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Age-related changes in sorbitol dehydrogenase activity of rat brain, liver, kidney and eye

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Sorbitol dehydrogenase (SDH) activity was measured in whole brain, different brain areas, liver, kidney and eye of old rats (23–26 months) and compared with matched animals (3 months). A significant increase in SDH activity was found in whole brain (34%) of old rats, as well as in all brain areas studied: brainstem (59%), hypothalamus (38%), cerebral cortex (35%), striatum (30%), midbrain (27%), hippocampus (27%) and cerebellum (19%). The increase in SDH activity in brain of old rats is due to V_{max}. These results do not favour an accumulation of sorbitol in brain of old rats. A significant decrease (36%) in SDH activity was found in kidney and no significant change in liver and eye of old rats when compared with young rats. Our previous and present results suggest that an accumulation of sorbitol might occur in some tissues of old rats, particularly in the eye, and cause cataract formation.

Two major forms (high-K_m and low-K_m) of aldehyde reductase (AR, EC 1.1.1.2) which are differentiated by their substrate specificity and sensitivity to inhibition by the anticonvulsant drug sodium valproate (DPA) (Turner & Whittle 1980, 1981; Whittle & Turner 1981) have been found in animal tissues (Turner & Tipton 1972; Branlant & Biellmann 1980; Turner & Whittle 1981; Turner et al 1982; Flynn 1982). They occur in certain tissues, particularly in lens which has another enzyme with very similar general properties (Hers 1960; Moonsammy & Stewart 1967; Hayman & Kinoshita 1965; Kinoshita et al 1963, 1968; Sheaff & Doughty 1976). This enzyme which is called aldose reductase (EC 1.1.1.21) has some affinity for D-glucose and other aldo-sugars (Sheaff & Doughty 1976).

Aldose reductase appears, on immunological and other criteria, to be identical with the low-K_m form of AR (Whittle & Turner 1981; Cromlish & Flynn 1983). Aldose reductase which catalyses the transformation of D-glucose to sorbitol is the first enzyme of the polyol pathway (Winegrad et al 1972) and has been implicated in the aetiology of the complications of diabetes (Gabbay 1973). The second enzyme is sorbitol dehydrogenase (SDH, EC 1.1.1.14) catalysing the transformation of sorbitol to fructose as well as the reverse reaction (Rehg & Torack 1977). SDH has been found in the liver and in other human and animal tissues (Gerlach 1965; Rehg & Torack 1977; Collins & Corder 1977; Jedziniak et al 1981).

Age-related changes in AR activity have been reported in several rat tissues (Cao Danh et al 1984a, c). A previous work also showed that aldose reductase increased in eye of old rats (Cao Danh et al 1984b). To date, little information is available concerning the variations of the activities of the enzymes of the polyol pathway; the present work was therefore undertaken to study the changes of SDH activity in whole brain, in different brain areas, in liver, kidney and eye of old rats compared with young rats.

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Materials and methods

All chemical products were purchased from Sigma, St Louis, MO, USA. Male, Wistar rats (Iffa Credo), aged 23–26 months, were compared with matched animals of 3 months.

Dissection of brain areas (cerebral cortex, striatum, cerebellum, midbrain, hippocampus, hypothalamus and brainstem) was carried out as described by Glowinski & Iversen (1966). Animals were decapitated, brains, different brain areas after dissection, liver, kidney and eye were immediately weighed and homogenized in an ultra-Turrax in 5 volumes of 60 mM sodium phosphate buffer (pH 6·2). The homogenates were then centrifuged in a type JA20-1 rotor at 10 000*g* for 20–30 min in a Beckman J2-21 model centrifuge. The supernatants were used to measure the activity of SDH. SDH activity was assayed according to the method of Rehg & Torack (1977). The reaction mixture consisted of the enzyme source (0·25–0·70, 0·02–0·10, 0·03–0·10 and 1·5–3·5 mg protein/assay for brain, liver, kidney and eye, respectively), 0·2 μmol NADH, 500 μmol D-fructose and 60 μmol sodium phosphate buffer (pH 6·2) in a total volume of 1 ml. Incubation in the absence of substrate was carried out for 5–10 min at 25 °C. The reaction was started by the addition of substrate. In these conditions, SDH activity increased linearly with time until 5 min.

SDH activity was determined spectrophotometrically by following the rate of NAD formation at 340 nm, using a Beckman model 25 spectrophotometer. Protein concentration was determined by the method of Lowry et al (1951), with bovine serum albumin as standard.

For kinetic analysis K_m and V_{max} of each experiment were calculated using a non-linear least-squares procedure.

Results

Changes in SDH activity with age in rat whole brain. As shown in Table 1, a significant increase of SDH activity was found in whole brain of old rats.

Table 1. SDH activity in whole brain, liver, kidney and eye of young and old rats. SDH activity is expressed as nmol NADH oxidized min^{-1} (mg protein)$^{-1}$, n = 6–13. *$P <$ 0·001, Student's *t*-test or Wilcoxon test.

Tissue	Young rats mean ± s.e.m.	Old rats mean ± s.e.m.	$\frac{\text{Old}}{\text{Young}} \times 100$ mean ± s.e.r.
Brain	5·61 ± 0·12	7·52 ± 0·31	134 ± 6*
Liver	146·43 ± 8·87	159·00 ± 12·00	109 ± 8
Kidney	93·32 ± 3·83	59·80 ± 5·97	64 ± 7*
Eye	1·75 ± 0·04	1·78 ± 0·07	102 ± 4

Kinetic analysis of SDH in brain of young and old rats. In young rats, the K_m and V_{max} values (mean ± s.d., n = 4) of D-fructose (12·5–200 mM) reduction by brain SDH are 50·23 ± 12·37 mM and 8·20 ± 0·75 nmol NADH oxidized min^{-1} (mg protein)$^{-1}$. The corresponding values in old rats are 40·18 ± 5·22 mM and 9·90 ± 1·15 nmol NADH oxidized min^{-1} (mg protein)$^{-1}$.

Statistical analysis shows that the V_{max} of SDH in brain homogenate supernatants of old rats is significantly higher than that of young rats ($P < 0·05$, Student's *t*-test, n = 4), whereas the K_m values do not differ significantly.

Changes in SDH activity with age in different brain areas. Fig. 1 shows that a significant increase in SDH activity was found in all brain areas studied: brainstem (59%), hypothalamus (38%), cerebral cortex (3%), striatum (30%), midbrain (27%), hippocampus (27%) and cerebellum (19%).

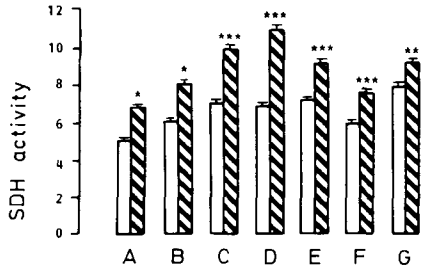

FIG. 1. SDH activity in different brain areas of young (open columns) and old (hatched columns) rats. n = 6; *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$, Student's *t*-test or Wilcoxon test. Key: A, cerebral cortex; B, striatum; C, hypothalamus; D, brainstem; E, midbrain; F, hippocampus; G, cerebellum. Units of SDH activity are nmol NADH oxidized min^{-1} mg $protein^{-1}$ ± s.e.m.

Changes in SDH activity with age in liver, kidney and eye. As shown in Table 1, a significant decrease (36%) in SDH activity was found in kidney, but no significant change in enzyme activity was found in liver and eye of old rats compared to young rats.

Discussion

The present results indicate that SDH activity increases in brain of old rats, although only the fructose-to-sorbitol direction has been followed. Moreover, in our previous work, no significant change in aldose reductase activity was observed in brain of old rats (Cao Danh et al 1984a, c). Two enzymes make up the sorbitol pathway: aldose reductase and SDH. The accumulation of sorbitol causes cataract formation, cerebral oedema and neuropathy (O'Brien & Schofield 1980). These results suggest that an increase in brain sorbitol concentration should not be involved in cerebral oedema in old rats.

Anatomical (Scheibel & Scheibel 1975; Brizzee et al 1976) and biochemical (Dhopeshwarkar & Mead 1975) alterations with age have been reported in the brain. According to Vernadakis (1975), a reduction in neuron number is compensated for by gliosis. An age-related increase in the activity of enzymes such as glutamine synthetase (Norenberg 1979; Norenberg & Martinez-Hernandez 1979; Martinez-Hernandez et al 1977; Cao Danh et al 1985) and monoamine oxidase B (Strolin Benedetti & Keane 1980; Fowler et al 1980; Cao Danh et al 1983; Lewitt et al 1982) both localized in glial cells has been found. In contrast, a decrease with age in the activity of enzymes localized in neurons (Raine 1976) such as tyrosine hydroxylase, glutamate decarboxylase, choline acetyltransferase, monoamine oxidase A, has been observed (Gottfries et al 1975; Strolin Benedetti & Keane 1980; Lai et al 1981). Although the localization of SDH in the different brain cells is unknown, the increase of SDH in the brain of old rats might be an adaptive phenomenon.

Our present results show that a significant decrease in SDH activity with age was found in rat kidney but no significant change in liver and eye. Due to the impermeability of most cell membranes to sorbitol, the accumulation of this metabolite establishes an osmotic imbalance that ultimately causes the development of a number of pathological conditions in various tissues such as diabetic cataractogenesis in lens, diabetic neuropathy in peripheral nerve, cerebral oedema in brain (O'Brien & Schofield 1980). In our previous study, a significant increase in aldose reductase was found in eye and a significant decrease in kidney of old rats (Cao Danh et al 1984b, c). These results suggest that an accumulation of sorbitol might occur in the eye and possibly in the kidney of old rats and cause cataract formation and nephropathy.

Care has to be taken to extrapolate results on enzymes of the sorbitol pathway from animal lenses to those from human lenses, as human lenses have been found to contain limited levels of aldose reductase and high levels of polyol dehydrogenase relative to the animal lenses (Jedziniak et al 1981). Moreover, juvenile normal human lenses seem to contain substantially higher levels of aldose reductase than do senile lenses (Jedziniak et al 1981).

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