Polyol Pathway in Aorta: Regulation by Hormones

Abstract. *Aldose reductase is present in human and rabbit aortas and provides a mechanism whereby hyperglycemia can alter the metabolism of the arterial wall. Aortic sorbitol concentration is regulated by ambient glucose concentration and is increased by epinephrine, isoproterenol, dibutyryl-3',5'-adenosine monophosphate, ouabain, and angiotensin II.*

Aldose reductase (Alditol NADP oxidoreductase, E.C. 1.1.1.21) catalyzes the reduction of a number of aldose sugars to their respective polyol derivatives. In mammalian tissues this reaction is essentially irreversible (*1*). Aldose reductase has been thought to have a restricted distribution and, aside from its role in the synthesis of fructose from glucose in the seminal vesicles, its physiological function and usual substrates are unknown (*1*). Aldose reductase has high Michaelis constants (K_m) for glucose and galactose which suggests that these hexoses may only be fortuitous substrates. However, their polyol derivatives (sorbitol and dulcitol) accumulate within the lens in diabetes and galactosemia and are thought to be responsible for the development of cataracts (*2*). The intracellular accumulation of polyols was shown by Kinoshita and his co-workers to be accompanied by an increased water content of the lens, a decreased rate of active transport of amino acids, and a decreased ability to maintain normal intracellular sodium and potassium concentrations (*2*). These effects were attributed to the osmotic consequences of polyol accumulation since these compounds cross cell membranes very slowly; however, a secondary defect in myoinositol metabolism may contribute to their production (*2, 3*). The presence of aldose reductase in a tissue in which the intracellular transport of glucose is not rate-limiting thus provides the basis of a pathological mechanism; elevated concentrations of glucose in the plasma could increase intracellular concentration of glucose, resulting in increased sorbitol formation with consequences similar to those in the lens (*2*).

We have isolated and partially purified aldose reductase from human and rabbit thoracic aorta, tissues in which glucose transport is not rate-limiting (*4*), and have demonstrated that the sorbitol content of the aortic wall is determined, in part, by the ambient glucose concentration. These observations provide a mechanism by which hyperglycemia might lead to a derangement in the metabolism of the arterial wall and contribute directly to the development of the vascular lesions associated with diabetes mellitus. We have also observed that the sorbitol content of the aortic wall is subject to hormonal regulation. This finding suggests that the polyol sequence may play an important and previously unsuspected role in the metabolism of mammalian cells.

The sorbitol content of whole thoracic aorta from male New Zealand rabbits (1.5 kg) was determined by fluorometric enzymatic assay with sorbitol dehydrogenase (L-iditol: NAD oxidoreductase E.C. 1.1.1.14) by use of neutralized perchloric acid filtrates of tissue that had been quickly flushed free of blood and frozen in liquid nitrogen. The assay was carried out in 1.0 ml of glycine-NaOH buffer (0.05*M*), *p*H 9.6, containing nicotinamide adenine dinucleotide (NAD) (0.1 m*M*) and 0.68 unit of sorbitol dehydrogenase from sheep liver (Boehringer) previously dialyzed to remove glycerol. The sorbitol content of normal rabbit thoracic aorta is 13.7 ± 0.8 nmole/g (wet weight) ($N = 6$), while the plasma sorbitol concentration is consistently less than 3.0 nmole/ml ($N = 12$). This suggests that sorbitol is either synthesized or concentrated in the aortic wall. Tubular segments of rabbit thoracic aorta comprised of only intima and media provide a suitable system for studies in vitro (*4*). When aortic tissue is incubated with increasing concentrations of glucose, there is a progressive increase in the tissue sorbitol content (Fig. 1). When paired samples of aortic tissue were incubated for 2 hours with 5 m*M* and 50 m*M* glucose, the mean increase in sorbitol

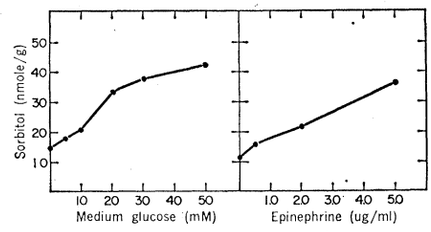

Fig. 1. Pooled samples (from six rabbits) of aortic tissue incubated in Krebs-bicarbonate buffer, *p*H 7.4; the gas phase was 5 percent CO_2 in air. (Left) Samples incubated for 2 hours; (right) glucose medium concentration, 5 m*M*; samples were first incubated for 30 minutes, then transferred to fresh medium containing glucose (5 m*M*) and epinephrine, and incubated for 30 minutes.

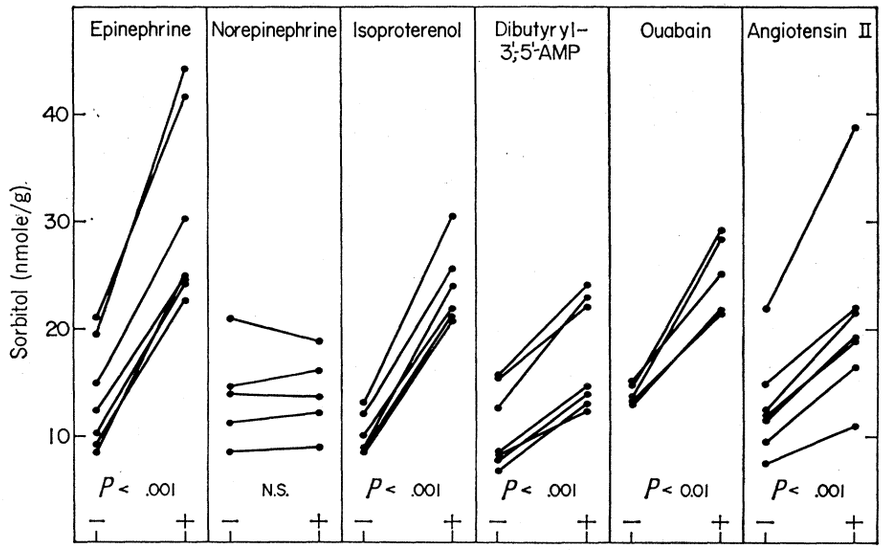

Fig. 2. Paired samples of rabbit thoracic aorta were first incubated in Krebs-bicarbonate buffer, *p*H 7.4, for 30 minutes; the gas phase was 5 percent CO_2 in air; the glucose concentration was 5 m*M*; samples were transferred to fresh medium and one of each pair was incubated as a control (—) and the other with the agent indicated (+) for 30 minutes, except in experiments with ouabain (1 hour) and angiotensin II (2 hours). The concentration of epinephrine was 2 μg/ml; of norepinephine, 2 μg/ml; of DL-isoproterenol, 4 μg/ml; of dibutyryl-3',5'-AMP, $10^{-4}M$; of ouabain, $10^{-5}M$; and of angiotensin II, 1 μg/ml.

content at the higher concentration was 14.1 ± 2.5 nmole/g ($N = 6$, $P < .01$). The ambient glucose concentration thus appears to regulate the sorbitol content of the aortic wall.

Aldose reductase was isolated from rabbit thoracic aorta and from fresh autopsy specimens of human thoracic aorta. Frozen tissue (200 g) from 400 rabbits or pooled human specimens was minced and homogenized in a Virtis tissue homogenizer at 2°C in phosphate buffer (5 mM), pH 6.8, containing 2-mercaptoethanol (1 mM). The homogenates were centrifuged at 20,000g for 30 minutes at 2°C. The protein content of the supernatant was determined (*5*), and twice the dry weight of aged calcium phosphate gel was added. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. The protein precipitating in the 40 to 80 percent saturated ammonium sulfate fraction was dissolved in a small volume of phosphate buffer (5 mM), pH 6.8, and passed through a column of Sephadex G-50 that had been equilibrated with the same buffer. The protein was then applied to a column (2.5 by 25 cm) of diethylaminoethylcellulose (DEAE) equilibrated with phosphate buffer (5 mM), pH 6.8. The column was washed with 200 ml of the same buffer and developed with 750 ml of a linear gradient of phosphate buffer, pH 6.8, which increased from 5 mM to 25 mM.

Two peaks of nicotinamide adenine dinucleotide phosphate (NADP) polyol dehydrogenase activity were recovered. The first peak, which had the characteristics of aldose reductase, eluted between 16 and 19 mM of phosphate. When assayed in phosphate buffer (67 mM), pH 6.2, aldose reductase from rabbit aorta had the following K_m's: with D-xylose as substrate, 29 mM; with D-glucose, 130 mM; with potassium D-glucuronate, 28 mM; and with D-glucuronolactone, 2 mM. The K_m with NADPH as substrate was 0.015 mM, and no activity was observed with NADH. The specific activity of the rabbit aortic aldose reductase was 72 international units per gram. The aldose reductase preparations used in these studies were subsequently subjected to column chromatography on a column (2.5 by 80 cm) of Sephadex G-100 equilibrated with phosphate buffer (5 mM), pH 6.8, and developed with the same buffer. Aldose reductase activity was associated with a single peak of protein. The K_m's of aldose reductase isolated from human aorta were, with xylose as substrate, 10 mM; with glucose, 180 mM; with glucuronate, 70 mM; with glucuronolactone 2 mM; and with NADPH, 0.007 mM. The comparable K_m values for aldose reductase isolated from rabbit lens by the same technique were for xylose, 69 mM; glucose, 300 mM; glucuronate, 33 mM; glucuronolactone, 6 mM; and NADPH, 0.04 mM. The second peak of NADP polyol dehydrogenase activity eluted from the DEAE-cellulose column had the characteristics of L-gulonate: NADP oxidoreductase (E.C. 1.1.1.19) with a K_m glucose in excess of 2M for both the human and rabbit aortic enzymes.

Activity of sorbitol dehydrogenase was demonstrated in the supernatant fraction of rabbit thoracic aortic homogenates (1:1, weight: volume; 0.25M sucrose; centrifuged at 100,000g for 30 minutes at 2°C). Activity was assayed fluorometrically in 1.0 ml of glycine–NaOH buffer (50 mM), pH 9.6, containing NAD (0.4 mM) and sorbitol (50 mM). The initial velocity was linearly related to the volume of supernatant added over the range of 0.005 to 0.015 ml. In four such experiments the rates of sorbitol oxidation were 11.3, 13.3, 15.2, and 10.2 nmole min^{-1} g^{-1} (wet weight). Both enzymes of the polyol pathway are therefore present in the aorta.

L-Epinephrine added in vitro increased the aortic sorbitol content of paired aortic samples incubated with glucose (5 mM) and produced linear increments over the range of 0.5 to 5.0 μg/ml (Figs. 1 and 2). This effect was reproduced by the beta receptor stimulator isoproterenol, but not by the alpha receptor stimulator norepinephrine (Fig. 2). Dibutyryl-3′,5′-adenosine monophosphate also increased aortic sorbitol content when added in vitro (Fig. 2). In addition, it was observed that ouabain and angiotensin II increased the sorbitol content of aortic tissue incubated in vitro with glucose (5 mM) (Fig. 2). In the same system, prostaglandins E_1, E_2, and $F_{1\alpha}$ had no significant effect when added in concentrations of 1 μg/ml. Moreover, these prostaglandins did not inhibit the effect of epinephrine (2 μg/ml) added in vitro.

The mechanism by which these agents produce their effects on aortic sorbital content has not been clearly established. Since glucose-6-phosphatase is not present in the aortic wall (*4*), increased phosphorylase activity cannot materially increase the free intracellular glucose concentration. The effect of epinephrine on aortic sorbitol content can be demonstrated in the absence of oxygen.

The demonstration of the polyol pathway in the aortic wall provides the first evidence of a mechanism by which hyperglycemia can directly alter the metabolism of the arterial wall. In addition, these hormonal effects raise the more fundamental question of the normal function of aldose reductase in tissues other than the seminal vesicle.

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Centromeres in Human Meiotic Chromosomes

Abstract. *Two heterochromatic spots were observed in most bivalents of human spermatocytes at the late diplotene stage from six individuals. It is our belief that these dark staining bodies are the centromeres of the meiotic chromosomes.*

The size of the chromosome and position of the centromere are the basis for mitotic chromosome karyotyping (*1*). Recently we suggested a similar procedure for karyotyping meiotic chromosomes according to the size of the bivalents (*2*). At that time we were not able to locate the centromere.