3 cm into the right jugular vein under a ketamine cocktail anaesthesia (ketamine 50 mg/ml, diazepam 5 mg/ml, and atropine 1 mg/ml, 5/4/1, by vol) administered intraperitoneally (0.3 ml/200 g). The catheter was channelled around the neck and exited through the skin at the vertex of the head. The rats were allowed to recover from the anaesthesia and all the other procedures were carried out with animals in conscious and unrestrained conditions. By means of a peristaltic pump (Minipuls II, Gilson) attached to the catheter a continuous infusion of either bidistilled water or 50% glucose was administered at the rate of 35 ml/day to each rat, corresponding to 55–60 mg · kg^{-1} · min^{-1}).

Euglycemic clamp

Each rat was subjected to 2 euglycemic-hyperinsulinemic clamps. The first one was performed one day after cannulation and was named clamp-0 h. The second clamp (clamp-72 h) was performed just after 72 h of infusion with either bidistilled water or 50% glucose. In this way, each animal was used as its own control and only the rats in which both clamps (0 and 72 h) were successfully performed, were used. The clamps were performed always between 10:00 and 12:00 h, which corresponds to the postabsorptive period of the rats.

A two way interconnector connected to two infusion pumps (Precidor Infusion Pumps Type 5003, Infors HT) was adapted to the catheter placed at the jugular vein. A constant infusion of human monocomponent insulin (Actrapid from Novo, Copenhagen) at the rate of 0.8 IU/h/kg was given through one of the pumps while a variable amount of 20% glucose was infused through the second pump. Small blood samples (5 μl) were collected every 5 min from the tip of the tail to measure the glycaemia immediately by means of an immobilized glucose oxidase method (Reflolux II M from Boehringer-Mannheim). Adjustments of the exogenous glucose infusion rate

were made to maintain euglycemia, and a steady-state glucose infusion was normally achieved at 45 min after starting the clamp experiment. At 0, 30, 60 min and at the end of the clamp (120 min) 200 μl of blood was also collected in order to measure plasma insulin (*Heding* 1972).

Calculations

During the clamp, the glucose appearance rate (R_a) equals the sum of the endogenous glucose production plus the amount of infused glucose. Once a steady state is achieve in the glucose infusion, the rate of glucose utilization (R_d) is equal to the rate of glucose appearance (R_a). Since, under conditions of hyperinsulinemia, the endogenous production of glucose is practically nil (*Burnol, Leturque, Ferré* and *Girard* 1983), it is assumed that at the steady-state the rate of glucose infusion (R) is equal to the rate of glucose utilization (R_d), and was used as an insulin sensitivity index. The insulin sensitivity index (S_{ip}) proposed by *Ader* and *Bergman* (1987) was also measured. This index represents the increase in glucose clearance caused at steady state by an increase in plasma insulin, and is calculated as the ratio of the increase in glucose utilization measured during the clamp (ΔR_d) plus the product of glucose concentration during the clamp (G) times the increase in plasma insulin (ΔI) and body weight (bw): $S_{ip(clamp)} = \Delta Rd/G \cdot \Delta I \cdot bw$.

The statistical comparison between the groups was done by the Student's t-test. A paired t-test for the mean of two samples was used for comparison between the indices obtained in the 2 clamps performed to each animal. All values are expressed as means ± SEM.

Results

After collecting blood for glucose and insulin determinations under basal conditions, the rats were subjected to an euglycemic hyperinsulinemic clamp. After clamping the rats were divided into two different groups. In one of the groups, called the control group, the rats received a continuous intravenous infusion of bidistilled water for 72 h at the rate of 35 ml/h, whereas in the other group the rats received an intravenous infusion of 50% glucose at the same rate for the same period. Daily food intake of the rats receiving the bidistilled water infusion did not change during the infusion period, and averaged 16.3 ± 1.1 g/day, whereas food intake in those receiving the glucose infusion declined to 6.5 ± 0.5 g/day (p < 0.001). As shown in Table 1 and Fig. 1, plasma glucose values increased during the 1st day in the rats receiving the 50% glucose infusion, whereas at the 2nd and 3rd day of infusion they were similar to those found in the control rats. However, as shown in Table 1 and Fig. 1, plasma insulin levels increased very intensively on the 1st day of the infusion in the rats receiving the 50% glucose, and although the values tended to decline thereafter they always remained above those in the rats receiving the bidistilled water infusion until the 3rd day.

After 3 days of the respective infusions animals were again subjected to an euglycemic hyperinsulinemic clamp. Fig. 2 shows the blood glucose and plasma insulin levels during the clamp performed after 3 days of infusion. Blood glucose was clamped at the same level in both groups of animals and was kept constant during the clamp. As also shown in Fig. 2 plasma insulin levels at 0 time appeared slightly but significantly higher in

Table 1 Plasma glucose and plasma radioimmunoassayable insulin during the continuous intravenous infusion during 3 days with either bidistilled water or 50 % glucose (35 ml/day) in the conscious rat.

|	Control	50 % Glucose	p
Plasma glucose (mM) day 0	8.1 ± 0.3	7.1 ± 0.2	< 0.05
Plasma glucose (mM) day 1	7.0 ± 0.0	8.2 ± 0.1 **	< 0.001
Plasma glucose (mM) day 2	7.5 ± 0.2	7.8 ± 0.8	NS
Plasma glucose (mM) day 3	9.0 ± 0.3	8.3 ± 0.5	NS
Plasma insulin (pM) day 0	50 ± 10	72 ± 7	NS
Plasma insulin (pM) day 1	51 ± 6	833 ± 25 ***	< 0.001
Plasma insulin (pM) day 2	109 ± 25 *	558 ± 136 **	< 0.01
Plasma insulin (pM) day 3	118 ± 42	327 ± 112 *	NS

All values are means ± SEM of 7 – 8 rats/group
p = statistical comparison between rats receiving bidistilled water (controls) and glucose infusions. Asterisks correspond to the statistical comparisons versus values at day 0 within each group (*p < 0.05; **p < 0.01; ***p < 0.001).
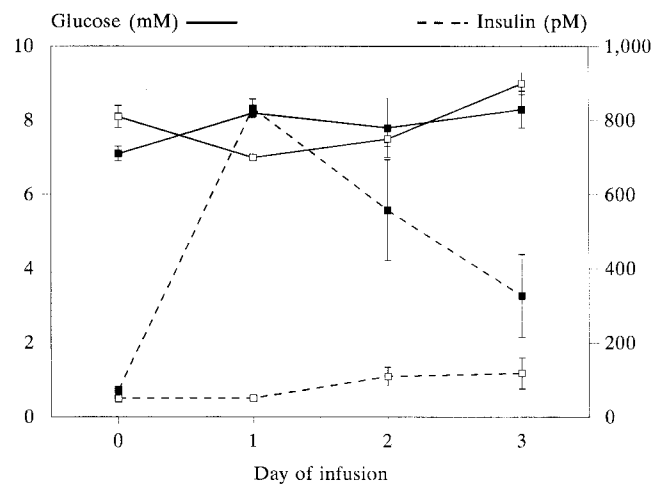
Fig. 1 Plasma glucose (-) and RIA-insulin (- -) levels during the continuous intravenous infusion for 3 days with either bidistilled water (□) or 50 % glucose (■) in the conscious rat. Data correspond to those shown in Table 1.

those rats that had previously received the 72 h glucose infusion than in those that received the bidistilled water. Plasma insulin levels increased progressively in both groups during the first 60 min of the clamp, remaining stable thereafter.

As shown in Fig. 3, neither glucose utilization rate (R) nor the insulin sensitivity index (S_{ip}) differed in the rats which had received the infusion with the medium (bidistilled water) for 72 h and their basals (time 0). On the contrary, in the rats infused with 50% glucose for 72 h, both indexes appeared significantly lower than in either the same animals studied before
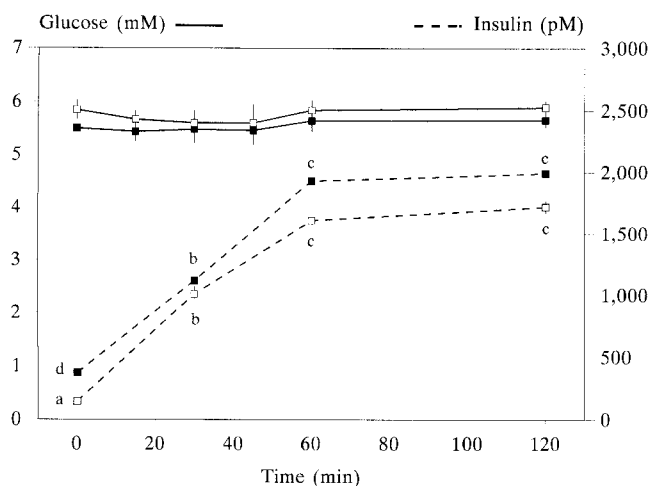
Fig. 2 Blood glucose levels (-) and plasma RIA-insulin (- -) during the euglycemic-hyperinsulinemic clamp performed in conscious rats after 72 h of continuous infusion with either bidistilled water (□) or with 50 % glucose (■). Values are means ± SE of 4 animals per group. No statistical differences were observed between the groups in the values of blood glucose. Letters correspond to statistical comparisons in insulin levels: The same letter means no statistical difference between the groups, whereas different letters indicate significant difference $(p < 0.05)$.

receiving any treatment (time 0) or in those infused with the medium.

Discussion

Two major conclusions can be reached with the present study. The first is methodological and based on the fact that 3 days of intravenous infusion with bidistilled water did not affect the two indexes of insulin sensitivity studied, the glucose utilization rate (R) and insulin sensitivity index (S_{ip}) in the conscious rat. Additionally, blood glucose and plasma insulin remained stable during bidistilled water infusion as compared to the same animals studied under basal conditions. It may then be concluded that neither the surgical process nor the experimental design used affected the glucose/insulin interactions in the animals. The second conclusion relating to the hyperinsulinemia developed as a result of the 72 h infusion with 50 % glucose and the decline in the insulin sensitivity indexes found in these animals. Such decline indicates that the glucose infusion treatment caused a state of insulin resistance. The present experimental design is comparable to that used by Hager, Jochen and Kalkhoff (1991) who also demonstrated decreased insulin sensitivity in rats subjected to a 72 h infusion with glucose. The difference between the two studies resides in the fact that while Hager, Jochen and Kalkhoff (1991) used a condition where hyperinsulinemia coincided with hyperglycemia, in our rats glycemia remained at the basal level. We may therefore conclude that prolonged endogenous hyperinsulinemia without hyperglycemia seems to be sufficient to trigger insulin resistance. Conditions of prolonged hyperinsulinemia caused by insulin administration have also been shown to induce a decreased insulin sensitivity previously (Whittaker, Alberti, York and Singh 1979), although there are studies showing the opposite effect (Wardzala, Hirshman, Pofcher, Horton, Mead, Cushman and Horton 1985). The difference between these studies showing opposite insulin effects resides in the dose, the duration of the treatment, the degree of blood glucose change and even on the nature of the tissue studied. Investigations of the development of insulin resistance in the genetically obese Zucker rat (fa/fa) have indicated that in the early development of this syndrome there is a phase of enhanced insulin sensitivity (Guerre-Millo, Wardzala and Lavau 1985). The mechanism through which hyperinsulinemia may cause opposite effects is complex. In the early development of hyperinsulinemia insulin may have had a growth-promoting effect, that would have increased the overall cell protein content, including the number of insulin receptors and glucose transporters as previously suggested (Guerre-Millo, Wardzala and Lavau 1985), whereas more prolonged (or intense) hyperinsulinemia could have been associated to the loss of glucose transporters (Hissin, Karnieli, Simpson, Salans and Cushman 1982) and other defects during the postinsulin receptor events (Hager, Jochen and Kalkhoff
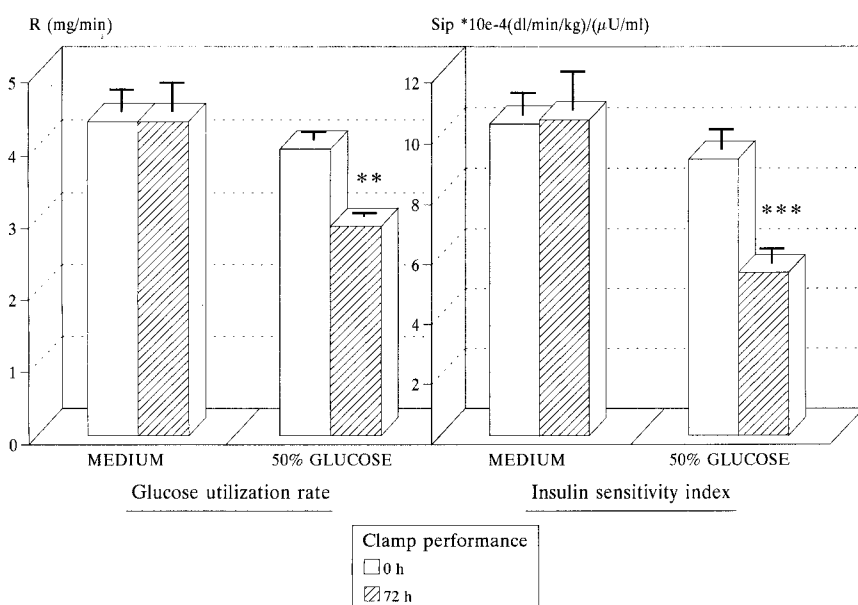
Fig. 3 Effect of a continuous intravenous infusion for 72 h with either bidistilled water (medium) of 50 % glucose (35 ml/day) on both the glucose utilization rate (R) and the insulin sensitivity index (S_{ip}) measured by the hyperinsulinemic euglycemic clamp in the conscious rat. Asterisks correspond to the statistical comparison between values of each group studied at 72 h after the correspnding infusion versus values of the same animals studied under basal conditions (clamp-0 h): $**p < 0.01$; $***p < 0.001$.

1991). Therefore, it seems, that chronic hyperinsulinemia can have different long-term effects on its own sensitivity; the present results show that conditions of moderate hyperinsulinemia are sufficient to develop an insulin resistance.

References

Ader, M., R. N. Bergman: Baillieres. Clin. Endocrinol. Metab. 1: 879–910 (1987)

Burnol, A.-F., A. Leturque, P. Ferré, J. Girard: Reprod. Nutr. Dev. 23: 429–435 (1983)

DeFronzo, R. A., D. Simonson, E. Ferrannini: Diabetologia 23: 313–319 (1982)

Foss, M. C., F. V. Vlachokosta, L. N. Cunningham, T. T. Aoki: Diabetes 31: 46–52 (1982)

Garvey, W. T., J. M. Olefsky, J. Griffen, R. F. Hamman, O. G. Kolterman: Diabetes 34: 222–234 (1985)

Guerre-Millo, M., L. Wardzala, M. Lauvau: J. Biol. Chem. 260: 2197–2201 (1985)

Hager, S. R., A. L. Jochen, R. K. Kalkhoff: Am. J. Physiol. 260: E353–E362 (1991)

Heding, L. G.: Diabetologia 8: 260–266 (1972)

Hissing, P. J., E. Karnieli, I. A. Simpson, L. B. Salans, S. W. Cushman: Diabetes 31: 589–592 (1982)

Kolterman, O. G., R. S. Gray, J. Griffen, J. Burstein, J. Insel, J. A. Scarlett, J. M. Olefsky: J. Clin. Invest. 68: 957–969 (1981)

Rossetti, L. D., D. Smith, G. I. Shulman, D. Papachristou, R. A. DeFronzo: J. Clin. Invest. 79: 1510–1515 (1987)

Trimble, E. R., G. C. Weir, A. Gjinovci, F. Assimacopoulos-Jeannet, R. Benzi, A. E. Renold: Diabetes 33: 444–449 (1984)

Wardzala, L. J., M. Hirshman, E. Pofcher, E. D. Horton, P. M. Mead, S. W. Cushman, E. S. Horton: J. Clin. Invest. 76: 460–469 (1985)

Whittaker, J., K. G. Alberti, D. A. York, J. Singh: Biochem. Soc. Trans. 7: 1055–1066 (1979)

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