Lenticular Rubidium Uptake and Plasma Renin Activity in Weanling Cataract-Prone Salt-Sensitive Rats

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Our earlier studies of cataracts in Dahl salt-sensitive (DS) rats suggested the possibility of altered lens ion transport as a contributing factor in cataractogenesis in this genetic model. We also observed that those weanling DS rats with the greatest pressor response to a high salt diet eventually developed cataracts, and that changes in salt intake modified cataract formation. In the present studies, we measured lens $^{86}$Rb uptake as an index of sodium-potassium adenosine triphosphatase [(Na$^+$,K$^+$)-ATPase] activity in weanling DS rats before the development of cataracts or sustained hypertension. Additionally, plasma renin activity was measured to indirectly assess our hypothesis that the difference between cataract-prone DS rats and DS rats unlikely to develop cataracts might be a difference in degree of salt sensitivity. At the age of 4 weeks, 50 DS and 25 salt-resistant (DR) rats were given a high sodium diet for 2 weeks, at which time the rats were divided into three groups based on the systolic blood pressure response, that is, cataract-prone DS rats with systolic blood pressure equal to or greater than 155 mm Hg, DS rats unlikely to develop cataracts with systolic blood pressure less than or equal to 125 mm Hg, and DR rats. Lens and aqueous humor Na$^+$ and K$^+$, lens dry weight, and water content were not significantly different among the three groups of weanling rats. Plasma renin activity was lowest in cataract-prone DS rats and low in DS rats unlikely to develop cataracts when compared with values in DR rats. Lens ouabain-sensitive $^{86}$Rb uptake was increased in cataract-prone DS rats but not in DS rats unlikely to develop cataracts as compared with values in DR rats. Ouabain-insensitive $^{86}$Rb uptake was not significantly different among the three groups. The positive correlation between systolic blood pressure and lens ouabain-sensitive $^{86}$Rb uptake in weanling cataract-prone DS rats, and the fact that the lowest plasma renin activity was in this group suggest that salt sensitivity might be greatest among rats that eventually develop cataracts. These data also show altered lens ion transport before cataracts or sustained hypertension in cataract-prone DS rats, and raise the possibility of a generalized disturbance in passive or active ion transport as an initiating defect in cataracts associated with salt-sensitive hypertension. (Hypertension 1990;15(suppl I):I-144–I-148)
markedly higher in cataract-prone DS than in DS rats unlikely to develop cataracts and DR rats, whereas lenticular potassium concentration was decreased in both cataract-prone DS and DS rats unlikely to develop cataracts as compared with DR rats. These data suggested the possibility of altered sodium-potassium transport in the lens, ciliary ridge epithelia, or both. Lenticular ouabain-sensitive $^{86}$Rb uptake in adult hypertensive cataract-prone DS rats was decreased before lens opacification as compared with values in hypertensive DS rats unlikely to develop cataracts and control DR rats.6

Furthermore, these latter studies were based on our repeated observation that DS rats that eventually develop cataracts during sustained hypertension consistently demonstrate a markedly increased pressor response to 2 weeks of a high sodium diet initiated at weanling age.1,6 This high sodium regimen is used to induce the development of hypertension in DS rats, without altering the course of longitudinal blood pressure development in control DR rats. Because decreased lenticular rubidium uptake was observed only in those DS rats with the higher initial pressor response to sodium loading at weanling age, these data are consistent with the hypothesis that cataractogenesis in DS rats might be related to the hypertensive process and that decreased lens sodium-potassium adenosine triphosphatase [(Na$^+$,K$^+$)-ATPase] activity might precede lens opacification.

In the present study, we measured lenticular $^{86}$Rb uptake in the presence and absence of ouabain, and plasma renin activity in weanling cataract-prone DS rats before the development of either sustained hypertension or lens opacification. Age-matched DS rats unlikely to develop cataracts and control DR rats were also studied. In addition, aqueous humor sodium and potassium concentrations, as well as lenticular content of sodium, potassium and water, and lenticular dry weight were assessed in separate groups of weanling rats. In this way, we evaluated possible lenticular abnormalities suggested by our earlier data that might play an initiating role in cataractogenesis and our hypothesis that salt sensitivity might be greatest among rats that eventually develop cataracts.

Methods

A total of 50 female DS and 25 female DR rats were placed on a high sodium diet beginning at the age of 4 weeks. Systolic blood pressure (SBP) was measured weekly to establish the pressor response to this high sodium regimen. Each measurement was based on 10 consecutive determinations in conscious restrained rats by tail-cuff plethysmography. At the age of 6 weeks, the rats were divided into three groups, that is, cataract prone DS rats with SBP equal to or greater than 155 mm Hg, DS rats unlikely to develop cataracts with SBP less than or equal to 125 mm Hg, and control DR rats with SBP less than or equal to 95 mm Hg. Lens transparency was assessed through slit-lamp microscopy. Eight rats from each of the three groups were decapitated, and blood samples were collected for the determination of plasma renin activity. Lenses were immediately dissected using a posterior approach for measurements of $^{86}$Rb uptake. Lenses were transferred to vials containing Dulbecco’s Modified Eagle’s Medium, pH 7.4, at 37°C for a 5-hour equilibration period. Afterwards, ouabain was added to vials such that one lens from each rat was incubated in 1 mmol/l ouabain for measurement of ouabain-insensitive uptake, whereas total uptake was measured in the contralateral lens. Ouabain-sensitive $^{86}$Rb uptake was calculated as the difference between total and ouabain-insensitive uptake. These values were expressed as $^{86}$Rb lens/medium ratio for 25 mg lens wet weight. The lens distribution of [H]insulin was simultaneously measured to correct for extra-cellular distribution of $^{86}$Rb. Lenses were incubated for 2 hours in the presence of these radioisotopes. Lenses and aliquots of incubation media were then transferred to vials containing liquid scintillation fluid and were counted the next day. Values were corrected for $^{86}$Rb counts reflected in the [H]window as well as for slight quenching attributed to lenses and media. Six additional rats from each of the three groups were killed for the determination of aqueous humor sodium and potassium concentrations as well as lenticular content of sodium, potassium and water, and lenticular dry weight. The aqueous humor and lenses were prepared and analyzed as previously described.1

Statistical analysis of the data was done using Student’s t test between groups, and p values less than or equal to 0.05 were considered significant. All values in the study are expressed as the arithmetic mean±SEM. All experimental procedures were approved by the local institutional review board for animal care and use; procedures were performed in accordance with the guidelines of the National Institutes of Health, The American Physiological Society, and the Association for Research in Vision and Ophthalmology resolution.

Results

In agreement with our previous studies, slit-lamp microscopy revealed lenticular transparency not only in control DR and DS rats classified as unlikely

| Table 1. Systolic Blood Pressure in Weanling “Cataract-Prone” Salt-Sensitive Rats |
|------------------------|------------------|------------------|------------------|
| Group                  | Systolic blood pressure (mm Hg) |
|------------------------|------------------|------------------|------------------|
| Cataract-prone DS      | 154±2*†          |                  |                  |
| DS                     | 102±3*           |                  |                  |
| DR                     | 87±7             |                  |                  |

Values are mean±SEM. DS, Dahl salt-sensitive rats; DR, Dahl salt-resistant rats. *p<0.01 as compared with corresponding values in DS. †p<0.05 as compared with corresponding values in DS.
to develop cataracts but also in cataract-prone DS rats. As illustrated in Table 1, by definition, weanling cataract-prone DS rats had higher levels of SBP than age-matched DS rats classified as unlikely to develop cataracts and control DR rats after 2 weeks on a high sodium diet. It should be noted that these data were based on our identification criteria of subgroups of DS rats and, consequently, only demonstrate that selection of such subgroups was appropriate. Additionally, those weanling DS rats classified as unlikely to eventually develop cataracts also demonstrated increased SBP as compared with control DR rats. As shown in Table 2, plasma renin activity was lower in weanling cataract-prone DS rats than in DS rats unlikely to develop cataracts and control DR rats. Also, plasma renin activity was lower in weanling DS rats unlikely to develop cataracts as compared with values in DR rats.

As might be anticipated on the basis of our previous observations, lenticular dry weight and water content were not different among cataract-prone DS, DS unlikely to develop cataracts, and control DR rats when evaluated at weanling age after 2 weeks’ exposure to a high sodium diet.

Additionally, no differences were observed in aqueous humor sodium and potassium concentrations (Figure 1) or in lenticular sodium and potassium content (Figure 2) among the three groups of weanling rats studied.

Total lenticular $^{86}$Rb uptake, illustrated in Figure 3, was increased in cataract-prone DS rats as compared with corresponding values in both DS rats unlikely to develop cataracts and control DR rats. No difference in total lens $^{86}$Rb uptake was observed, however, between these two latter groups. Ouabain-insensitive $^{86}$Rb uptake was simi-
lar among the three groups of rats studied such that the increase in total lens $^{86}$Rb uptake in cataract-prone DS rats reflected a selective increase in ouabain-sensitive $^{86}$Rb uptake in this group of rats before sustained hypertension and lens opacification (Figure 3). Both ouabain-insensitive and ouabain-sensitive $^{86}$Rb uptake were similar between DS rats unlikely to develop cataracts and control DR rats during the present study.

**Discussion**

The classification of DS rat subgroups as cataract-prone and unlikely to develop cataracts was not arbitrary in the present study, but instead based on extensive studies in our laboratory. The differences between SBPs in these two groups of weanling rats not only show appropriate choice of criteria for the classification of DS rat subgroups but also reflect the heterogeneity of salt-sensitivity reported in DS rats of the Brookhaven strain. Additionally, the lower plasma renin activity in weanling cataract-prone DS rats as compared with DS rats unlikely to develop cataracts is consistent with a greater volume expansion and an increased initial pressor response to a high sodium diet in those DS rats that eventually develop cataracts. These data support our hypothesis that cataract-prone DS rats might be more salt sensitive than DS rats that do not develop cataracts.

The similarity in aqueous humor of both sodium and potassium concentrations among the three groups of rats studied at weanling age might indicate that altered lenticular ionic transport in weanling cataract-prone DS rats is not accompanied by altered aqueous humor electrolyte composition. Alternatively, inasmuch as the lens is an avascular tissue, these data might reflect normal ciliary ridge epithelial ion transport in cataract-prone DS rats in the weanling rat. Our data do not, however, exclude the participation of altered ciliary ridge epithelial ion transport in the eventual changes observed in aqueous humor electrolyte composition described in adult hypertensive DS rats with cataractous lesions. This is supported by the fact that ciliary ridge (Na$^+$,K$^+$)-ATPase activity not only influences aqueous dynamics but is also largely responsible for the composition of aqueous humor secreted into the anterior lenticular chamber.

Selectively increased ouabain-sensitive lenticular $^{86}$Rb uptake in cataract-prone DS rats presumably reflects increased lens (Na$^+$,K$^+$)-ATPase activity in this subgroup of DS rats before both the development of sustained hypertension and lens opacification, which might reflect an intrinsic lens ion transport defect. We believe that such an ion transport defect could lead to an adaptive increase in lenticular (Na$^+$,K$^+$)-ATPase activity in the weanling cataract-prone DS rats.

The absence of measurable changes in lens and aqueous humor of sodium and potassium at this age, with increased lens (Na$^+$,K$^+$)-ATPase activity, suggests the possibility that high lens activity of this enzyme might effectively compensate a possible change in lenticular electrolyte content in the weanling rat.

The facts that lenticular and aqueous humor ionic composition was altered in adult cataract-prone DS rats and that the (Na$^+$,K$^+$)-ATPase activity was decreased rather than increased, as in the weanling rat, suggest the possibility of a reduced electrical gradient across lens fiber membranes in adult hypertensive DS rats before cataract formation. The injurious effects of prolonged lens depolarization on cellular metabolism might explain the decrease in active lenticular ion transport in the adult cataract-prone DS rats. Although specific measurements of sodium and potassium permeability were not done in the present study, a defect in lenticular ionic membrane permeability is consonant with data in other tissues in models of genetic and experimental hypertension, such as the spontaneously hypertensive rat and deoxycorticosterone-salt hypertension. It is yet unclear if such a postulated increase in lens epithelial permeability, fiber sodium permeability, potassium permeability, or all three might represent a primary membrane
defect or an abnormality secondary to, for example, decreased membrane fiber calcium binding. Nonetheless, the present data cannot distinguish these possibilities. Additionally, preliminary studies showing increased renal \((\text{Na}^+,\text{K}^+)-\text{ATPase}\) activity in weaning DS rats\(^{11}\) suggest that the same defect, which at the kidney can participate in the development of salt-sensitive hypertension, might at the lens participate in cataractogenesis. Thus, altered ion transport might represent a link between mechanisms involved in cataractogenesis associated with salt-sensitive hypertension and the hypertensive process.

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References


KEY WORDS • salt-sensitive hypertension • crystalline lens • aqueous humor • sodium-potassium ATPase • plasma renin activity • sodium • potassium
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