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Effects of Chlorothiazide on Glucose Utilization, Glycogen Content, and Lactic Acid Production of Aorta[1] (39573)

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Benzothiadiazine drugs are widely utilized, both as diuretic agents and for the treatment of hypertension, but the cellular mechanisms responsible for these actions are not known. Thiazides also alter carbohydrate metabolism in both patients and experimental animals (1, 2). *In vitro* effects of chlorothiazide on glucose utilization in fat and liver have been demonstrated (3, 4). Similarly, the rate of utilization of glucose by dog aorta is reduced when chlorothiazide is added to the incubating solution (5). When the chlorothiazide concentration in the medium is increased, there is a decrease in the glucose disappearance rate from the medium (5). The following study delineates in greater detail additional changes in carbohydrate metabolism of dog aorta which are brought about by chlorothiazide, with special attention to glycogen and lactic acid.

Materials and methods. Mongrel dogs of both sexes weighing 18 to 27 kg were anesthetized with 20 to 30 mg of sodium pentobarbitol/kg body weight iv and exsanguinated, and the abdominal aorta was removed and placed in ice-cold isotonic saline solution. The aorta was cleaned of fat and adventitia, blotted, and 250- to 300-mg portions of tissue were placed in tared flasks previously gassed with 5% CO_2–95% O_2 mixture. Each flask contained 5 ml of Krebs' bicarbonate buffer solution (6) and was kept briefly in ice until ready for incubation at 37° for 2 hr.

In experiment I, the incubating medium also contained 11.1 m*M* glucose; in experiment II, there was no glucose in the medium, and in experiment III, the medium contained 1.2 m*M* lactic acid. In all experiments, one-half of the flasks contained 1.0 m*M* chlorothiazide in the medium. Glucose determinations on medium before and after incubation were done by the glucose oxidase method (reagents from Worthington Biochemical Corp.). Lactic acid contents were done on the medium at the beginning and end of incubation by ultraviolet spectroscopy (Sigma Technical Bulletin No. 826). Glycogen content of tissue at the end of incubation was determined by its hydrolysis to glucose (7) which was then measured by the glucose oxidase method. Glycogen was calculated from glucose by the Morris conversion factor of 1.11 (8). Statistical significance was evaluated by Student's *t* test.

Results. In experiment I in which the medium contained glucose in eight studies, each composed of 5 to 10 control and chlorothiazide-containing flasks, mean glucose utilization of aorta was significantly reduced by the presence of 1 m*M* chlorothiazide in the medium (Table I). Mean net lactic acid production by aorta also was reduced significantly by the presence of 1 m*M* chlorothiazide in 11 studies, each composed of five to nine flasks (Table I). In five studies in which glycogen content of aorta was determined, the presence of 1 m*M* chlorothiazide in the medium resulted in a significantly lower mean glycogen content of the tissue at the end of incubation (Table I).

In experiment II in eight studies, each composed of four to eight control and chlorothiazide-containing flasks, mean net lactic acid production by aorta (in this system not containing glucose as substrate) was not reduced by the presence of 1 m*M* chlorothiazide (Table I). It should be noted that even in the absence of chlorothiazide the lack of glucose in the medium caused a considerable reduction in the net lactic acid production by aorta compared to that of aorta in medium containing 11.1 m*M* glucose ($P < 0.001$). In four studies in which glycogen content of aorta was determined at the end

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TABLE I. NET GLUCOSE UTILIZATION, LACTIC ACID PRODUCTION, AND GLYCOGEN CONTENT OF AORTA WITHOUT AND WITH CHLOROTHIAZIDE IN THE MEDIUM.[a]

Experiment	Without chlorothiazide			With chlorothiazide[b]		
	Glucose (μmole/g aorta/2 hr)	Lactic acid (μmole/g aorta/2 hr)	Glycogen (μg/g aorta)	Glucose (μmole/g aorta/2 hr)	Lactic acid (μmole/g aorta/2 hr)	Glycogen (μg/g aorta)
I. Glucose in medium	30.0 ± 0.81 (54)	46.6 ± 0.84 (67)	878 ± 64 (30)	23.3* ± 0.84 (52)	42.7** ± 0.83 (71)	603** ± 55 (32)
II. No glucose in medium		24.7 ± 0.54 (46)	150 ± 6.1 (24)		25.5 ± 0.63 (45)	156 ± 10 (26)
III. Lactic acid in medium		26.7 ± 0.63 (39)	177 ± 16 (27)		24.2** ± 0.66 (37)	184 ± 21 (27)

[a] Values expressed as Mean ± SE. Number of flasks is in parentheses.
[b] Significantly different from medium without chlorothiazide: * $P < 0.001$; ** $P < 0.01$.

of incubation in medium not containing glucose, the presence of chlorothiazide resulted in no consistent change (Table I). As was the case with lactic acid production, in medium not containing chlorothiazide, the absence of glucose resulted in the glycogen content of the aorta at the end of incubation being greatly reduced ($P < 0.001$), as compared to that found with aorta incubated in glucose-containing medium.

In experiment III in which lactic acid was added to the medium in six studies, each composed of five to nine control and chlorothiazide-containing flasks, mean net lactic acid production by aorta was significantly lower in the presence of chlorothiazide (Table I). Note that all of these values represent an increase in lactic acid content of the medium at the end of incubation. In five studies in which glycogen content of aorta was determined at the end of incubation in medium containing 1.2 m*M* lactic acid, the presence of chlorothiazide resulted in no significant difference (Table I).

Discussion. Chlorothiazide interferes with the utilization of glucose by aorta as evidenced by the decreased rate of disappearance of glucose from the medium. When chlorothiazide is present in medium containing glucose, at the end of incubation there is both a decreased concentration of lactic acid in the medium and less glycogen present in the tissue. However, when no glucose is in present in the medium, so that final glycogen content of aorta is reduced and lactic acid production is decreased, then the presence of chlorothiazide does not significantly alter either tissue glycogen content or net lactic acid production of aorta. The major action of chlorothiazide appears to be interference with the rate of utilization of glucose by the cells. This could be due to a slowing of the entry of glucose into the cells or a partial block of one or more metabolic steps within the cell.

It is known that the administration of thiazides to patients causes a reduction in circulating insulin-like activity (9). The *in vitro* experiments presented here, along with previous studies of fat tissue (3), indicate that in addition to having an effect on insulin-like activity chlorothiazide also acts at the cellular level independently of the insulin effect to decrease the rate of utilization of glucose. Furosemide has been shown to have similar *in vivo* and *in vitro* effects on glucose metabolism (10). Recently Jung and Mookerjee (11) have demonstrated that furosemide, and to a lesser degree chlorothiazide, interferes with the transport of glucose into red blood cells. This may well be the case with aorta and could explain the findings in this study. However, interference with enzymatic activities in the metabolic pathway of glucose can also play a role.

In cell-free systems, furosemide inhibits lactate formation from fructose-1,6-diphosphate and glucose-6-phosphate (12). This inhibitory action of furosemide appears to involve glyceraldehyde-3-phosphate dehydrogenase (12, 13). It does not seem likely, however, that the alterations in carbohy-

drate metabolism of aorta brought about by chlorothiazide are due primarily to inhibition of an enzymatic process. A better possibility would appear to be that chlorothiazide has a major effect on the entry of glucose into the cells.

Summary. Chlorothiazide in the incubating medium significantly reduces net glucose utilization, glycogen content, and net lactate production of normal dog aorta. When no glucose is present in the incubating medium, these actions of chlorothiazide are not evident. When 1.2 m*M* lactic acid is present in place of glucose as substrate, net lactic acid production of aorta is decreased by chlorothiazide and glycogen content is unaltered.

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