Zeitschrift für Ernährungswissenschaft

Journal of Nutritional Sciences · Journal des Sciences de la Nutrition

Band 18 Heft 1 März 1979

Z. Ernährungswiss. 18, 1–7 (1979)

ISSN 0044-264 X

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Serum mineral changes due to exogenous ATP and certain trace elements in experimental diabetes

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With 3 tables

(Received June 5, 1978)

Production of experimental diabetes by intravenous injection of dithizone indicated a connection between insulin synthesis and the metabolism of trace elements. Blocking trace elements in the organism led to hypoinsulinism and in turn the disturbance of insulin synthesis led to excretion from the organism of trace elements. Hyperglycemia, glucosuria and metalluria produced during dithizone diabetes were also prevented by insulin (29).

Simultaneous administration of zinc chloride (2 mg/kg s.c. twice daily for 7 days) prevented cadmium chloride induced hyperglycaemia and decreased glucose tolerance (12).

We have also reported that the intravenous injection of zinc chloride (1 mg/kg) immediately before and fifteen minutes after alloxan or dithizone prevented the usual hyperglycemia observed 24 hours after induction of diabetes. Histological examination showed that the islets of these animals which were injected with zinc were intact and their beta cells stained normally. The intravenous injection of manganese chloride prevented any marked rise of blood glucose, without protect ing the islets. Chromium and cobalt chloride lowered the blood glucose level to a certain extent (24).

ATP resulted also in a significant reduction in blood sugar in female rats examined on the 10th day after induction of alloxan diabetes (24).

The aim of the present study is to evaluate the changes in serum zinc, copper, iron, calcium, magnesium, potassium and sodium under the protective effect of ATP and certain trace elements such as zinc, manganese, chromium and cobalt on the diabetes induced by alloxan or dithizone.

Material and methods

Sprague dawley rats bred in the laboratory, weighing 200–250 g and fed stock diet ad libitum, were used throughout.

Fasted rats were rendered diabetic by alloxan by intraperitoneal injection of freshly prepared alloxan monohydrate solution in a dose of 150 mg/kg of body weight. In case of dithizone diabetes, rats were rendered diabetic, after 24 hours fast by slow intravenous injection of freshly prepared dithizone solution in a dose of 200 mg/kg of body weight (22).

The rats in this study were divided into seven groups, control group comprised 15 normal rats. Diabetic group comprised 15 alloxanized and 15 dithizonized rats. Groups 3, 4, 5 and 6 comprised each 20 rats injected intravenously with either zinc, manganese, chromium or cobalt chloride solution to a dose 1 mg of the salt/kg body weight immediately before and 1 mg 15 minutes after alloxan or dithizone.

Group 7 represents the effect of ATP on alloxan diabetes. ATP (Richter) was given by the intraperitoneal route of administration one hour before alloxan in the dose of 30 mg/rat.

Twenty-four hours after injection, the animals were killed by decapitation. Blood samples were collected from the heart. A portion of the blood was used for estimation of blood glucose. The remaining portion was allowed to clot and the serum was used for analysis of minerals.

The method of Nelson's modification of *Somogyi's* (20) was used for determination of blood sugar. Serum zinc and copper were determined by the method of *Sinaha* and *Gabrieli* (21). Serum iron, potassium and sodium were estimated by the method published in *Backman*, Analytical Method by Atomic Absorption Spectrophotometer. Serum calcium and magnesium were determined using the method of *Willis* (27).

Results and discussion

In health, zinc is one of the most uniform biochemical constituents of blood and that increased metabolic activities induced a marked and immediate fall in blood zinc (5).

A significant quantity of zinc is present in normal pancreas, and the content of zinc is greatly reduced in the pancreas of patients with diabetes mellitus (26).

Zinc administration seems to stimulate glucose uptake (17) as well as an increase in plasma insulin like activity (ILA) and seems to enhance the hypoglycemic action of insulin itself (15).

The hypoglycemic effect of manganese has been reported by many workers (18, 8). It may act peripherally on utilization of glucose, or through glucagon, inhibiting its release or interfering with its glycogenolytic action (8).

It is known that chromium increases the utilization of glucose in laboratory animals that have been subjected to a chromium-deficient diet (9).

Sugar transport across the cell membrane is one site of action of chromium. The mode of action of chromium appears to be closely related to that of insulin (13).

The effect of cobalt ions on the metabolism of glucose by adipose tissue was studied with ^{14}C labeled glucose by *Padmaker* and *Lazarow* (14). Cobalt ions enhanced glucose uptake, its oxidation to CO_2 and its incorporation into fat pad lipids, thus stimulating the action of insulin.

In in-vivo studies the intravenous injection of high dose of cobalt chloride (25–50) mg/kg body weight caused a transitory hyperglycemia in normal rabbits (10). This is probably caused by a rapid selective injury to the alpha-cells of the pancreatic islets, releasing glucagon. The same authors however found that cobalt had no effect on the diabetogenic action of alloxan.

In ATP-treated rats (2 mg i.v./rat) the much faster clearance of blood sugar after glucose injection was associated with a significant increase in

insulin secretion. ATP could also increase insulin secretion from the still functioning beta cells in the partially alloxan diabetic rats (23).

In the present study, serum zinc, copper, iron and magnesium in alloxan-treated animals were significantly higher than in normal, while the level of serum calcium, sodium and potassium were lower than normal.

However in the dithizone diabetic animals, the levels of serum zinc, iron and potassium were higher than normal, while those of serum calcium and sodium were lower, copper and magnesium levels were unchanged.

These results are in agreement with previous data obtained by our laboratory (6, 7).

Wolff et al. (28) reported an increase in serum zinc after dithizone. This was followed by a greater urinary output of zinc. The iron content of the serum and urine showed the same pattern as found for zinc, but no significant change was found in copper, cobalt and magnesium (28). Serum sodium and calcium were reduced, while potassium was raised (1).

Table 1. Effect of certain inorganic elements in protecting the onset of Alloxan diabetes in rats.

		Control	Alloxan	Effect of intravenous injection of			
				Zinc	Manganese	Chromium	Cobalt
		(15)	(15)	(10)	(10)	(10)	(10)
Blood glucose	mg%	116.7	450.9	110.5	114.0	269.5	196.5
	S.D ±	18.8	123.4	11.8	15.6	48.6	33.9
	P			0005	0005	0025	0005
Zinc	µg% M	131.7	225.8	174.4	117.7	194.9	173.5
	S.D ±	15.9	74.3	27.9	15.3	21.9	11.7
	P			Insig.	0.0005	Insig.	Insig.
Copper	µg% M	116.9	182.8	172.5	132.6	167.8	166.7
	S.D ±	20.1	40.5	17.8	12.4	20.5	9.3
	P			Insig.	0.0005	Insig.	Insig.
Iron	µg% M	132.1	170.5	165.2	161.5	167.1	177.6
	S.D ±	19.8	38.3	23.1	32.9	28.8	27.7
	P			Insig.	Insig.	Insig.	Insig.
Calcium	mg% M	7.55	5.95	6.38	5.43	4.89	4.57
	S.D ±	0.78	0.94	1.11	0.89	0.77	0.84
	P			Insig.	Insig.	0.025	0.01
Magnesium	mg% M	3.92	5.65	5.57	5.60	5.59	6.14
	S.D ±	0.44	1.10	0.61	0.80	0.60	0.62
	P			Insig.	Insig.	Insig.	Insig.
Potassium	mg% M	23.28	19.88	20.01	21.66	19.67	19.55
	S.D ±	2.19	1.35	1.30	1.90	1.57	1.00
	P			Insig.	0.05	Insig.	Insig.
Sodium	mg% M	225.3	180.8	219.4	210.5	185.1	190.0
	S.D ±	10.8	13.8	7.9	8.8	11.5	16.4
	P			0.0005	0.0005	Insig.	Insig.

Many authors reported increased serum zinc due to alloxan diabetes. The islet cells have the distinctive property of concentrating zinc, and the injury of these cells leads to a release of zinc in serum (11).

As shown in tables 1 and 2, the administration of zinc salt in alloxan or dithizone diabetic rats prevented the appearance of hyperglycemia.

Since hyperglycemia in diabetic rats is associated with hyperzincemia (tables 1 and 2), one would expect that the normal blood sugar which was obtained in the zinc-protected animals would have been associated with a normal serum zinc. But this was not so because the protective agent was zinc itself and this brought about a rise per se in serum zinc, not associated with any hyperglycemia.

Similarly there was no return of serum zinc to the normal level when insulin was used. *Rasin* (19) reported that insulin lowered blood zinc level directly after administration in diabetic dogs, but the zinc level increased again after few hours.

Injection of zinc chloride in alloxan or dithizone diabetic rats led to normalization of the decreased sodium. The mean serum sodium in alloxan or dithizone diabetes was considerably lower than normal. The drop in serum sodium was due to diuresis which followed the diabetic state. After

Table 2. Effect of certain inorganic elements in protecting the onset of dithizone diabetes in rats.

		Control (15)	Dithizone (15)	Effect of intravenous injection of Zinc	*Manganese*	*Chromium*	*Cobalt*
Blood glucose	mg% M	116.7	231.9	111.7	120.0	179.7	174.8
	S.D ±	18.8	79.2	19.8	27.7	27.3	32.3
	P			.0005	.0005	0.05	0.05
Zinc	µg% M	131.7	214.8	290.8	185.3	233.9	203.9
	S.D ±	15.9	39.8	74.7	34.1	30.0	34.6
	P			0.025	Insig.	Insig.	Insig.
Copper	µg% M	116.9	105.3	113.6	108.1	104.3	131.6
	S.D ±	20.1	18.3	14.7	8.4	15.7	10.5
	P			Insig.	Insig.	Insig.	0.025
Iron	µg% M	132.1	184.8	185.6	196.5	179.9	205.9
	S.D ±	19.8	31.6	27.8	20.1	34.9	25.3
	P			Insig.	Insig.	Insig.	Insig.
Calcium	mg% M	7.55	6.02	4.84	5.82	5.32	6.41
	S.D ±	0.78	1.20	0.52	0.67	0.57	0.74
	P			0.01	Insig.	Insig.	Insig.
Magnesium	mg% M	3.92	3.49	5.19	4.15	4.36	3.89
	S.D ±	0.44	0.64	0.39	0.55	0.41	0.46
	P			0.0005	0.05	0.005	Insig.
Potassium	mg% M	23.28	25.58	30.13	25.97	26.96	26.50
	S.D ±	2.19	2.37	2.47	1.11	1.75	1.80
	P			0.0025	Insig.	Insig.	Insig.
Sodium	mg% M	225.3	195.31	210.3	205.9	206.6	205.6
	S.D ±	10.8	15.8	8.9	13.7	11.2	7.9
	P			0.05	Insig.	Insig.	Insig.

treatment with zinc, the sodium level tends to normalize. This may be due to the relief of the diabetic state as shown from the lowering of the glucose levels (tables 1 and 2).

Manganese plus alloxan led to a normalization of blood glucose, serum zinc, copper, potassium and sodium. In the case of dithizone plus manganese, magnesium was raised while the other elements were unchanged when compared to animals injected with dithizone only.

In the case of chromium and cobalt, serum sodium dropped because hyperglycemia and consequent polyuria still occur despite the injection of chromium and cobalt chloride.

Adenosine triphosphate can form complexes with ions as copper and magnesium (4). The chelation of metal ions by ATP can affect serum minerals.

It has also been shown that the red cell ATP as activity and the active transport of ions are correlated (16).

Pretreatment with adenosine triphosphate led to a normalization of zinc, copper, magnesium, sodium and potassium. While in case of calcium and iron there was no significant change from that found in alloxan diabetes (table 3).

Table 3. Effect of ATP in protecting the onset of alloxan diabetes in rats.

Item		Control	Alloxan	Alloxan + ATP
Zinc	µg% Mean	131.7	225.8	135.2
	S.D ±	15.9	74.3	18.7
P: C vs	A + ATP			Insig.
A vs	A + ATP			0.005
Copper	µg% Mean	116.9	182.8	126.4
	S.D ±	20.1	40.5	20.6
P: C vs	A + ATP			Insig.
A vs	A + ATP			0.005
Iron	µg% Mean	132.1	170.5	166.1
	S.D ±	19.8	38.3	10.4
P: C vs	A + ATP			0.005
A vs	A + ATP			Insig.
Calcium	mg% Mean	7.55	5.95	5.50
	S.D ±	0.78	0.94	0.36
P: C vs	A + ATP			0.05
A vs	A ± ATP			Insig.
Magnesium	mg% Mean	3.92	5.65	3.80
	S.D ±	0.44	1.10	0.59
P: C vs	A + ATP			Insig.
A vs	A + ATP			0.005
Potassium	mg% Mean	23.28	19.88	22.15
	S.D ±	2.19	1.35	1.15
P: C vs	A + ATP			Insig.
A vs	A + ATP			0.005
Sodium	mg% Mean	225.3	180.8	220.5
	S.D ±	10.8	13.8	12.2
P: C vs	A + ATP			Insig.
A vs	A + ATP			0.005

The high level of serum copper in alloxan diabetes may be due to the toxic effect of alloxan on the liver, which is the source of copper (7). ATP administration ameliorated this effect.

Alloxan and dithizone diabetic rats showed an increase in serum iron. This may be due to hemolysis of erythrocytes (6, 7). ATP has no effect on the elevated serum iron in alloxan diabetic rats.

The normalization of the decreased serum sodium and potassium under the influence of ATP may be due to decreased diuresis.

Talaat et al. (25) found that exogenous ATP is associated with a significant decrease in the rates of potassium loss in the phrenic diaphragm preparation. They postulated that ATP exercises its beneficial effect on the exchange of potassium ions by acting on the cell membranes.

ATP also lowered the elevated serum magnesium in alloxan diabetic rats. That is the case with insulin, a slow simultaneous intravenous injection of insulin and glucose caused a decrease in the magnesium content of blood plasma (2). *Binet* (2) suggested that in diabetic animals insulin acts on the cell membrane promoting the entry of magnesium from the surrounding tissue fluids, thereby creating optimum conditions within the cell for the metabolism of glucose and ATP, and hence its decrease in the serum after insulin injection. Thus ATP administration seems to have an insulin-like effect, it acts on the cell membrane promoting the entry of magnesium and glucose.

Moreover the ionic movements across the beta cell membrane are important in insulin secretion by the beta cell and in maintaining it (3).

Thus the present results are in accord with our previous suggestion that ATP and certain trace elements are essential for both insulin secretion as well as glucose utilization by various tissues (24).

Summary

The intravenous injection of zinc or manganese chloride immediately before and 15 minutes after alloxan or dithizone prevented the usual hyperglycemia observed 24 hours after induction of diabetes.

Injection of zinc chloride in alloxan diabetes led to normalization of sodium while zinc, copper, iron and magnesium remained high and calcium and potassium remained low as in alloxan.

In case of dithizone diabetes, the administration of zinc salt led to an increase in serum zinc, magnesium and potassium and to a decrease in serum calcium while the sodium level returned to normal.

Manganese plus alloxan led to a normalization of serum zinc, copper, potassium and sodium. In the case of dithizone plus manganese only magnesium was raised while the other elements were unchanged when compared to animals injected with dithizone only.

Chromium and cobalt chloride lowered the blood glucose to a certain extent however it did not affect most of the elements. The same changes occurred in all elements as with alloxan or dithizone alone.

Pretreatment with ATP led to a normalization of serum zinc, copper, magnesium, sodium and potassium, while in case of iron it remained high and calcium remained low as that found in alloxan diebetic rats.

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