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Blood Pressure Changes Induced by Chronic Insulin Treatment in Wistar Rats

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Summary

Hyperinsulinaemia may play a causal role in the development of hypertension in obese hypertensives. However, experimental evidence supporting this statement is inappropriate. The main purpose of this study was to evaluate the chronic effects of insulin administration on blood pressure, total-body glucose metabolism and urinary catecholamine excretion. After 10 weeks of insulin injection blood pressure was substantially increased in insulin-treated animals compared to those treated with saline (125±2 vs 108±2 mm Hg, $p<0.001$). There were no differences in glycaemia, plasma triglyceride levels and free fatty acid levels between these two groups. Plasma level of corticosterone was increased in both insulin-treated and saline-treated rats as compared to untreated animals suggesting that the level of stress was similar in both injected groups. The urinary excretion of norepinephrine and dopamine was increased in the insulin-injected group by about 120 % and 310 %, respectively. Our data clearly indicate that long-term insulin administration increased blood pressure but the underlying mechanisms remain to be elucidated.

Key words
Insulin – Blood pressure – Glycaemia – Urinary catecholamines

Introduction

Much evidence indicates that hyperinsulinaemia is the link between diabetes and hypertension. Moreover, obese individuals are more likely to develop hypertension than non-obese ones and these subjects are frequently insulin-resistant and hyperinsulinaemic (Dustan 1983). The body weight reduction in such patients is often accompanied by decreases in blood pressure and insulin resistance, suggesting that insulin may mediate the blood pressure increase associated with obesity (Olefsky *et al.* 1974, Reisen *et al.* 1978). Perhaps more evidence for the role of insulin resistance and hyperinsulinaemia in the regulation of blood pressure can be derived from the study of Krotkiewski *et al.* (1979). These authors demonstrated that blood pressure could be lowered in obese individuals by physical training, even without changes in body weight, but only in those who were hyperinsulinaemic and hypertriglyceridaemic before the training program had started.

Acute administration of large doses of insulin elevates blood pressure in conscious dogs (Liang *et al.* 1982). In addition, it has been demonstrated that an acute insulin infusion decreases renal sodium excretion (De Fronzo 1983) and increases the activity of sympathetic nervous system (Landsberg and Krieger 1989, Liang *et al.* 1982). Similar results have been published by Brands *et al.* (1991) in Wistar rats receiving infusions of insulin for 5 days. A consistent rise of blood pressure and insulin resistance as well as hyperinsulinaemia were also seen in rats which were fed a high-carbohydrate diet (Hwang *et al.* 1990) and these blood pressure changes were attenuated by exercise training (Reaven *et al.* 1988).

To our knowledge, there is no experimental evidence about the chronic effects of insulin administration lasting for several weeks on blood pressure. We therefore evaluated the blood pressure response, total-body glucose metabolism as well as the metabolism of catecholamines in Wistar rats after insulin administration for 10 weeks.

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Methods

Male Wistar rats (200±10 g, VELAZ, Prague) were fed standard rat chow containing 170 mmol NaCl/kg. Tap water was given *ad libitum* and the animals were maintained on a 12 h light/dark cycle. The rats were divided randomly into three groups – intact controls, saline-injected rats and insulin-injected rats. Insulin-injected animals received a daily s.c. injection of insulin (Lente Insulin, Novo, Bagsvaerd, Denmark) for 10 weeks. Doses of the insulin-zinc suspension were gradually increased in three-day-intervals: 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 20.0 U/kg b.w. during three weeks. The highest dose was administered for additional 7 weeks. The saline-injected group was similarly treated with the same volume of saline. Both insulin and saline were injected between 1200 h and 1300 h. Diurnal changes of plasma glucose were determined on day 21, i.e. 3 days after the achievement of the highest insulin dose. In the 9th week of the experiment, urine samples were collected after 5 days of adaptation in metabolic cages. Urinary catecholamines and monoamine metabolites were determined by HPLC with electrochemical detection as described previously (Hušek 1990).

Systolic blood pressure was measured in conscious rats once a week by tail-cuff method. Blood pressure was recorded by the same person and at the same time of the day. At least 5 readings were performed in each session for each animal.

Insulin administration was terminated 36 h before the end of the experiment. All animals were deprived of food for 18 h before blood collection. Plasma glucose was measured using the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). The triglycerides concentration was determined by means of a commercial enzymatic kit (Lachema, Brno, Czech Republic) and free fatty acid concentration by the microcolorimetric method (Noma *et al.* 1973). Insulin was measured by competitive radioimmunoassay (Nakagawa *et al.* 1973) and anti-insulin antibodies by precipitation of ^{125}I-insulin antibody complexes (Gerbitz and Krammler 1978). Plasma corticosterone was assayed by competitive radioimmunoassay (Hampl *et al.* 1987).

Results are presented as means±S.E.M. unless otherwise stated. Statistical analysis was performed by Student's t-test. Probability values less than 0.05 were considered statistically significant.

Results

The systolic blood pressure of insulin-treated rats was significantly increased ($p<0.001$) compared to both saline-injected and intact control rats (Table 1). Daily injection of insulin for 10 weeks did not influence the body weight of insulin-injected animals in comparison with both control groups.

Table 1
Body weight (BW) and systolic blood pressure (SBP) of intact control, saline-treated and insulin-treated Wistar rats at the end of the experiment

	Control (n=12)	Saline (n=12)	Insulin (n=12)
BW (g)	515±16	532±14	528±13
SBP(mm Hg)	105±3	108±2	125±2*

Data are means ± S.E.M.
** $p<0.001$ compared to control and saline-treated groups*

Table 2
The metabolic parameters in control, saline-treated and insulin-treated Wistar rats at the end of the experiment

	Control (n = 12)	Saline (n = 12)	Insulin (n = 12)
Glycaemia (mmol/l)	4.97±0.20	5.33±0.1	26.02±0.15*+
Triglyceride (mmol/l)	0.52±0.23	0.53±0.2	40.59±0.26
FFA (mmol/l)	0.76±0.04	0.75±0.04	0.77±0.05
Insulin (mU/l)	30.1±2.7	29.1±2.8	30.4±3.2
Corticosterone (nmol/l)	453±71	950±98*	968±166*

Data are means ± S.E.M., FFA – free fatty acids,
*Statistical significance * $p<0.01$ from control group, + $p<0.01$ from saline-treated group*

Table 3
Plasma glucose variation in rats after insulin (20 U/kg b.w.) or saline injection applied between 12.00 h and 13.00 h

Time after injection (h)	0	4	8	12	20
Saline	5.16 ±0.35	6.35 ±0.51	7.84 ±0.97	8.36 ±0.85	6.23 ±0.37
Insulin	6.31 ±0.48	5.21 ±0.41	6.72 ±1.15	8.02 ±1.11	6.78 ±0.50

Data are means ± S.E.M.

Table 2 summarizes the experimental data at the end of the experiment. It can be seen that after the interruption of insulin administration, the glycaemia in insulin-treated group was significantly higher when compared to both controls and saline-treated rats. No differences were seen in fasting values of insulin, triglycerides and free fatty acids. Plasma levels of corticosterone were increased in both insulin- and saline-treated groups compared to untreated animals.

Even though the insulin doses used in this study were rather high, diurnal variations of plasma glucose were not significantly different between insulin-treated and saline-treated rats (Table 3). Plasma glucose levels decreased under basal levels at 4 h after insulin injection but recovered to the preinjection levels 8 h after injection. Due to a gradual increasing of insulin doses during the first three weeks of the experiment no severe hypoglycaemia or death of animals were observed.

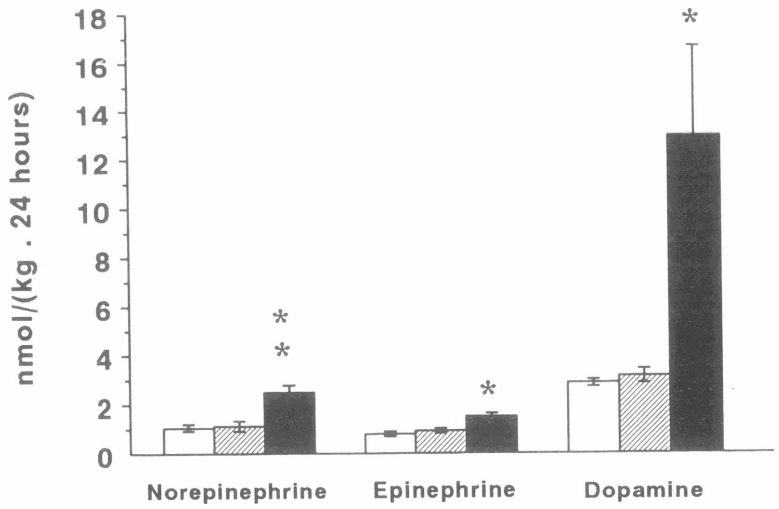

Fig. 1
Urinary excretion of catecholamines in control (open columns), saline-treated (hatched columns) and insulin-treated (full columns) Wistar rats. * and ** – significantly different ($p<0.05$ and $p<0.01$, respectively) from both control groups.

Urinary excretion of norepinephrine was increased by 120 % and that of dopamine by 310 % in insulin-treated rats in comparison with the other two groups (Fig. 1). The excretion of monoamine metabolites is shown in Fig. 2. Urinary vanilmandelic acid was increased by 66 % and 3,4-dihydroxyphenylacetic acid by 90 % in insulin-treated rats compared to the intact controls. The excretion of 5-hydroxyindolacetic acid (a serotonin metabolite) was also increased by 60 % in the insulin-treated group. Control and saline-treated groups did not differ in urinary excretion of these metabolites.

Discussion

Though several previous studies have suggested a relationship between hypertension and hyperinsulinaemia, there have been only a few studies in which the long-term effects of insulin on blood pressure have been directly evaluated. These studies were conducted in dogs or rats with different results.

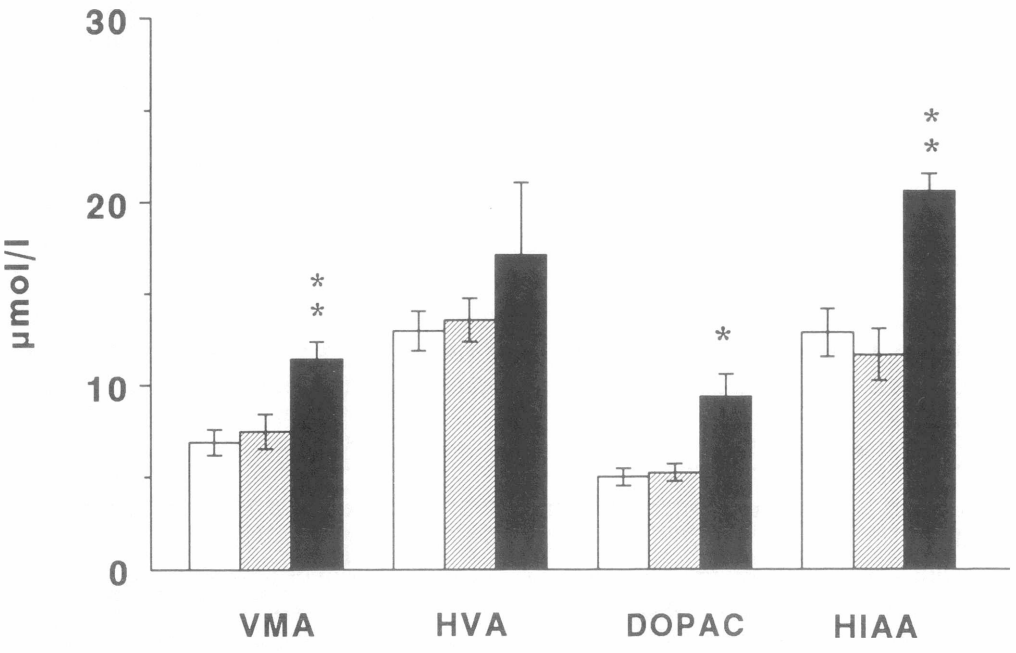

Fig. 2
Urinary excretion of monoamine metabolites in control (open columns), saline-treated (hatched columns) and insulin-treated (full columns) Wistar rats. VMA – vanilmandelic acid, HVA – homovanilic acid, DOPAC – 3,4-dihydroxyphenylacetic acid, HIAA – 5-hydroxyindolacetic acid. * and ** - significantly different ($p<0.01$ and $p<0.001$, respectively) from both control groups.

Hall *et al.* (1990) have shown that insulin infusion in conscious chronically instrumented dogs for 7 to 28 days highly increased plasma insulin concentration but plasma glucose was maintained within the normal range whereas mean arterial pressure was even decreased. On the other hand, a 5-day intravenous infusion of insulin and glucose into Sprague-Dawley rats increased both plasma insulin concentrations and mean arterial pressure (Brands *et al.* 1991). The results of our study clearly demonstrated that 10 weeks of insulin injection significantly elevated systolic blood pressure in Wistar rats. This was not true in the study of Bunag *et al.* (1991) who found that the blood pressure of conscious Wistar rats remained unchanged after 12 days of insulin injection. These discrepancies might be explained by the concept that hypertension is related to insulin resistance rather than to hyperinsulinaemia *per se*. It appears that hyperinsulinaemia, at least a short-term one, is insufficient to increase blood pressure unless it is accompanied by peripheral insulin resistance. Thus, it is not surprising that insulin infusion does not always raise blood pressure because of the different duration of its application. Moreover, possible strain differences should also be considered because Buchanan *et al.* (1992) demonstrated the dissociation of hypertension and peripheral insulin resistance in spontaneously hypertensive rats.

However, the cause-and-effect relationship between insulin and chronic increase of blood pressure remains to be evaluated. One possible mechanism whereby hyperinsulinaemia may increase blood pressure is stimulation of the sympathetic nervous system (Landsberg and Krieger 1989, Young and Landsberg 1977). There is some evidence that the changes in sympathetic nervous activity were closely related to the changes in plasma insulin concentration. Using the insulin/glucose-clamp technique, Rowe *et al.* (1981) found that insulin caused a dose-related increase in plasma norepinephrine levels. The results of our present study are in good agreement with the above mentioned results, because the urinary excretion of norepinephrine as well as of dopamine was highly increased in long-term insulin-injected rats. On the other hand, Hall *et al.* (1990) did not observe any chronic effects of hyperinsulinaemia on plasma catecholamines in dogs although previous short-term studies have suggested that insulin can stimulate catecholamine excretion even when plasma glucose is maintained constant (Liang *et al.* 1982).

There are other possible mechanisms by which insulin could influence blood pressure. The alterations of cation transport might be one of them. Since sodium pump is insulin-sensitive (Moore and Rabovsky 1979), its decreased activity during the reduced tissue insulin sensitivity could increase intracellular calcium and thus to enhance the contractility of vascular smooth muscle cells. Nevertheless, direct insulin effects on the reactivity of vascular smooth muscle cells were also reported (Zemel *et al.* 1990a). Moreover, cell culture studies have shown that insulin itself is a potent mitogenic factor in cultured arterial smooth muscle cells (Pfeifle and Ditschuneit 1981) and a cellular growth regulator of hepatocytes (Steiner *et al.* 1977). Sato *et al.* (1989) clearly

demonstrated that one year of insulin treatment resulted in the thickening of the aortic intima in all insulin-treated rats. If hyperisulinaemia could also cause the alterations at the level of resistance vessels and thus modulate the total peripheral resistance remains to be evaluated. In addition, some recent studies suggested that increased vascular resistance and reactivity in insulin-resistant states may result from an impaired vascular smooth muscle response to insulin (Zemel *et al.* 1990a, 1990b, 1991).

In conclusion, our results clearly demonstrated that long-term insulin treatment increases systolic blood pressure. This blood pressure rise might be partially caused by alterations of sympathetic nervous system because catecholamine metabolism was altered in insulin-treated rats but not in saline-treated ones. Other underlying mechanisms of long-term insulin effects remain to be elucidated.

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References

BUCHANAN T.A, SIPOS G.F, MADRILEJO N., LIU CH., CAMPESE V.M.: Hypertension without peripheral insulin resistance in spontaneously hypertensive rats. *Am. J. Physiol.* **262:** E14–E19, 1992.

BRANDS M.W., HILDEBRANDT D.A., MIZELLE H.L., HALL J.E.: Sustained hyperinsulinemia increases arterial pressure in conscious rats. *Am. J. Physiol.* **250:** R764–R768, 1991.

BUNAG R.D., KRIZSAN-AGBAS, D., ITOH H.: Sympathetic activation by chronic insulin treatment in conscious rats. *J. Pharmacol. Exp. Ther.* **259:** 131–138, 1991.

DE FRONZO R.A.: The effects of insulin on renal sodium metabolism. *Diabetologia* **21:** 165–171, 1983.

DUSTAN H.P.: Mechanisms of hypertension associated with obesity. *Ann. Intern. Med.* **98:** 860–864, 1983.

GERBITZ, K.D., KRAMMLER W.: Method for rapid quantitation and chracterization of insulin antibodies. *Clin. Chem.* **24:** 890–894, 1978.

HALL J.E, COLEMAN T.G, MIZELLE H.L, SMITH M.J.: Chronic hyperinsulinemia and blood pressure: interaction with catecholamines? *Hypertension* **15:** 519–527, 1990.

HAMPL R., BIČÍKOVÁ M., MOTLÍK K., STÁRKA L.: Effect of Etomidate on adrenal function in rats. *Endocrinol. Exp.* **21:** 229–235, 1987.

HUŠEK P.: Simple approach for reversal of the epinephrine-norepinephrine elution order in ion-pair reversed-phase liquid chromatography. *J. Chromatogr.* **532:** 394–399, 1990.

HWANG I.-S., HO H., HOFFMAN B.B., REAVEN G.M.: Fructose- induced insulin resistance and hypertension in rats. *Hypertension* **15:** 519–527, 1990.

KROTKIEWSKI M., MANDROUKAS K., SJOSTROM L., SULLIVAN L., WETTERQVIST, H., BJORNTORP P.: Effects of long-term physical training on body fat, metabolism, and blood pressure in obesity. *Metabolism* 28: 650–658, 1979.

LANDSBERG L., KRIEGER D.R.: Obesity, metabolism, and the sympathetic nervous system. *Am. J. Hypertens.* **2:** 1255–1325, 1989.

LIANG C.S., DOHERTY J.V., FAILLACE R., MACKAWA K., ARNOLD S., GAVIAS, H., HOOD W.B.Jr.: Insulin infusion in conscious dogs: effects on systemic and coronary hemodynamics, regional blood flows and plasma catecholamines. *J. Clin. Invest.* **69:** 1321–1336, 1982.

MOORE R.D., RABOVSKY J.L.: Mechanism of insulin action on resting membrane potential of frog skeletal muscle. *Am. J. Physiol.* **236:** C249–C254, 1979.

NAKAGAWA S., NAKAYAMA H., SASAKI T.: A simple method for the determination of serum free insulin levels in insulin-treated patients. *Diabetes* **22:** 590–596, 1973

NOMA A., OKABE H., KITA M.: A new colorimetric microdetermination of free fatty acids in serum. *Clin. Chim. Acta* **43:** 317–320, 1973

OLEFSKY J., REAVEN G.M., FARQUHAR J.W.: Effect of weight reduction on obesity studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J. Clin. Invest.* **53:** 64–76, 1974.

PFEIFLE B., DITSCHUNEIT H.: Effect of insulin on growth of cultured human arterial smooth muscle cells. *Diabetologia* **20:** 155–158, 1981.

REAVEN G.M., HO H., HOFFMAN B.B.: Attenuation of fructose induced hypertension in rat by exercise training. *Hypertension* **12:** 129–132, 1988.

REISEN E., ABEL R., MODAN M., SILVERBERG D.S., ELIAHOU H.E., MODAN B.: Effect of weight loss without salt restriction on the reduction of blood pressure in overweight hypertensive patients. *N. Engl. J. Med.* **298:** 1–6, 1978.

ROWE J.W., YOUNG J.B., MINAKER K.L., STEVENS A.L., PALLOTTA J., LANDSBERG L.: Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* **30:** 219–225, 1981.

SATO Y., SHIRAISHI S., OSHIDA Y., ISHIGURO T., SAKAMOTO N.: Experimental atherosclerosis-like lesions induced by hyperinsulinism in Wistar rats. *Diabetes* **38:** 91–96, 1989.

STEINER D.F., CHAN S.J., TERRIS S., HOFMANN C.: Insulin as cellular growth regulator. In: *Hepatotrophic Factor.* Porter R., Whelan. J. (eds), Amsterdam, Elsevier, 1977, pp. 217–228.

YOUNG J.B., LANDSBERG L.: Stimulation of the sympathetic nervous system during sucrose feeding. *Nature* **269:** 615–617, 1977.

ZEMEL M.B., REDDY S., SHEHIN S.E, LOCKETTE W., SOWERS J.R.: Vascular reactivity in Zucker rats: role of insulin resistance. *Vasc. Med. Biol.* **2:** 81–85, 1990a.

ZEMEL M.B., SOWERS J.R., SHEHIN S.E., WALSH M.F., LEVY J.: Impaired calcium metabolism associated with hypertension in Zucker obese rats. *Metabolism* **39:** 704–708, 1990b.

ZEMEL M.B., REDDY S., SOWERS J.R.: Insulin attenuation of vasoconstrictor responses to phenylnephrine in Zucker lean and obese rats. *Am. J. Hypertens.* **4:** 537–539, 1991.

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