Effects of Benzothiadiazine Drugs on Carbohydrate Metabolism

By JOHN M. WELLER AND PAUL E. BORONDY

The administration of chlorothiazide both to man and to rats results in hyperglycemia following a glucose load. Its administration to rats causes a decreased rate of glucose utilization by their adipose tissue in vitro. Chlorothiazide added directly to the incubating medium also decreases the rate of glucose utilization of rat adipose tissue. These effects of chlorothiazide are most likely due to both a direct action of the drug on tissue glucose metabolism and an indirect effect which is the result of chlorothiazide causing a decrease in the level of serum insulin-like activity which in turn diminishes the activity of insulin-dependent enzymes involved in glucose metabolism.

HYPERGLYCEMIA has been reported in association with the administration of benzothiadiazine drugs.[1-4] It has been stressed that these compounds can cause exacerbation of existing or potential diabetes mellitus,[5-10] however, their hyperglycemic action has been noted even in normal individuals. The following studies were carried out to delineate further the alterations of carbohydrate metabolism produced by benzothiadiazines.

METHODS AND RESULTS

Glucose Tolerance Tests on Patients

Two studies were carried out on patients.* In the first, 10 patients ages 35 to 65 were selected at random from those attending the hypertension clinic. Criteria for selection were that hypertension was not complicated by renal, cardiac or central nervous system involvement and that each patient had been on 0.5 Gm. chlorothiazide p.o. twice a day plus oral potassium chloride (1.0 Gm. t.i.d.) for no less than 6 months. Two patients had previously diagnosed diabetes mellitus controlled by diet alone. Each patient was placed on a 3 day preparatory diet containing 300 Gm. carbohydrate, 90 Gm. protein and 2600 calories a day. On the fourth day a 3-hour glucose tolerance test was performed with the patient in the fasting state. The glucose load administered orally after obtaining a fasting blood sugar was 1.75 Gm./Kg. ideal body weight. Following the initial glucose tolerance test all patients were taken off of medication for one week. Each patient then followed the same 3-day preparatory diet prior to the second glucose tolerance test which was done on the seventh day off drug and in a fashion identical to the first. All blood sugar determinations were done on capillary blood by the Somogyi-Nelson method.[11]

No patient showed significant elevation of fasting blood sugar in association with chlorothiazide therapy (table 1). One hour after carbohydrate ingestion the mean blood glucose of patients taking chlorothiazide was 167 mg./100 ml. (79 mg./100 ml. above the fasting value), while the mean blood glucose when patients were not taking thiazide

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From Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan.

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Table 1.—*Blood Glucose Values during Oral Glucose Tolerance Tests on Patients*

Patient	On Chlorothiazide (Time in hours)						Off Chlorothiazide (Time in hours)					
	0	½	1	1½	2	3	0	½	1	1½	2	3
T. A.	95	123	134	114	106	115	85	108	117	113	109	99
A. S.	89	174	168	156	106	101	95	117	120	130	118	95
W. B.	82	131	137	122	113	92	86	144	112	108	106	99
M. S.*	90	172	234	264	243	200	93	240	226	194	187	178
J. S.	82	160	160	102	95	100	94	114	140	102	99	96
M. M.	85	129	101	101	111	105	83	98	100	104	103	100
O. B.	90	120	200	202	161	159	90	140	135	132	104	100
M. A.	94	218	208	180	151	102	97	180	208	198	124	99
L. B.	85	135	132	86	98	97	100	129	115	109	113	92
H. P.*	90	148	200	224	200	90	83	124	158	150	147	126
Mean	88	151	167	155	138	116	91	139	143	134	121	108
±S.D.	4.3	29	40	57	47	34	5.8	40	14	34	26	25

*Patients with previously diagnosed diabetes mellitus controlled by diet alone.
All glucose values expressed as mg./100 ml. blood.

was 143 mg./100 ml. (52 mg./100 ml. above fasting). The difference between these means is significant ($p < 0.02$). If the 2 known diabetic patients (M. S. and H. P.) are eliminated, the difference between mean blood glucose levels at this 1 hour time is still significant ($p < 0.05$).

In the second study, 10 individuals ages 25 to 40, both normotensive and hypertensive, who were not on benzothiadiazine drugs were selected. After a 3-day glucose tolerance preparatory diet, an intravenous glucose tolerance test was carried out. The glucose load was 1.5 Gm./Kg. body weight given over 3 to 5 minutes. Venous blood was used for blood sugar determinations. Chlorothiazide was then given in a dose of 0.5 Gm. p.o. twice daily. On the seventh day, again after the preparatory diet, the intravenous glucose tolerance test was repeated. Diet and analyses of blood sugars were identical to those of the first study. At all times, both when fasting and after the administration of the glucose load, mean values of blood sugars were higher when subjects were on chlorothiazide than when not taking drug. Differences between means, however, were not significant.

Glucose Tolerance Tests on Rats

Two studies were done on rats. In the first, 3 groups of rats, each containing 12 to 15 animals, had oral glucose tolerance tests carried out by giving 1.75 Gm. glucose/Kg. body weight through a stomach tube. Blood was obtained by clipping the tail. During the following week the first group of rats ate Purine Chow, the second ate similar food with placebo tablets incorporated, and the third ate similar food with 100 mg. chlorothiazide/Kg. rat/day incorporated. At the end of a week, oral glucose tolerance tests were repeated. Results are shown in table 2. Mean blood glucose values for normal and placebo groups were significantly different at fasting ($p < 0.01$) and 2-hour times ($p < 0.05$). Mean blood glucose values of chlorothiazide rats differed significantly from both placebo and control groups ($p < 0.01$ at all times except 2 hours when $p < 0.05$).*

In the second study, 3 groups of rats, each containing 9 to 11 animals, had oral glucose tolerance tests carried out as previously. They were then given in drinking water for 2 weeks/Kg. rat/day, respectively, 100 mg. chlorothiazide, or 100 mg. diazoxide, or 50 mg. chlorothiazide plus 50 mg. diazoxide. At the end of this time glucose tolerance tests were repeated. Although mean blood glucose values of animals given drugs were consistently higher than before drug administration, the differences did not reach statisti-

*We are indebted to M. A. Schork, Ph.D., for this contrast-multiple comparison analysis.

Table 2.—*Blood Glucose Values during Oral Glucose Tolerance Tests on Rats*

Group	Time in Hours				
	0	½	1	1½	2
Normal (12)					
Mean	72	119	111	99	90
±S.D.	5.7	15	12	10	11
Placebo (12)					
Mean	80	121	113	103	100
±S.D.	3.4	11	7.1	11	5.2
Chlorothiazide (15)					
Mean	87	142	127	112	102
±S.D.	7.6	9.3	9.8	6.0	10

Number of animals in parenthesis. All glucose values expressed as mg./100 ml. blood.

cal significance. Rats receiving the combination of chlorothiazide and diazoxide did not show as consistent an elevation of mean blood glucose values.

Glucose Metabolism of Adipose Tissue

Five studies were done on nonfasting male Sprague-Dawley rats weighing 250 to 350 Gm. In any one study rats' weights were approximately the same. In each study one group of rats was given chlorothiazide (Lyodiuril) 100 mg./Kg. body weight daily in drinking water for 14 to 18 days while another group drank tap water. Rats were decapitated and epididymal fat pads removed, placed in a buffer solution at 37 C., cut into pieces which were placed in tared flasks containing 3 ml. modified Krebs' bicarbonate buffer solution with 200 mg. glucose/100 ml. at 4 C., previously equilibrated with 5 per cent CO_2–95 per cent O_2 at room temperature for 5 minutes. In the first 2 studies the peripheral 5 mm. of each fat pad were cut into 3 pieces, in the third it was cut into 4 pieces, and in the last 2 studies into 8 pieces. Four pieces of fat pad, each from a different rat, but from the chlorothiazide group or the control group, and totalling approximately the same weight, were in each flask. Some media also contained chlorothiazide (0.25 mg./ml.) and/or crystalline insulin (0.25 units/ml.).* Flasks were then incubated at 37.5 C. for 2 hours with gentle shaking. Flasks plus tissues were weighed after incubation. Media glucose determinations before and after incubation were done by the Nelson-Somogyi method.[11]

The rate of decrease of glucose in the incubating medium brought about by the epididymal fat pads of rats given chlorothiazide and of control rats is given in table 3. Also shown are the effects of the presence or absence of chlorothiazide and insulin in the medium. In the groups of control rats, adding chlorothiazide to the medium reduced the rate of glucose disappearance due to these normal rats' fat pads in 2 of 3 experiments ($p < 0.01 > 0.001$). Similarly adding chlorothiazide to medium containing insulin reduced the rate of glucose utilization of normal rats' fat pads in 2 of 3 experiments ($p < 0.01 > 0.001$ and $< 0.02 > 0.01$). It should be noted that adding insulin to the medium increased the glucose disappearance rate caused by normal rats' fat pads in 7 of 8 experiments, i.e., both in the presence of and the absence of added chlorothiazide in the medium ($p < 0.001$ in 4, $< 0.05 > 0.02$ in 2, and $< 0.01 > 0.001$).

In the groups of rats receiving chlorothiazide orally for 2 weeks prior to sacrifice, the following effects were noted: the glucose disappearance rate brought about by chlorothiazide rats' fat pads was reduced, as compared to that of normal rats, in all 3 experiments in which insulin, as well as chlorothiazide, was absent from the medium ($p < 0.001$,

*Kindly supplied by Eli Lilly and Co.

Table 3.—*Glucose Disappearance Rates from Media Containing Epididymal Fat Pads of Rats Given Chlorothiazide and of Control Rats*

Exp. No.	Chlorothiazide Rats (Composition of medium)				Control Rats (Composition of medium)			
	With Insulin With Thiazide	No Insulin With Thiazide	With Insulin No Thiazide	No Insulin No Thiazide	With Insulin With Thiazide	No Insulin With Thiazide	With Insulin No Thiazide	No Insulin No Thiazide
1	1.06 ±0.015 (9)	0.88 ±0.22 (9)					2.52 ±0.43 (6)	1.52 ±0.23 (6)
2	2.11 ±0.51 (6)	1.30 ±0.29 (6)					3.04 ±1.19 (6)	2.22 ±0.52 (6)
3	1.68 ±0.34 (10)	1.36 ±0.24 (10)	1.79 ±0.49 (10)	1.32 ±0.25 (10)	3.75 ±1.38 (10)	2.54 ±0.87 (10)	4.40 ±0.94 (10)	2.46 ±0.71 (10)
4	1.25 ±0.33 (20)	0.70 ±0.34 (20)	2.31 ±0.89 (20)	1.49 ±0.80 (20)	1.96 ±0.65 (20)	1.07 ±0.37 (20)	2.91 ±1.01 (20)	2.10 ±1.17 (20)
5	1.64 ±0.55 (18)	0.93 ±0.45 (18)	2.32 ±0.71 (18)	1.65 ±0.59 (18)	2.53 ±0.87 (18)	1.51 ±0.81 (18)	3.40 ±1.22 (18)	2.81 ±1.35 (18)

Values expressed as the mean ± S.D. of the rate of decrease of glucose (mg./Gm. wet tissue/2 hrs.) from the medium. The number of separate determinations, i.e., incubating flasks, is shown in parenthesis.

$< 0.01 > 0.001$ and $< 0.05 > 0.02$) and in 2 of the 3 in which insulin was added to the medium ($p < 0.001$ and $< 0.01 > 0.001$). Adding only chlorothiazide to the medium resulted in further reduction of the rate of glucose utilization of these chlorothiazide rats' fat pads in 2 of 3 experiments. ($p < 0.001$), while adding chlorothiazide to medium containing insulin also further reduced the rate of decrease of glucose by chlorothiazide rats' fat pads in 2 of 3 experiments ($p < 0.01 > 0.001$ and < 0.001). Adding insulin to the medium increased the glucose utilization rate of chlorothiazide rats' fat pads in 7 of 8 experiments, i.e., both in the presence of and the absence of added chlorothiazide in the medium ($p < 0.001$ in 3, $< 0.01 > 0.001$ in 3, and $< 0.05 > 0.02$).

These alterations in glucose metabolism resulting from chlorothiazide administration to the rats and from addition of chlorothiazide to the incubating media are summarized for experiments 3, 4 and 5 in figure 1. The large standard deviations of the means are due to variability between experiments. The variability is much smaller in any one experiment (see table 3).

Discussion

The glucose tolerance tests done on patients show that long-term chlorothiazide therapy (6 or more months) results in a decreased rate of glucose utilization following carbohydrate loading. Indeed, a single dose of a benzothiadiazine compound appears to be sufficient to cause an immediate relative hyperglycemia.[3] In view of the reported reversal of the thiazide effect by potassium supplementation,[12] it is of interest that the patients on long-term chlorothiazide therapy who received potassium supplements showed a greater effect of the thiazide on carbohydrate metabolism than did the

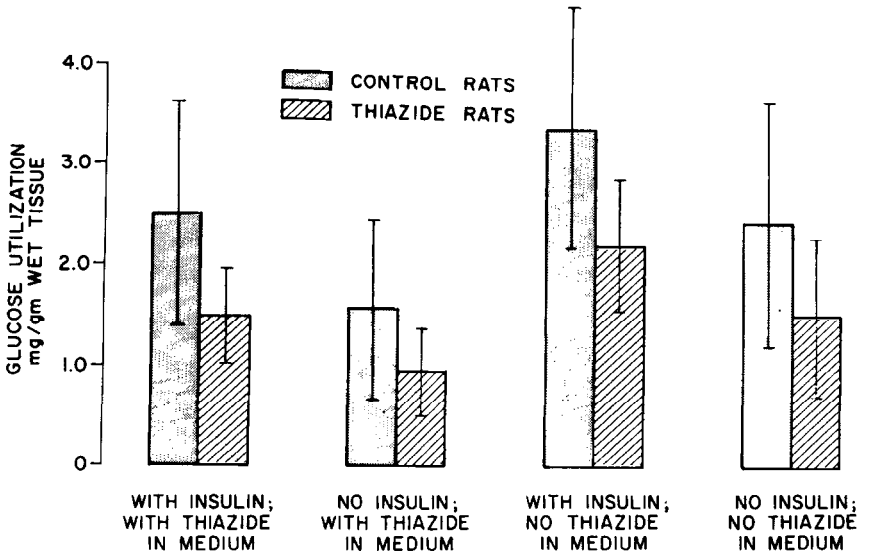

Fig. 1.—The mean rates of glucose disappearance, each of 48 determinations (experiments 3, 4, and 5) expressed as mg. glucose/Gm. wet weight of epididymal fat pad/2 hours, of control rats (grey columns) and of rats given chlorothiazide (diagonally-lined columns), when incubated with or without insulin and/or chlorothiazide in the medium (as shown below the columns). Standard deviations of the means are indicated by lines in the centers of columns.

patients on short-term therapy who did not take added potassium. The fact that the hyperglycemic action of benzothiadiazine drugs can be demonstrated as soon as 2 hours after a single dose of thiazide[3] indicates that significant potassium loss is not a requirement for its effect on carbohydrate metabolism.

The glucose tolerance tests on rats suggest that the hyperglycemic action of chlorothiazide can be demonstrated even in the normal animal, i.e., in which a prediabetic state presumably is not present. Other investigators have also found that chlorothiazide has a hyperglycemic effect in the rat,[13] however, in one recent study this action was not evident despite large doses of chlorothiazide and even the addition of a potassium deficient diet.[14] The administration of a carbohydrate load would seem to be useful in making the hyperglycemic action of thiazides more apparent. Other thiazides, and especially diazoxide, also have been reported to cause hyperglycemia in the rat.[15,16] In the present study diazoxide appeared to have an action quite similar to that of chlorothiazide. No synergism was evident when these 2 drugs were given together.

The experiments with rat epididymal fat pad show both in vivo and in vitro effects of chlorothiazide. When the drug is given to the rat, the animal's incubated fat pad demonstrates a reduced rate of glucose disappearance from the medium. This occurs both when insulin is and is not added to the medium, although adding insulin increases the disappearance rate of glucose when the fat pad from the chlorothiazide rat is present as it does

when that from the normal rat is incubated. Adding chlorothiazide to the medium further reduces the rate of utilization of glucose brought about by the epididymal fat pads of rats given chlorothiazide. This same effect on glucose metabolism of adding chlorothiazide to the medium is seen with normal rats' fat pads. With both normal and chlorothiazide-treated rats' fat pads, however, insulin is able to increase the rate of glucose disappearance even when chlorothiazide is in the incubating medium.

The hyperglycemia due to thiazides might result from: (1) interference with glucose transport into the cell, (2) decrease in the rate of glucose phosphorylation, (3) decrease in the rate of formation of glycogen, (4) decrease in the rate of lipogenesis, (5) increase in the rate of glycogen breakdown, or (6) increase in the rate of formation of glucose from protein. The evidence suggests that more than one of these mechanisms is playing a role. It has been reported that the benzothiadiazine drugs decrease serum insulin-like activity.[17,18] Such a decrease would lead to a diminished rate of transport of glucose into those cells where this requires insulin. That interference with the glucose transport system is not the entire cause of thiazide hyperglycemia is shown by studies on rat liver homogenates in which glucokinase, which is involved in glucose utilization, and dihydroxyacetone-kinase enzyme activities are reduced by the administration of chlorothiazide to rats.[19] These enzymes are insulin-dependent, so that a lowering of serum insulin levels would decrease their activities. That either a diminution in the rate of formation of glycogen or an increase in glycogen breakdown results from thiazide administration is supported by the finding that diazoxide decreases liver glycogen content.[15] It is of interest that tolbutamide administration abolishes the hyperglycemic effect of thiazides.[13,16] This might be due to tolbutamide increasing serum insulin-like activity sufficiently to cause an increase in activity of insulin-dependent enzymes. If in thiazide-treated patients the decrease in serum insulin-like activity is due to the thiazide interfering with the insulin-release mechanism, tolbutamide must overcome this interference. An additional action of thiazide drugs is necessitated by the observation that chlorothiazide added directly to the incubating medium decreases glucose utilization of epididymal fat pad. This points to a direct tissue effect; its exact site of action is unknown.

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John M. Weller, A.B., M.D., Professor of Internal Medicine, University of Michigan Medical School, Ann Arbor, Mich.

Paul E. Borondy, B.S., Research Assistant, University of Michigan Medical School, Ann Arbor, Mich.