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The Acute Effect of Insulin on Renal Haemodynamics and Protein Excretion in Diabetics

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Summary. The effect of IV injection of 7 to 8 I. U. of insulin on renal haemodynamics and on urinary excretion of beta-2-microglobulin and of albumin was examined in 5 juvenile diabetics. Plasma glucose decreased from a mean value of 250 mg/100 ml to 117 mg/100 ml during the first 85 min after insulin. None of the patients had symptoms of hypoglycaemia and plasma adrenaline did not increase. There was no change in arterial blood pressure after insulin whereas pulse rate increased from 66/min to a maximum of 75/min. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were decreased by 9 per cent and 13 per cent, respectively, during the first 90 min after insulin ($2p < 0.01$). There was also a statistically significant decrease in urine flow and urine secretion of several electrolytes, while filtration fraction remained almost constant. IV insulin decreased urinary excretion of beta-2-microglobulin and increased albumin excretion ($2\ p < 0.05$). The albumin excretion induced by insulin is most likely due to increased amounts of filtered albumin, the mechanism of which remains unexplained.

Key words: Albumin, beta-2-microglobulin, blood glucose, cardiovascular, glomerular filtration rate, insulin, proteinuria, pulse rate, renal plasma flow.

Insulin, apart from its effects on metabolism and on ion fluxes, has a marked acute effect on the cardiovascular system.

Intravenous injection of insulin in diabetics induces hypovolaemia [1]. Arterial blood pressure is maintained by an increase in sympathetic nervous activity, as demonstrated by a rise in plasma noradrenaline [1, 2, 3], an increase in pulse rate [1, 4], and peripheral vasoconstriction [1]. However, in diabetic patients with neuropathy and in sympathectomized patients intravenous insulin may result in a fall in arterial blood pressure [5, 6, 7]. The cardiovascular effects of insulin are most pronounced when the subjects are in the feet-down, tilted position and are not due to hypoglycaemia [1, 4].

The aim of the present study was to examine the effects of insulin on renal plasma flow (RPF), glomerular filtration rate (GFR) and urinary excretion rates of beta-2-microglobulin and of albumin in diabetics.

Patients

Five male juvenile diabetic out-patients were studied after an overnight fast. Their mean ages were 28 years and the duration of diabetes averaged 8 years. None of the patients had clinical signs of peripheral neuropathy. Pertinent clinical data are shown in Table 1. They were all treated with insulin twice a day and had their last dose of insulin 24 hours before the investigation. They drank 20 ml of water every 20 min during the experiment, starting 1 hour before the clearance procedure. The subjects were also asked to drink one glass of water in the morning before leaving home. Informed consent was obtained from all subjects.

Table 1. Pertinent clinical data in 5 male, juvenile diabetics

Pat.	Age Years	Body weight, fraction of ideal	Diabetes duration Years	Retinopathy	Usual Insulin dose (IU/24 h)
1	36	0.97	7	–	60
2	23	0.92	10	–	56
3	28	1.09	5	–	48
4	29	1.18	8	few red dots	60
5	26	0.97	9	–	76

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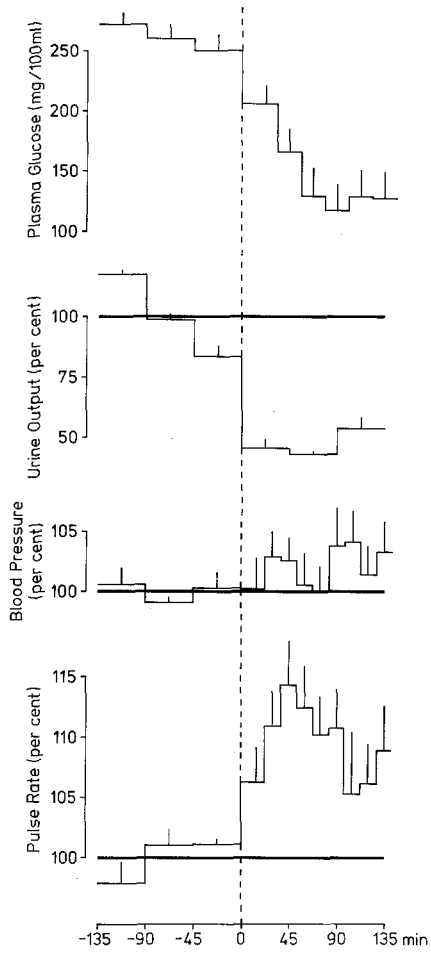
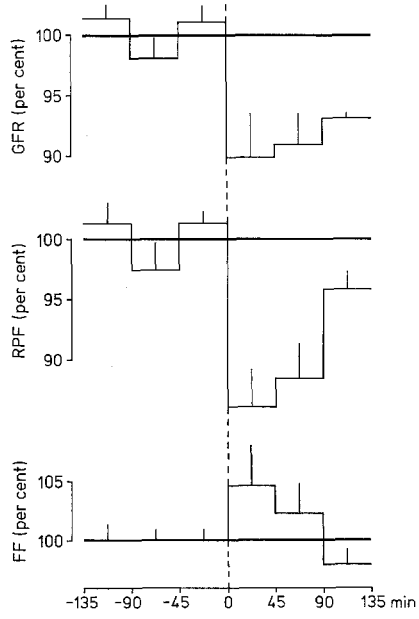


Fig. 1. Plasma glucose (mg/100 ml) and percentage changes from mean value of pre-insulin period in urine output, blood pressure and pulse rate after intravenous insulin administration. Results are mean values ± SEM



Procedure

The investigations were started at 8 a. m. Following a resting period of 1½ hours in the supine position, GFR, RPF, urinary albumin, and beta-2-microglobulin were measured in three 45 min clearance periods before and three periods after insulin. Seven or eight I. U. of insulin (Actrapid®, Novo) were given intravenously during the first minute of the fourth clearance period. The patients rested in the supine position during the whole experiment, standing up only to void.

Methods

GFR and RPF were measured by constant infusion techniques using ^{125}I-iothalamate as filtration marker and the clearance of ^{131}I-hippuran for measuring plasma flow [8]. A total of 50 ml were infused during an experiment. Urinary concentration of albumin and beta-2-microglobulin (Phadebas® β_2-micro Test) was measured by radioimmunoassay [9, 10]. Heart rate and auscultatory blood pressure were measured in the middle of each 45 min period before insulin and every 15 min in the periods after insulin injection. Plasma glucose was determined by an ortho-toluidine method in venous blood taken in the middle of each period; after insulin it was also measured 5 min before the termination of each period. The concentrations of sodium, potassium, and phosphate were measured by standard methods in plasma samples taken in the middle of each period as well as in urine samples. Plasma adrenaline was measured in two patients before and 45 min after injection of insulin [11, 12].

Student's t-test for paired samples was used for judging the significance of differences between mean values. A five per cent limit was employed. In the text the dispersion around a mean value at a certain point of time is given by the standard deviation (SD), whereas that of an average difference between different points of time is given by the standard error of the mean (SEM).

Results

The mean plasma glucose concentration before injection of insulin was 250 ± 31 mg/100 ml (mean ± SD). IV injection of insulin resulted in a fall in plasma glucose of 87 ± 11 mg/100 ml (mean ± SEM) during the first 45 min period and of 137 ± 22 mg/100 ml during the first two periods. No changes occurred in the last 45 min period (Fig. 1). Resting heart rate was increased by 7.3 ± 1.5 beats/min during the first two periods after insulin ($2p = 0.0077$, Fig. 1). Arterial blood pressure increased, but not significantly. Despite the constant water intake of 1 ml/min the urine output declined by 17 ± 3%/45 min between the three clearance periods before insulin. The fall in urine output of 54 ± 3% from the mean pre-insulin level in the first period after insulin was, however, highly significant even after correction for this trend ($2p = 0.0031$, Fig. 1).

◀ **Fig. 2.** Percentage changes from mean value of pre-insulin period in renal function after intravenous insulin administration. Results are mean values ± SEM

Table 2. Urinary excretion of albumin (μg/min) and of beta-2-microglobulin (ng/min) before and after insulin injection in juvenile diabetics

Patients		Pre-insulin periods			Post-insulin periods		
		0–45	45–90	90–135	0–45	45–90	90–135
					min		
1	Albumin	5.2	4.5	4.6	4.3	8.0	5.4
	beta-2-microglobulin	86	157	88	22	65	118
2	Albumin	4.8	4.6	4.3	5.4	8.8	9.5
	beta-2-microglobulin	172	134	77	47	28	74
3	Albumin	5.1	4.1	3.8	4.3	3.9	11.3
	beta-2-microglobulin	175	116	145	59	29	107
4	Albumin	14.3	12.2	16.8	21.8	64.4	20.7
	beta-2-microglobulin	10	6	10	3	1	35
5	Albumin	18.6	10.6	7.1	40.6	23.9	
	beta-2-microglobulin	85	69	62	55	69	

No significant changes were observed between the three periods after insulin.

GFR and RPF were constant in the three pre-insulin periods. GFR fell from a basal value of 133 ± 14 ml/min by 14 ml/min in the first post-insulin clearance period and remained low throughout the next two periods (Fig. 2). The mean fall of 12.2 ± 1.9 ml/min during the first two post-insulin periods was highly significant ($2p = 0.0033$). In the same two periods RPF was decreased by 67 ± 4 ml/min ($2p = 0.000067$) from a baseline value of 532 ± 60 ml/min, but had risen again by 48 ± 10 ml/min ($2p = 0.016$) in the last clearance period (Fig. 2). The filtration fraction remained constant throughout the experiments (basal value 0.250 ± 0.011), except for a small decrease during the last period (0.018 ± 0.005), ($2p = 0.035$).

Predictably, serum potassium and serum phosphate decreased after insulin by 0.5 ± 0.1 mmol/l ($2p = 0.0088$) and 0.5 ± 0.1 mg/100 ml ($2p = 0.011$), serum sodium was unchanged (+ 1.3 ± 0.5 mmol/l, $2p = 0.090$), and the urinary excretion rates of all three electrolytes were reduced in the post-insulin clearance periods by 50 ± 4% ($2p = 0.00030$), 75 ± 3% ($2p = 0.000017$), and 37 ± 12% ($2p = 0.036$), respectively.

The individual values of albumin and beta-2-microglobulin excretion rates appear in Table 2. After insulin, albumin excretion was increased by 92 ± 28% ($2p = 0.029$), whereas beta-2-microglubulin excretion rate was decreased in the first two periods by 55 ± 20 ng/ml ($2p = 0.0499$) (59 ± 12%, $2p = 0.0070$) followed by an increase of 53 ± 9 ng/ml ($2p = 0.011$). The two diabetics (Nos. 4 and 5) with the highest basal albumin excretion rate showed both an absolutely (21 ± 1 versus 2.2 ± 0.6 μg/ml) and a relatively (157 ± 10 vs. 49 ± 14%) larger increase in albumin excretion after insulin than the other three patients ($2p = 0.00026$ and 0.012, respectively). The increase in albumin excretion was usually seen within a single clearance period rather than uniformly throughout all three post-insulin periods, giving rise to a significantly greater relative intraindividual variation after insulin than in the pre-insulin periods ($F = 7.8$, $2p = 0.00088$). The distribution of individual maxima were, however, different within the three post-insulin periods, for which reason only the mean values were considered above.

Plasma adrenaline averaged 0.07 ng/ml in two subjects before injection of insulin and was unchanged 45 min after injection of insulin.

There was no correlation between changes in blood glucose after injection of insulin and changes in any of the haemodynamic and renal function parameters mentioned above.

Discussion

The present study shows that IV injection of insulin results in changes in renal haemodynamics and in urinary excretion of albumin and beta 2-microglobulin. These changes may be explained by the increase in sympathetic nervous activity which occurs after insulin or may be due to a direct effect on renal function of insulin or of glucose.

It is well-established that IV injection of insulin results in increases sympathetic nervous activity which most probably is due to hypovolaemia [1]. Insulin decreases both plasma volume and the intravascular pool of albumin. It has recently been shown that insulin increases the number of micropinocytotic vesicles in endothelial cells in muscle capillaries in rats with experimental diabetes [13]. These findings suggest that the hypovolaemic effect of insulin may in part be due to an increased transfer

of plasma out of the vascular compartment. The cardiovascular effect of insulin is not due to hypoglycaemia [1, 4], since the effect occurs even when the blood glucose concentration does not decline below normal fasting values. Furthermore, none of the patients examined in the present and in a previous study had symptoms of hypoglycaemia and plasma adrenaline did not increase [1].

No correlations were found between changes in blood glucose and haemodynamic changes observed after insulin either in the present study or in a previous one [1]. It should be emphasized, however, that the haemodynamic effects of insulin have not been shown to occur independently of a fall in blood glucose concentration, a question which is currently under investigation.

The effects of insulin on renal haemodynamics (i. e. the decrease in GFR and in RPF observed in the present study) are most likely explained by the concomitant increase in sympathetic nervous activity [1]. Both the decrease in RPF and in GFR and the increase in sympathetic nervous activity are of a magnitude comparable to that seen in normal subjects in response to assuming the upright position [14]. The antidiuretic effect of insulin has been demonstrated previously [15]. It is at least partly independent of the fall in blood glucose concentration. It may be due to an increased secretion of the antidiuretic hormone [15, 16], probably elicited in response to insulin induced hypovolaemia.

The inhibitory effect of insulin on renal excretion rates of electrolytes [17, 18, 19] occurs at least partly independently of changes in blood glucose concentration [18, 19] and may explain the pronounced sodium retention occurring in diabetics when insulin treatment is instituted. It may also partly explain the inhibitory effect of carbohydrate on sodium excretion during fasting. Insulin enhances tubular reabsorption of sodium by stimulating the active sodium pump [20].

Unexpectedly, insulin was found to have opposite effects on urinary excretion of beta-2-microglobulin and albumin. Excretion of beta-2-microglobulin was decreased whereas that of albumin was increased. Normally, beta-2-microglobulin is almost totally reabsorbed by the tubules. Therefore, the effect of insulin on the total tubular reabsorption of beta-2-microglobulin must be relatively small, while the effect on urinary excretion rate of beta-2-microglobulin was marked. As mentioned above, insulin increases the number of micropinocytotic vesicles as well as the ratio between free and attached vesicles in endothelial cells in muscle capillaries in diabetic rats [13]. Tubular reabsorption of beta-2-microglobulin is dependent on the formation of vesicles. It is possible that insulin by changing the physical properties of the tubular cell membrane may increase vesicular transport by enhancing the production of vesicles.

Insulin increased urinary excretion of albumin. This effect was most probably due to increased filtered amounts of albumin after insulin, because the changes in beta-2-microglobulin suggest that tubular reabsorption of protein was increased. The effect was most pronounced in patients who had the highest basal urinary excretion rate of albumin. It is well-known that other procedures which influence renal haemodynamics, e. g. exercise, may lead to an increased albumin excretion [21] especially in diabetics who have elevated basal excretion rates of albumin (C. E. Mogensen: Unpublished observations). This abnormality induced by exercise is probably due to a defective glomerular filter which cannot withstand the increased filtration pressure developed during exercise. The increase in albumin excretion induced by insulin may also be due to a defective glomerular filter. The mechanism is, however, likely to be different from that of exercise, as insulin did not increase the filtration fraction.

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