Inhibitory Effect of Chlorothiazide on Glucose Utilization by Aorta[1] (34367)

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The antihypertensive action of the natriuretic thiazide drugs has been known for more than a decade. During the immediate period after initiation of therapy with chlorothiazide, the decreases which occur in plasma and extracellular fluid volumes are undoubtedly important factors in lowering the blood pressure (1). With continued administration of the drug, however, these volumes return toward normal (2). At this later stage of treatment the antihypertensive effect is related to a decrease in peripheral vascular resistance; the mechanism responsible for bringing this about is unknown.

A close congener of chlorothiazide, diazoxide, although it is a renal sodium retainer, is a potent antihypertensive agent, having a direct action resulting in peripheral vasodilation (3). Both chlorothiazide and diazoxide alter glucose utilization in man and experimental animals. The administration of chlorothiazide to man or to rats results in relative hyperglycemia after a glucose load (4). This hyperglycemic effect of the benzothiadiazine drugs was noted soon after their introduction (5). One means by which these agents bring about this aberration of glucose metabolism is by reducing the level of serum insulin-like activity (6). In addition, however, chlorothiazide, or the combination of chlorothiazide and diazoxide, also has a direct action on peripheral tissue, *i.e.*, it causes a decrease in the *in vitro* rate of utilization of glucose by normal rats' adipose tissue when added to the incubating medium (7, 8). The effect of chlorothiazide on glucose utilization by arterial smooth muscle cells is not known. It is possible that the action which chlorothiazide and diazoxide both exert on carbohydrate metabolism may be related to their shared antihypertensive effect. The following studies were carried out to determine the effect of chlorothiazide on the *in vitro* rate of glucose utilization by normal dog aorta when the drug is added directly to the incubating medium.

Methods. Mongrel dogs anesthetized with 20–30 mg/kg body weight of sodium pentobarbital intravenously were exsanguinated, the abdominal cavity opened, and thoracic and abdominal aorta removed and placed in ice-cold normal saline solution. The aorta was cleaned of fat and adventitia, blotted, and a total of about 300 mg of pieces of tissue that ranged from 0.5 to 1.0 mm in thickness was placed in chilled media in each of several stoppered tared flasks. Total weight of tissue in each flask initially was approximated by use of a torsion balance. Each flask contained 5.0 ml of ice-cold Krebs' bicarbonate buffer solution, previously equilibrated with 5% CO_2–95% O_2, to which 200 mg glucose per 100 ml had been added. Two series of experiments were done. In the first, the glucose utilization rate of aorta was determined with 1.0 m*M* chlorothiazide per liter of medium and compared with no drug in the medium. In the second series, the rate of utilization of glucose was determined with 0.01 and 1.0 m*M* chlorothiazide per liter of medium and compared with no drug. Flasks were gassed with 5% CO_2–95% O_2 and incubated at 37° for 2 hr with gentle shaking. Accurate weight of each flask plus tissue was obtained after incubation. Media glucose determinations before and after incubation were done by the glucose oxidase method.[2]

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[2] Glucostat, Worthington Biochemical Corporation.

TABLE I. Glucose Disappearance Rates with and without Chlorothiazide in the Medium.[a]

Expt. no.	Chlorothiazide 1.0 m*M*/liter (mean ± SD)	No chlorothiazide (mean ± SD)	*p*
1	4.53 ± 0.35 (8)	5.38 ± 0.57 (7)	<0.01>0.001
2	4.45 ± 0.24 (9)	5.18 ± 0.56 (8)	<0.01>0.001
3	1.75 ± 0.27 (8)	2.05 ± 0.20	<0.05>0.02
4	1.37 ± 0.17 (8)	2.36 ± 0.51	<0.001

[a] Mean values express the rate of decrease of glucose from the medium as mg glucose/g wet tissue /2 hr. Numbers in parentheses denote the number of separate incubating flasks.

Results. In the first series, the glucose disappearance rate of dog aorta as determined in four experiments is shown in Table I. In each experiment the normal rate of glucose utilization without drug present in the medium was compared with the rate found when 1.0 m*M* chlorothiazide per liter medium was present. It is important that each experiment contain such a normal control, as the variability between experiments is large, whereas the variability is much smaller in any one experiment. Chlorothiazide caused a significant decrease in the rate of utilization of glucose in all four experiments. In the second series, in each of three experiments, the glucose disappearance rate of dog aorta was determined when the chlorothiazide concentrations in the medium were 0.01 and 1.0 m*M* and compared with the rate found in the absence of chlorothiazide. The results of these experiments are shown in Table II. Increasing the concentration of chlorothiazide in the medium resulted in a decrease in the rate of glucose utilization by the aorta. The mean glucose disappearance rate when 0.01 m*M* chlorothiazide was present in the medium was 70% of the normal rate, while the mean rate when 1.0 m*M* chlorothiazide was present was 51% of the normal rate.

Discussion. The diabetogenic effect of the thiazide drugs has been well established, but its mechanism remains unclear. One reason for this hyperglycemic action of the thiazides is that they reduce the level of circulating plasma insulin-like activity (6). This in turn reduces the activities of insulin-dependent enzymes such as liver glucokinase and dihydroxyacetone-kinase (9). That there is also a direct inhibitory effect of thiazides on carbohydrate metabolism of tissues is shown by the fact that the rate of utilization of glucose *in vitro* by normal rat fat is reduced when chlorothiazide is added to the incubating medium (7). Furosemide, an anthranilic acid derivative of benzothiadiazine, exerts a similar direct action (10).

These studies demonstrate that chlorothiazide has an *in vitro* effect on the carbohydrate metabolism of aorta which is similar to its action on fat tissues. Both rat adipose tissue and dog aorta have their *in vitro* rates of utilization of glucose reduced when chlorothiazide is added to the incubating medium. Both tissues demonstrate an increasing degree of inhibition of the rate of glucose utilization with increasing concentrations of chlorothiazide in the medium.

These alterations in carbohydrate metabolism which are brought about by the thiazide drugs might interfere with energy transfer in

TABLE II. Glucose Disappearance Rates with Varying Amounts of Chlorothiazide in the Medium.[a]

Expt. no.	Chlorothiazide 1.0 m*M*/liter (mean ± SD)	*p*	Chlorothiazide 0.01 m*M*/liter (mean ± SD)	*p*	No chlorothiazide (mean ± SD)
5	2.66 ± 0.43 (7)	<0.01>0.001	3.33 ± 0.24 (6)	<0.001	5.78 ± 0.26 (6)
6	2.45 ± 0.61 (7)	<0.001	3.75 ± 0.38 (8)	<0.01>0.001	4.46 ± 0.28 (7)
7	2.31 ± 0.55 (8)	<0.001	3.10 ± 0.14 (9)	<0.001	4.28 ± 0.40 (8)

[a] Mean values express the rate of decrease of glucose from the medium as mg glucose/g wet tissue/2 hr. Numbers in parentheses denote the number of separate incubating flasks.

the smooth muscle cells of the arterial wall. Diazoxide reduces the activity of 3′,5′-AMP-phosphodiesterase in heart and skeletal muscle (11). Although the arteriole may not respond in a fashion similar to aorta, it is possible that interference with energy transfer in arterial smooth muscle cells by thiazides might lead to a diminution in arteriolar tone, and thereby bring about a decrease in peripheral resistance.

Summary. The rate of utilization of glucose *in vitro* by normal dog aorta has been determined with various concentrations of chlorothiazide in the incubating Krebs' bicarbonate solution. Pieces of dog aorta totaling approximately 300 mg were incubated for 2 hr at 37° in 5.0 ml medium containing 200 mg glucose per 100 ml. Chlorothiazide added to the incubating solution caused a reduction in the rate of utilization of glucose by the aorta. As the chlorothiazide concentration in the incubating medium was increased there was a decrease in the glucose disappearance rate from the medium. At a chlorothiazide concentration of 1.0 m*M*, the mean rate of decrease of glucose from the medium was approximately one-half of the rate found when chlorothiazide was not present. This direct inhibitory effect of chlorothiazide on the utilization of glucose by dog aorta was evident even when the concentration of this drug was as low as 0.01 m*M*.

1. Wilson, I. M. and Freis, E. D., Circulation **20**, 1028 (1959).

2. Lauwers, P. and Conway, J., J. Lab. Clin. Med. **56**, 401 (1960).

3. Finnerty, F. A., Jr., Kakaviatos, N., Tuckman, J., and Magill, J., Circulation **28**, 203 (1963).

4. Weller, J. M. and Borondy, P. E., Metabolism **14**, 708 (1965).

5. Finnerty, F. A., Jr., *in* "Hypertension" (J. H. Moyer, ed.), p. 653. Saunders, Philadelphia (1959).

6. Samaan, N., Dollery, C. T., and Fraser, R., Lancet **2**, 1244 (1963).

7. Weller, J. M. and Borondy, M., Proc. Soc. Exptl. Biol. Med. **124**, 220 (1967).

8. Barnett, C. A. and Whitney, J. E., Metabolism **15**, 88 (1966).

9. Borondy, P. E. and Weller, J. M., Proc. Soc. Exptl. Biol. Med. **118**, 938 (1965).

10. Weller, J. M. and Borondy, M., Metabolism **16**, 532 (1967).

11. Schultz, G., Senft, G., Lesert, W., and Sitt, R., Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmakol. **253**, 372 (1966).

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