Experimental Physiology

Insulin sensitivity and glucose effectiveness estimated by the minimal model technique in spontaneously hypertensive and normal rats

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(Manuscript received 22 May 2000; accepted 25 August 2000)

This study was performed to compare glucose metabolism in anaesthetised spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) in an attempt to clarify whether this animal model of hypertension approximates the insulin-resistant state seen in human hypertension. With this aim the minimal model of glucose kinetics was applied to glucose and insulin data derived from a 12-sample, 120 min intravenous glucose tolerance test (IVGTT) performed in ten SHR and nine WKY rats under pentobarbital anaesthesia. This method provided two metabolic indices: the glucose effectiveness, S_G, which quantifies the ability of glucose *per se* to enhance its rate of disappearance and to inhibit hepatic glucose production, and the insulin sensitivity, S_I, which measures the ability of insulin to enhance plasma glucose disappearance and to inhibit hepatic glucose production. Systolic and diastolic arterial pressures in the SHR group were significantly higher ($P < 0.0005$) than in the WKY group. Mean S_G and S_I estimates from the SHR group ($S_G = 16.2\ (\pm 2.0) \times 10^{-2}$ dl min^{-1} kg^{-1} and $S_I = 12.5\ (\pm 1.9) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$) were not significantly different ($P > 0.05$) from mean estimates that characterised the WKY group ($S_G = 13.1\ (\pm 1.5) \times 10^{-2}$ dl min^{-1} kg^{-1} and $S_I = 15.8\ (\pm 4.3) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$). This result is in contrast with reported findings from humans in which insulin sensitivity is significantly reduced in the presence of hypertension. *Experimental Physiology* (2000) **85.6**, 775–781.

Hyperinsulinaemia, glucose intolerance and insulin resistance, defined as a reduced sensitivity to the effects of insulin action, have been observed in patients with essential hypertension (Modan *et al.* 1985; Ferrannini *et al.* 1987; Swislocki *et al.* 1989; Reaven, 1991; Natalucci *et al.* 1999). Despite a great scientific effort, the nature of the relationship between high blood pressure and these metabolic abnormalities is still unclear. To improve the understanding of the pathogenesis of insulin resistance and its link with high blood pressure, several studies have been performed in spontaneously hypertensive rats (SHR). SHR are generally considered to be the best available experimental model of essential hypertension because they have numerous similarities with this clinical pathology (Trippodo & Frohlich, 1981). In particular, in contrast to other rat models in which the hypertension is induced by surgical intervention (Pinto *et al.* 1998) or by intrauterine programming mechanisms (Langley-Evans, 1996; Langley-Evans *et al.* 1996), in SHR the hypertensive disease is genetically determined, as it is in humans.

Previous studies in SHR yielded contradictory results. A reduction of glucose tolerance and insulin action in the SHR, compared to the related control strain (Wistar Kyoto rats, WKY), was found in some studies (Mondon & Reaven, 1988; Gaboury *et al.* 1991; Hulman *et al.* 1993; Rao, 1993). In others, however, an increased insulin sensitivity (Tsutsu *et al.* 1989; Frontoni *et al.* 1992) or no evidence of insulin resistance (Frontoni *et al.* 1992; Buchanan *et al.* 1992*a*,*b*) was observed in SHR.

Direct and indirect methods were used to estimate insulin sensitivity indices in these previous investigations. The euglycaemic clamp technique (Buchanan *et al.* 1992*a*; Frontoni *et al.* 1992; Hulman *et al.* 1993; Rao, 1993) and the pancreatic suppression test (Narimiya *et al.* 1984; Mondon & Reaven, 1988; Gaboury *et al.* 1991) are typical direct methods. The interpretation of plasma insulin and glucose concentration measurements during an intravenous glucose tolerance test (IVGTT) is an indirect method (Tsutsu *et al.* 1989; Buchanan *et al.* 1992*b*) that allows quantitative determination of insulin

Publication of *The Physiological Society*

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sensitivity and glucose via the use of an appropriate model. Without using a model, as in the IVGTT interpretation of rat data by Tsutsu *et al.* (1989) and Buchanan *et al.* (1992*b*), only limited qualitative information about the processes affecting glucose disposal is produced.

It has been shown previously in humans and animals other than rats that reliable estimates of insulin sensitivity and glucose effectiveness indices can be determined by applying the minimal model to glucose and insulin data derived from an IVGTT (Finegood, 1997). The aim of this study is to adapt this approach to the SHR and WKY rats in order to address the issue as to whether the SHR approximates the insulin-resistant state seen in human hypertension.

METHODS

Animals

The study was performed in ten spontaneously hypertensive (SHR) and nine Wistar Kyoto (WKY) rats obtained from the Charles River Breeding Farms, NY, USA. They were housed in controlled conditions of temperature (21 ± 1 °C), humidity (60 ± 10 %) and lighting (lights on from 8.00 h–20.00 h) and received a standard rat chow containing 0.3 % sodium, with tap water *ad libitum*. The SHR and WKY rats were studied in matched pairs by age (12–15 weeks) and were anaesthetised with sodium pentobarbital (40 mg kg^{-1}, I.P.). The adequacy of the anaesthesia was assessed by monitoring the changes in heart rate (HR) and arterial pressure (AP) and by the state of the pupils. The experiments were performed in accordance with the Italian guidelines on animal experimentation (*Decreto Legislativo 27–01–1992 No 116, attuazione della direttiva N. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini specialistici*). The right femoral artery and vein were cannulated. The arterial cannula connected to a pressure transducer (Spectramed Statham P23XL) provided a record of AP through a Grass preamplifier (model 7P14A; Grass Instruments, Quincy, MA, USA). HR was monitored by the use of a Grass tachograph (model 7P4) triggered by lead II of the electrocardiogram. Rectal temperature was controlled and maintained at 37.5 ± 0.5 °C by a heating pad. The venous cannula was used for glucose injection. AP, ECG and HR were digitally recorded by an A/D converter (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK), stored on a PC and analysed by laboratory software (Spike 2, CED).

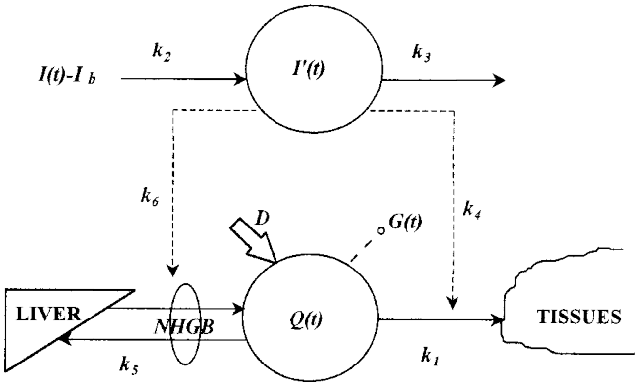

Figure 1

The single compartment minimal model of glucose disappearance (1CMM). NHGB is the net hepatic glucose balance; D represents the glucose dose (400 mg kg^{-1}); k_i coefficients are rate constant symbols characterising material fluxes (continuous lines) or control actions (dashed lines). $G(t)$ and $I(t)$ are glucose and plasma insulin concentrations at time t. I_b is baseline (end-test) insulin concentration. $I'(t)$ is insulin concentration at time t in a compartment remote from plasma. $Q(t)$ is plasma glucose mass at time (t).

Intravenous glucose tolerance test (IVGTT)

Experiments were performed after 12 h overnight fast. The starting time for the IVGTT protocol was 1 h after the catheterisation of the femoral artery and the femoral vein since a previous study by Rao (1992) showed that, in acutely operated rats, anaesthetised with pentobarbital, the stress response to surgery subsides within 1 h and that insulin sensitivity appears to be no different from that reported in chronically cannulated conscious rats. A glucose bolus of 400 mg kg^{-1} was injected over 1 min into the femoral vein (time 0). Two basal blood samples (200 μl) were taken from the arterial catheter at −5 and −2 min before the glucose injection. Ten additional blood samples were collected at 1, 2, 3, 5, 8, 15, 25, 40, 70 and 120 min after the injection. Plasma volume was replaced by controlled normal saline infusion. At the end of the experiments, the animals were killed by an overdose of sodium pentobarbital.

Assays

Blood was promptly centrifuged and glucose immediately measured by the glucose oxidase method with an automated glucose analyser. The remaining plasma was stored at −80 °C for later insulin determination. Insulin was measured by means of a commercially available rat insulin ELISA kit (Mercodia, Uppsala, Sweden). The sensitivity of the insulin assay was 0.07 μg l^{-1} with an 'inter' and 'intra' precision of 3.3 ± 0.1 % and 1.8 ± 0.3 %, respectively.

Data analysis

Measured values of insulin and glucose concentrations were analysed with the minimal model technique. The minimal model of glucose disappearance (Fig. 1) assumes a monocompartmental description of glucose kinetics during an IVGTT (Bergman *et al.* 1979; Finegood, 1997). Insulin exerts its action from an insulin compartment remote from plasma. The equations of the model are as follows:

$$\dot{Q}(t) = -[p_1 + X(t)]Q(t) + p_1 Q_b \qquad Q(0) = Q_b + D \quad (1a)$$

$$\dot{X}(t) = -p_2 X(t) + p_3[I(t) - I_b] \qquad X(0) = 0 \quad (1b)$$

$$G(t) = Q(t)/V \quad (1c)$$

where

$$X(t) = (k_4 + k_6)I'(t) \quad (2a)$$

$$p_1 = k_1 + k_5 \quad (2b)$$

$$p_2 = k_3 \quad (2c)$$

$$p_3 = k_2(k_4 + k_6) \quad (2d)$$

D (mg kg^{-1}) is the glucose dose; k_i coefficients ($i = 1, \ldots, 6$) are rate constants characterising either material fluxes (continuous lines in Fig. 1) or control actions (dashed lines in Fig. 1); $\dot{Q}(t)$ and $\dot{X}(t)$ are the first derivatives of $Q(t)$ and $X(t)$, respectively; $Q(t)$ is glucose mass (mg kg^{-1}) at time t, and Q_b is its baseline (end-test) steady-state value; $G(t)$ and $I(t)$ are plasma glucose (mg dl^{-1}) and insulin (μU ml^{-1}) concentrations at time t; $I'(t)$ is insulin concentration, at time t, in a compartment remote from plasma; G_b and I_b are baseline (end-test) glucose and insulin concentrations, respectively, computed as the mean of the last two to three points; V (dl kg^{-1}) is the distribution volume. From eqn (1*c*) we obtain $Q_b = G_b V$.

The minimal model provides indices of glucose effectiveness, S_G (dl min^{-1} kg^{-1}), and insulin sensitivity, S_I (dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$), which are defined as:

$$S_G = -\left.\frac{\partial \dot{Q}(t)}{\partial G(t)}\right|_{ss} = p_1 V \quad (3)$$

and

$$S_I = -\left.\frac{\partial^2 \dot{Q}(t)}{\partial I \partial G(t)}\right|_{ss} = \frac{p_3}{p_2} V. \quad (4)$$

The suffix *ss* in eqns (3) and (4), denotes steady state. Glucose effectiveness, S_G, quantifies the ability of glucose *per se* to enhance its rate of disappearance and to inhibit hepatic glucose production. Insulin sensitivity, S_I, measures the ability of insulin to enhance plasma glucose disappearance and to inhibit hepatic glucose production. It is important to note that S_G and S_I, at variance with the fractional (i.e. per unit volume) indices commonly expressed elsewhere, have the same units as the analogous glucose clamp indices, thus allowing a direct comparison (Caumo *et al.* 1999).

The free model parameters in eqns (1*a*)–(1*c*) are p_1, p_2, p_3 and V. *A priori* identifiability analysis for non-linear systems showed that the minimal model is uniquely identifiable in this parameterisation (Carson *et al.* 1983). The SAAM II software (SAAM Institute, University of Washington, Seattle, WA, USA) was used to estimate the model parameters with a non-linear least-squares estimation technique (Barrett *et al.* 1998) from glucose (G) and insulin (I) concentration data measured during an IVGTT. The insulin data were assumed to be without error. The errors associated with total glucose measurement were assumed to be random variables normally distributed, with zero mean and a constant 1.5 % coefficient of variation from reality. Weights were chosen to be optimally equal to the inverse of the measurement errors (Carson *et al.* 1983). Precision of parameter estimates were expressed as percentage coefficient of variation, $CV\% = SD_{p_i}/p_i \times 100$, where SD_{p_i} is the parameter standard deviation derived from the inverse of the Fisher information matrix and p_i is the related parameter estimate (Carson *et al.* 1983).

Statistical comparison of the differences between the two groups was performed by Student's paired t test. Values are means ± standard errors (S.E.M.). Variations were considered statistically significant when $P \leqslant 0.05$.

RESULTS

The mean (±S.E.M.) values of systolic (SAP) and diastolic (DAP) arterial pressure, and baseline concentrations of plasma glucose and insulin from SHR and WKY rats are presented in Table 1. In the SHR group, both SAP and DAP were significantly higher ($P < 0.0005$) than in the WKY group. The differences in fasting plasma glucose and insulin concentrations were not statistically significant ($P > 0.05$).

Figure 2 shows that, on average, the plasma glucose and insulin levels measured in the SHR group (●) and the WKY group (○) were similar throughout the entire IVGTT test. In both groups the plasma glucose and insulin levels reached the peak 1 min after the rapid injection of glucose and returned toward baseline values in two phases: a first rapid phase during the first 25 min and a second slower phase beyond the 25th minute.

Table 1. Baseline characteristics of SHR and WKY groups

	SHR ($n = 10$)	WKY ($n = 9$)	P
SAP (mmHg)	194 ± 5	158 ± 6	$P < 0.0005$
DAP (mmHg)	152 ± 5	118 ± 5	$P < 0.0005$
G (mg dl^{-1})	88.1 ± 6.1	92.6 ± 10.1	n.s.
I (μU ml^{-1})	20.5 ± 3.8	29.6 ± 4.3	n.s.

Values are means ± S.E.M. over n cases. SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto; SAP, systolic arterial pressure; DAP, diastolic arterial pressure. G and I are glucose and insulin plasma concentrations, respectively, measured before the IVGTT. n.s., not significantly different ($P > 0.05$).

Individual estimates of minimal model parameters, p_1, p_2, p_3 and V, determined in the WKY group and the SHR group are presented in Tables 2 and 3, respectively. The precision of the parameter estimates, CV%, is given in parentheses. Individual estimates of glucose effectiveness, S_G, and insulin sensitivity,

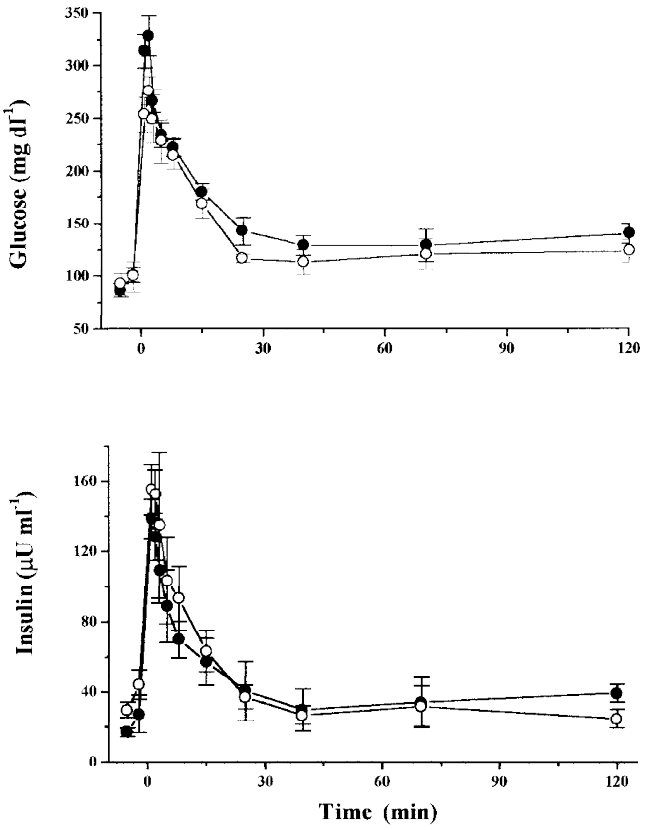

Figure 2

Top panel: plasma glucose concentrations during an intravenous glucose tolerance test (IVGTT) in the spontaneously hypertensive (SHR, ●) and Wistar Kyoto rats (WKY, ○). Bottom panel: plasma insulin concentrations during intravenous glucose tolerance test in the SHR (●) and the WKY rats (○). Values are expressed as means ± S.E.M.

Table 2. Estimates of minimal model parameters from Wistar Kyoto (WKY) rats

	$p_1 \times 10^2$ (min^{-1})	$p_2 \times 10^2$ (min^{-1})	$p_3 \times 10^5$ ($dl\ min^{-2}\ kg^{-1}$ $(\mu U\ ml^{-1})^{-1}$)	V ($dl\ kg^{-1}$)	$S_G \times 10^2$ ($dl\ min^{-1}\ kg^{-1}$)	$S_I \times 10^4$ ($dl\ min^{-1}\ kg^{-1}$ $(\mu U\ ml^{-1})^{-1}$)
1	7.27 (5.5)	40.2 (60)	6.64 (52)	1.93 (1.9)	14.0 (4.8)	3.19 (22)
2	8.98 (8.8)	12.1 (13)	7.27 (12)	2.08 (2.4)	18.7 (7.1)	12.5 (5.5)
3	2.5 (51)	15.5 (24)	7.97 (47)	2.26 (2.4)	5.65 (49)	11.6 (27)
4	2.41 (71)	7.05 (37)	5.10 (31)	2.94 (3.8)	7.09 (68)	21.3 (3.6)
5	5.98 (14)	12.3 (42)	19.0 (51)	3.00 (2.5)	17.9 (13)	46.1 (18)
6	7.49 (37)	16.0 (64)	5.07 (71)	1.67 (6.0)	12.5 (31)	5.29 (14)
7	5.79 (7.9)	1.57 (54)	0.83 (37)	2.71 (1.9)	15.7 (6.6)	14.4 (24)
8	4.91 (25)	11.4 (34)	4.43 (3.6)	2.16 (2.6)	10.6 (22)	8.40(6.7)
9	3.57 (57)	59.2 (38)	26.8 (44)	4.34 (4.9)	15.5 (55)	19.7 (13)
Mean ± S.E.M.	5.43 ± 0.76	19.5 ± 6.1	9.23 ± 2.75	2.56 ± 0.27	13.1 ± 1.5	15.8 ± 4.3

The percentage coefficient of variation (CV%), shown in parentheses, gives a measure of the precision of the parameter estimate. See Methods for definition of parameters p_1, p_2, p_3, V, S_G and S_I.

Table 3. Estimates of minimal model parameters from spontaneously hypertensive rats (SHR)

	$p_1 \times 10^2$ (min^{-1})	$p_2 \times 10^2$ (min^{-1})	$p_3 \times 10^5$ ($dl\ min^{-2}\ kg^{-1}$ $(\mu U\ ml^{-1})^{-1}$)	V ($dl\ kg^{-1}$)	$S_G \times 10^2$ ($dl\ min^{-1}\ kg^{-1}$)	$S_I \times 10^4$ ($dl\ min^{-1}\ kg^{-1}$ $(\mu U\ ml^{-1})^{-1}$)
1	0.14 (3.7)	1.58 (9.0)	0.66 (47)	1.35 (1.9)	18.7 (2.6)	5.64 (51)
2	8.59 (15)	13.6 (58)	7.01 (60)	2.27 (3.1)	19.5 (13)	11.7 (72)
3	8.62 (12)	3.66 (66)	1.64 (50)	1.46 (3.6)	12.6 (9.4)	6.54 (15)
4	4.78 (52)	18.1 (68)	9.74 (83)	1.84 (3.4)	8.81 (49)	9.92 (18)
5	3.40 (21)	3.63 (34)	2.26 (42)	2.1 (2.1)	7.26 (20)	13.1 (12)
6	6.25 (7.0)	38.3 (65)	22.2 (56)	2.44 (2.6)	15.3 (6.2)	14.2 (27)
7	10.4 (6.2)	2.56 (45)	1.38 (31)	2.08 (2.1)	21.7 (4.7)	11.3 (27)
8	7.15 (11)	6.39 (47)	1.40 (36)	4.00 (3.9)	28.6 (8.4)	8.79 (20)
9	7.75 (32)	7.98 (49)	9.12 (39)	2.32 (4.5)	18.0 (278)	26.6 (7.7)
10	5.22 (21)	5.03 (38)	3.84 (43)	2.23 (2.8)	11.6 (19)	17.0 (13)
Mean ± S.E.M.	6.23 ± 0.94	10.0 ± 3.5	5.92 ± 2.0	2.21 ± 0.23	16.2 ± 2.0	12.5 ± 1.9

CV%, shown in parentheses, gives a measure of the precision of the parameter estimate. See Methods for definition of parameters p_1, p_2, p_3, V, S_G and S_I.

S_I, derived from the minimal model are given in Table 2 for the WKY group and in Table 3 for the SHR group. These indices were estimated with an acceptable accuracy (in parentheses) in all circumstances. In the WKY group, the mean values (±S.E.M.) were $13.1\ (\pm 1.5) \times 10^{-2}$ dl min^{-1} kg^{-1} for S_G and $15.8\ (\pm 4.3) \times 10^{-4}$ dl min^{-1} kg^{-1} $(\mu U\ ml^{-1})^{-1}$ for S_I. In the SHR group S_G was $16.2\ (\pm 2.0) \times 10^{-2}$ dl min^{-1} kg^{-1} and S_I was $12.5\ (\pm 1.9) \times 10^{-4}$ dl min^{-1} kg^{-1} $(\mu U\ ml^{-1})^{-1}$. On average, the differences in S_G and S_I estimates observed in the two different groups were not statistically significant ($P > 0.05$). Also no significant difference was observed in the plasma glucose distribution volume, V (2.56 ± 0.27 dl kg^{-1} in the WKY group and 2.21 ± 0.23 dl kg^{-1} in the SHR group).

To assess the goodness of fit for glucose data we analysed weighted residuals, which are the difference between the data and model-predicted values, divided by the standard deviation of the data. In both the WKY group (top panel, Fig. 3) and the SHR group (bottom panel, Fig. 3) these residuals showed no systematic deviation and were consistent with the hypothesis that the measurement error was randomly distributed around zero.

DISCUSSION

An IVGTT protocol was set up to collect glucose and insulin data from anaesthetised SHR and WKY rats. The minimal model of glucose kinetics (Bergman *et al.* 1979; Finegood, 1997) was then used to estimate glucose effectiveness, S_G, and insulin sensitivity, S_I. The S_G index quantifies the glucose-dependent glucose disposal, whereas the S_I index quantifies the insulin-dependent glucose disposal. Previous reports showed a significant reduction of these indices in humans with essential hypertension (Modan *et al.* 1985; Ferrannini *et al.* 1987; Swislocki *et al.* 1989; Reaven, 1991; Natalucci *et al.* 1999). Such a reduction should be observed in SHR,

compared to a control strain, for this species to be used as an experimental model of metabolic disorder in hypertension.

Age-matched (12–15 weeks) SHR and WKY rats were used in the present study. At 12–15 weeks of age, hypertension is well established in SHR whereas body weight is only slightly different from that of the WKY strain. SAP and DAP in SHR is significantly higher ($P < 0.0005$) than in the WKY control strain (Table 1).

Estimates of S_G and S_I obtained from the WKY group were not significantly different ($P > 0.05$) from those determined in the SHR group (Tables 2 and 3). This suggests that the action of insulin and glucose *per se* on glucose metabolism is not impaired in SHR. Absence of alterations in glucose metabolism between these two species is in contrast with findings reported in humans in which a significant reduction in both peripheral tissue responsiveness to insulin and glucose-mediated glucose uptake was found in hypertensive subjects compared to normal (Modan *et al.* 1985; Ferrannini *et al.* 1987; Swislocki *et al.* 1989; Reaven, 1991; Natalucci *et al.* 1999). Thus, from the standpoint of glucose metabolism, the SHR does not appear to be an ideal model of human essential hypertension.

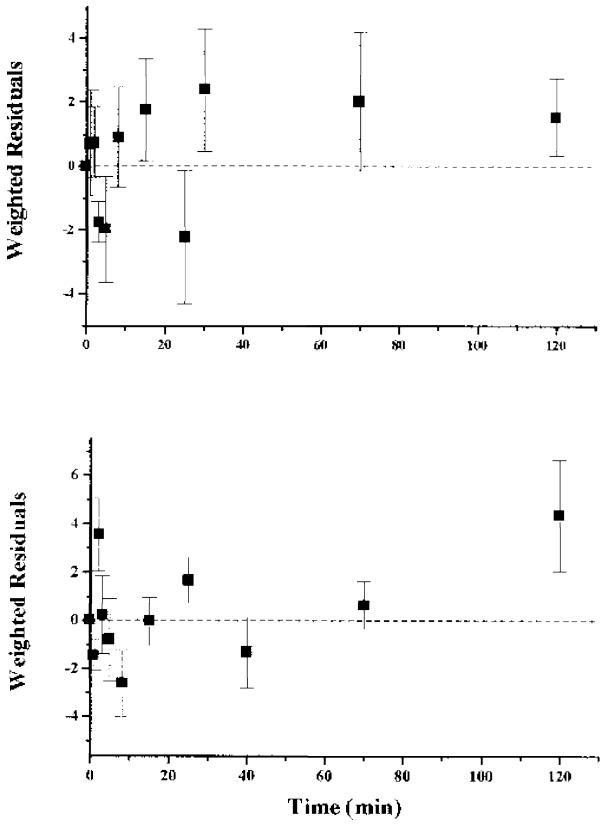

Figure 3

Top panel: mean weighted residuals of the minimal model of glucose kinetics (means ± S.E.M.) from the Wistar Kyoto (WKY) rats. Bottom panel: mean weighted residuals of the minimal model of glucose kinetics (means ± S.E.M.) from the spontaneously hypertensive rats (SHR).

The reliability of this result may be affected by the assertion of McArthur *et al.* (1999) that the IVGTT is inadequate to detect the effect of the acute insulin response to a glucose bolus on rat glucose disposal. This finding was inferred from the observation of no apparent acceleration of glucose disappearance when insulin was injected at any physiological dose (McArthur *et al.* 1999). To test whether this was true in our rats, we performed an IVGTT in which the glucose bolus (400 mg kg^{-1}) was followed by two insulin injections (0.05 U kg^{-1}), as in an insulin-modified IVGTT, 8 min and 120 min later, respectively. The IVGTT sampling schedule was modified as follows: one basal blood sample at −5 min before the glucose injection and 11 additional blood samples at 1, 2, 3, 5, 10, 13, 25, 40, 70, 120 and 125 min after the glucose injection. Figure 4 shows the comparison between glucose concentration measured during this insulin-modified IVGTT in SHR (○) and mean glucose concentration measured during the standard IVGTT in the SHR group (●). The evident acceleration of glucose disposal after the insulin injections (arrows) contradicts the experimental findings reported by McArthur *et al.* (1999) and justifies the use of the minimal model to interpret our IVGTT data. An explanation of this contradiction may be found in that our experiments were performed on anaesthetised rats whereas the data from McArthur *et al.* (1999) were from unanaesthetised rats. According to a recent study by Ahren & Pacini (1999) on the combined effects of glucagon-like peptide 1 (GLP-1) on the processes affecting glucose disposal showed that anaesthetised animals are more sensitive to the insulinotropic action of GLP-1, which might be explained by the circulating catecholamines, the levels of which are lower in anaesthetised mice than in unanaesthetised mice (Karlsson *et al.* 1994; Filipsson *et al.* 1998) and the action of which inhibits insulin secretion (Ahren & Karlsson, 1994).

That there was no significant variation in the S_I and S_G estimates found in our study is in agreement with findings by

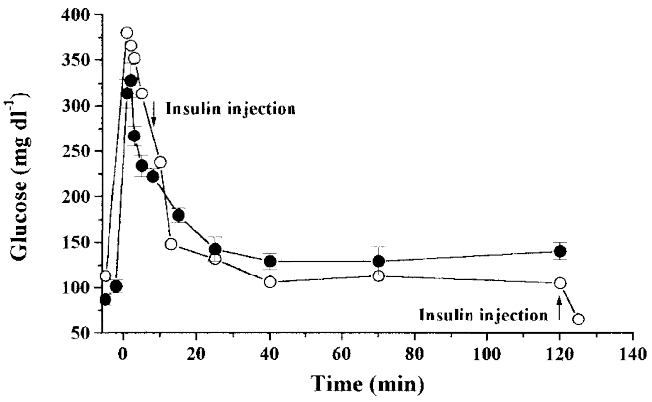

Figure 4

Comparison between glucose concentration measured during the insulin-modified intravenous glucose tolerance test (IVGTT) in a spontaneously hypertensive rat (SHR, ○) and mean glucose concentration measured during the standard IVGTT in the SHR group (●). Arrows indicate the additional insulin injections (8 and 120 min).

Buchanan *et al*. (1992*a*) and Frontoni *et al*. (1992). On the other hand, discrepancies were found with respect to the reports by Mondon & Reaven (1988), Tsutsu *et al*. (1989), Gaboury *et al*. (1991), Hulman *et al*. (1993) and Rao (1993). Buchanan *et al*. (1992*a*) measured the insulin sensitivity with the euglycaemic clamp technique in conscious mobile animals, 12 h fasted, whose age ranged between 8 and10 weeks. They showed a virtually identical insulin action on glucose metabolism in SHR and WKY rats and reported estimates of insulin sensitivity of about $10.4\ (\pm 2.7) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$ for SHR and of about $9.72\ (\pm 2.27) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$ for WKY rats, which are in agreement with the value of S_I estimated by us in anaesthetised SHR and WKY with the minimal model technique ($12.5\ (\pm 1.9) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$ for SHR and $15.8\ (\pm 4.3) \times 10^{-4}$ dl min^{-1} kg^{-1} (μU ml^{-1})$^{-1}$ for WKY rats). Also Frontoni *et al*. (1992), by using the euglycaemic glucose clamp technique, demonstrated that elevated blood pressure in conscious unstressed chronically catheterised SHR is associated with the normal ability of insulin to stimulate peripheral glucose uptake.

There may be several reasons for the discrepancies between our results and those reported by other authors (Mondon & Reaven, 1988; Tsutsu *et al*. 1989; Gaboury *et al*. 1991; Hulman *et al*. 1993; Rao, 1993). In particular the discrepancies between our results and those of Mondon & Reaven (1988) and Gaboury *et al*. (1991) may be due to the use of the insulin suppression test technique (IST) to assess insulin sensitivity in anaesthetised SHR and WKY rats. This methodology requires the infusion of pharmacological agents (i.e. glucose, insulin, adrenaline (epinephrine) and propanol or somatostatin infusion) that may produce different effects in SHR *vs*. WKY rats. Also the nutritional state of the animals at the time of the study was different from that in our study (12 h fast). Tsutsu *et al*. (1989) applied an IVGTT protocol to SHR and WKY rats and reported a slight (13 %) enhancement of glucose disappearance in SHR, despite slightly lower insulin levels in that group. However, these animals were studied at 22 weeks of age with WKY body weight being about 70 g higher than SHR body weight. The apparent enhancement of insulin sensitivity in SHR (faster glucose disappearance in the presence of lower insulin levels) may have been due to interstrain differences in weight and adiposity rather than to inherent differences in glucose metabolism. Moreover in this study the assessment of insulin sensitivity was indirect so that the separate effects of insulin on glucose production and disposal cannot be quantified. Hulman *et al*. (1993) and Rao (1993) showed a significant reduction in insulin-stimulated glucose uptake of SHR using the glucose clamp technique in conscious (Hulman *et al*. 1993) or anaesthetised (Rao, 1993) overnight-fasted rats. The source of the discrepancies between our results and those reported in these works is not clear but one of the most obvious differences between our study and those of Hulman *et al*. (1993) and Rao (1993) was that the animals were purchased from different commercial vendors. Kurtz & Morris (1987) documented important differences between growth and blood pressure patterns in WKY but not in SHR obtained from two of the largest suppliers of these animals (Taconic Farms, Germantown, NY, and Charles River Laboratories, Wilmington, MA, USA). The discrepancies between our results and those of Hulman *et al*. (1993) and Rao (1993) may represent another manifestation of the genetic variability of the WKY.

Glucose metabolism is not governed only by insulin-dependent actions. Also insulin-independent mechanisms contribute to the glucose disposal after glucose administration, mainly by insulin-independent glucose in the brain and in skeletal muscle and by suppression of liver glucose output during hyperglycaemia (Ader *et al*. 1985; Best *et al*. 1996). The glucose effectiveness, S_G, calculated by the minimal model quantifies both of these processes (Bergman *et al*. 1979; Ader *et al*. 1985; Finegood, 1997). Unfortunately, to the best of our knowledge, measures of glucose effectiveness in SHR and WKY rats have not previously been reported by others, thus a comparison with our results, to evaluate in depth the role of glucose effectiveness in this animal model of essential hypertension, is not possible.

In conclusion under the present experimental conditions, elevated blood pressure in SHR is associated with the normal ability of insulin and glucose *per se* to stimulate peripheral glucose uptake and comparable fasting insulin and glucose levels. This suggests that insulin resistance is not a primary metabolic defect in this genetic model of hypertension. Nevertheless, the possibility that nutrient-stimulated insulin levels are elevated in SHR remains to be explored. Thus, from the standpoint of glucose metabolism, the SHR does not appear to be an ideal model of human essential hypertension. Longitudinal studies are desirable to determine whether insulin resistance develops with ageing in the SHR.

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Acknowledgements

This work was supported in part by the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, COFIN 98).