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Further investigation on aldehyde reductase activity in ageing rat tissues

H. Cao Danh*, P. Pasquier-Béchet, M. Strolin Benedetti*, P. Dostert*†, *Centre de Recherche Delalande, 10 rue des Carrières, 92500 Rueil-Malmaison, France*

Aldehyde reductase (AR) activity was measured in whole brain, different brain areas, kidney and duodenum of 23–26 months old rats and then compared to matched animals of 3 months. No significant changes in AR activity were found in whole brain of old rats with D-glucose as substrate. A significant increase in AR activity in old rats was found in striatum (12%), cerebellum (26%), midbrain (6%), brainstem (19%) and hypothalamus (13%) with *p*-nitrobenzaldehyde, in striatum (14%), midbrain (10%), brainstem (52%), hypothalamus (9%) and hippocampus (20%) with D-glucuronate, in cerebellum (21%) with D-xylose, in striatum (43%), cerebellum (19%) and brainstem (22%) with DL-glyceraldehyde, and in brainstem (15%) with pyridine 3-aldehyde. A significant decrease in AR activity in old rats was found in midbrain (16%), brainstem (19%) and hypothalamus (19%) with D-xylose, and in midbrain (13%) with DL-glyceraldehyde. A significant decrease in AR activity in old rats was found in kidney with *p*-nitrobenzaldehyde (30%), D-xylose (31%), DL-glyceraldehyde (33%), pyridine 3-aldehyde (27%) and in duodenum with *p*-nitrobenzaldehyde (21%), but a significant increase was found in duodenum with pyridine 3-aldehyde (29%). Preliminary experiments show an increase (42%) of sorbitol dehydrogenase activity in whole brain of old rats. The present results clearly indicate that the AR activity increase in the brain of old rats is the high-K_m form of aldehyde reductase. However, as the activity of the enzyme responsible for the catabolism of sorbitol increases, its accumulation in the brain with age seems to be improbable; this is different from what happens in the eye where enzymatic changes with age appear to favour sorbitol accumulation, which is responsible for cataractogenesis.

The concentration of aldehydes is controlled by the activities of aldehyde metabolizing enzymes: aldehyde dehydrogenase (ALDH, EC 1.2.1.3) and aldehyde reductase (AR, EC 1.1.1.2) in mammalian tissues (Duncan & Sourkes 1974; Deitrich & Erwin 1975; Tipton et al 1977, 1981; Ryle & Tipton 1981; Tabakoff et al 1972; Anderson et al 1976). AR appears to play a number of distinct functions in cellular metabolism (Tipton et al 1977; Turner & Whittle 1981). Multiple forms of AR have been found in animal tissues from several species (Tipton et al 1981; Flynn 1982).

Two major forms (high-K_m and low-K_m) of AR have generally been considered in liver and brain. Both forms catalyse the reduction of a wide variety of substrates including aromatic and aliphatic aldehydes, aldo sugars, ketones and steroids with 17-aldol side chains. However, despite overall similarities they can be differentiated on the basis of substrate and coenzyme specificity and by their sensitivity to various inhibitors. For instance, the low-K_m form in contrast to the high-K_m enzyme can use NADH to a limited extent (Turner & Tipton 1972) and is also relatively insensitive to sodium valproate (Turner & Whittle 1980). Kinetic and specificity studies have indicated that only the low-K_m enzyme is involved in the reduction of biogenic aldehydes (Anderson et al 1976; Whittle & Turner 1981). This enzyme appears, on immunological and other criteria, to be identical with aldose reductase (Whittle & Turner 1981; Cromlish & Flynn 1983), the enzyme responsible for the reduction of glucose to sorbitol in the sorbitol pathway present in several tissues (Moonsammy & Stewart 1967; O'Brien & Schofield 1980). The second enzyme of the sorbitol pathway 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). However, SDH catalyses the reduction of ketose sugars at much higher concentrations than polyols.

Age-related changes in the enzyme producing aldehydes, monoamine oxidase (MAO), and in enzymes catabolising aldehydes, ALDH and AR, have been reported (Strolin Benedetti & Keane 1980; Cao Danh et al 1983a, b, 1984).

Our previous results showed a significant increase of AR in the brain with *p*-nitrobenzaldehyde and D-glucuronate, and in the liver with D-glucuronate, DL-glyceraldehyde and pyridine 3-aldehyde. In the heart of old rats, a significant increase in enzyme activity was found with *p*-nitrobenzaldehyde and DL-glyceraldehyde and a significant decrease with D-xylose. It was suggested that the AR activity increase in brain of old rats was the high-K_m form (Cao Danh et al 1984).

The present work was carried out to confirm our previous results and to collect information from other tissues of old rats. AR activity was measured in whole brain, in different brain areas, in kidney and in duodenum of old rats and compared with that of young rats. Preliminary experiments have also been carried out to measure the SDH activity in whole brain from young and old rats.

* Present address: Laboratoires Fournier, Centre de Recherche, 50 rue de Dijon, Daix 21121 Fontaine-lès-Dijon, France.
† Correspondence.

Table 1. AR activity in different brain areas of young and old rats. In our conditions (0·1–2·0 mg protein/assay) AR activity increased linearly with time for 5 min. AR activity is expressed as nmol NADPH oxidized min^{-1} mg $protein^{-1}$ ± s.e.m. (n = 4–6).

Substrate (μmol/assay)	Rats	Striatum	Cerebellum	Midbrain	Brainstem	Hypo-thalamus	Hippo-campus	Cerebral cortex
p-Nitrobenzaldehyde (0·5)	Young	3·79 ± 0·01	2·94 ± 0·09	4·85 ± 0·06	5·04 ± 0·06	3·27 ± 0·03	3·44 ± 0·12	3·50 ± 0·08
	Old	4·24 ± 0·05	3·71 ± 0·27	5·12 ± 0·04	6·02 ± 0·29	3·71 ± 0·15	3·83 ± 0·15	3·46 ± 0·07
D-Glucuronate (10)	Young	1·69 ± 0·06	2·78 ± 0·06	2·57 ± 0·03	2·43 ± 0·08	2·47 ± 0·08	2·32 ± 0·08	1·79 ± 0·03
	Old	1·92 ± 0·03	3·01 ± 0·08	2·83 ± 0·09	3·69 ± 0·10	2·68 ± 0·04	2·75 ± 0·04	1·88 ± 0·03
D-Xylose (100)	Young	1·07 ± 0·03	1·00 ± 0·02	1·02 ± 0·07	1·28 ± 0·01	1·06 ± 0·06	0·94 ± 0·05	0·67 ± 0·03
	Old	1·02 ± 0·06	1·22 ± 0·06	0·85 ± 0·03	1·04 ± 0·04	0·86 ± 0·02	0·93 ± 0·04	0·71 ± 0·03
DL-Glyceraldehyde (5·0)	Young	1·54 ± 0·02	1·92 ± 0·06	2·42 ± 0·01	1·38 ± 0·02	2·15 ± 0·10	2·07 ± 0·07	1·63 ± 0·05
	Old	2·21 ± 0·04	2·29 ± 0·03	2·10 ± 0·08	1·68 ± 0·08	1·96 ± 0·18	2·11 ± 0·06	1·74 ± 0·06
Pyridine 3-aldehyde (2·0)	Young	3·13 ± 0·07	3·45 ± 0·18	2·92 ± 0·08	4·56 ± 0·10	4·53 ± 0·13	2·19 ± 0·06	2·78 ± 0·05
	Old	3·14 ± 0·07	3·88 ± 0·05	2·91 ± 0·11	5·24 ± 0·06	4·85 ± 0·06	2·12 ± 0·09	2·86 ± 0·07

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 and whole brains, different brain areas after dissection, kidney and duodenum were immediately weighed and homogenized (Potter–Elvehjem) in 9 volumes of 0·1 M sodium phosphate buffer (pH 7·0) containing 0·32 M sucrose and 1% Triton X-100 for the determination of AR activity. The homogenates were then centrifuged in a type R40 rotor at 105 000*g* for 60 min in a Spinco–Beckman model L5 ultracentrifuge. Supernatants were used to measure AR activity.

AR activity was assayed according to the method of Reyes & Erwin (1977) as described in our previous work (Cao Danh et al 1984).

AR activity was determined with one substrate (D-glucose) in whole brain and with 4 or 5 substrates (*p*-nitrobenzaldehyde, D-glucuronate, D-xylose, DL-glyceraldehyde and pyridine 3-aldehyde) in different brain areas, in kidney and duodenum of old rats compared with matched animals of 3 months. The substrate and protein concentrations used are shown in detail in Results.

For the determination of SDH activity, brains were homogenized (Ultra-Turrax) in 5 volumes of 60 mM sodium phosphate buffer (pH 6·2). Homogenates were then centrifuged in a type JA20-1 rotor at 10 000*g* for 20–30 min with a Beckman J2-21 model centrifuge. Supernatants were used to measure SDH activity. SDH activity was assayed according to the method of Rehg & Torack (1977). The reaction mixture consisted of the enzyme source (1·0–3·0 mg protein per assay), 0·2 μmol NADH, 500 μmol 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. Reaction was started by the addition of substrate. Under these conditions SDH activity increased linearly with time for 5 min. AR or SDH activity was determined spectrophotometrically by following the rate of NADP or NAD formation at 340 nm. Protein concentration was determined by the method of Lowry et al (1951), with bovine serum albumin as standard.

Statistical analyses were performed on the experimental data, using the Student's *t*-test when the hypothesis of equal variance was valid as evaluated by the Fisher test, and the Wilcoxon non-parametric test when this hypothesis was rejected. Symbol for level of significance has been presented near the ratio (old/young × 100) (Tables 2, 3, 4), although the statistical tests comparing young vs old rats were performed with

Table 2. Comparison of AR activity in different brain areas from young and old rats. (Young rat brain activity = 100%.)

	Old/young × 100 (mean ± s.e.r.)						
Substrate (μmol/assay)	Striatum	Cerebellum	Midbrain	Brainstem	Hypo-thalamus	Hippo-campus	Cerebral cortex
p-Nitrobenzaldehyde (0·5)	112 ± 1***	126 ± 5***	106 ± 1*	119 ± 6*	113 ± 5*	111 ± 6	99 ± 3
D-Glucuronate (10)	114 ± 4**	108 ± 4	110 ± 4*	152 ± 6***	109 ± 4**	120 ± 5***	105 ± 3
D-Xylose (100)	95 ± 6	121 ± 6**	84 ± 6*	81 ± 3***	81 ± 5*	99 ± 7	107 ± 7
DL-Glyceraldehyde (5·0)	143 ± 3***	119 ± 4***	87 ± 3***	122 ± 3***	91 ± 9	102 ± 4	107 ± 5
Pyridine 3-aldehyde (2·0)	100 ± 3	112 ± 6	100 ± 5	115 ± 3**	107 ± 3	97 ± 5	103 ± 3

Student's *t*-test or Wilcoxon test: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$, n = 4–6.

Table 3. AR activity in kidney of young and old rats. In our conditions, AR activity increased linearly with time for 5 min. AR activity is expressed as nmoles NADPH oxidized min^{-1} mg $protein^{-1}$ (n = 5–6).

Substrate (μmol/assay)	Protein (mg/assay)	Young rats (mean ± s.e.m.)	Old rats (mean ± s.e.m.)	$\frac{\text{Old}}{\text{Young}} \times 100$ (mean ± s.e.r.)
p-Nitrobenzaldehyde (0·5)	0·07–0·17	22·20 ± 0·34	15·50 ± 1·70	70 ± 8***
D-Glucuronate (10·0)	0·14–0·22	27·70 ± 0·57	24·72 ± 2·71	89 ± 10
D-Xylose (100·0)	1·00–1·40	2·68 ± 0·26	1·82 ± 0·27	69 ± 8***
DL-Glyceraldehyde (5·0)	0·13–0·25	13·22 ± 0·31	9·14 ± 1·08	67 ± 12*
Pyridine 3-aldehyde (2·0)	0·04–0·10	22·46 ± 0·39	16·32 ± 1·67	73 ± 8***

*$P < 0·05$, ***$P < 0·001$, Student's *t*-test or Wilcoxon test.

the appropriate population mean (±s.e.m.) values (Tables 1, 3, 4).

Results

Changes in AR activity with age in rat whole brain. As shown in Fig. 1, no significant difference in AR activity was found with D-glucose as substrate. However, a significant increase in SDH activity was found in whole brain of old rats.

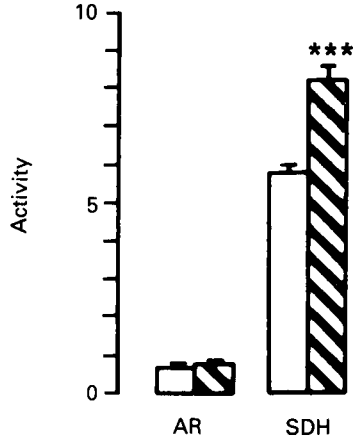

FIG. 1. AR and SDH activities in whole brain of young (open columns) and old (hatched columns) rats (n = 6). Substrate concentrations: D-glucose 400 mM (AR), D-fructose 500 mM (SDH). Ordinate: activity (nmol NADPH or NADH oxidized min^{-1} (mg prot.)$^{-1}$ ± s.e.m.) ***$P < 0·001$, Student's *t*-test or Wilcoxon test.

Changes in AR activity with age in different brain areas. As shown in Tables 1 and 2, a significant increase in AR activity of old rats was found in striatum, cerebellum, midbrain, brainstem and hypothalamus with *p*-nitrobenzaldehyde, in striatum, midbrain, brainstem, hypothalamus and hippocampus with D-glucuronate, in cerebellum with D-xylose, in striatum, cerebellum and brainstem with DL-glyuceraldehyde, and in brainstem with pyridine 3-aldehyde, whereas a significant decrease of AR activity of old rats was found in midbrain, brainstem with DL-glyceraldehyde, and in brainstem midbrain with DL-glyceraldehyde.

Changes in AR activity with age in rat kidney. As shown in Table 3, a significant decrease in AR activity was found with *p*-nitrobenzaldehyde, D-xylose, DL-glyceraldehyde and pyridine 3-aldehyde, whereas no significant decrease in enzyme activity was found with D-glucuronate in the kidney of old rats.

Changes in AR activity with age in rat duodenum. Table 4 shows that in the duodenum of old rats AR activity decreased with *p*-nitrobenzaldehyde, increased with

Table 4. AR activity in duodenum of young and old rats. In our conditions, AR activity increased linearly with time for 5 min. AR activity is expressed as nmoles NADPH oxidized min^{-1} mg $protein^{-1}$ (n = 5–6).

Substrate (μmol/assay)	Protein (mg/assay)	Young rats (mean ± s.e.m.)	Old rats (mean ± s.e.m.)	$\frac{\text{Old}}{\text{Young}} \times 100$ (mean ± s.e.r.)
p-Nitrobenzaldehyde (0·5)	0·25–0·27	9·35 ± 0·33	7·35 ± 0·36	79 ± 5***
D-Glucuronate (10·0)	0·60–0·90	3·79 ± 0·05	3·67 ± 0·12	97 ± 4
DL-Glyceraldehyde (5·0)	0·50–0·60	2·48 ± 0·18	2·92 ± 0·04	118 ± 9
Pyridine 3-aldehyde (2·0)	0·10–0·30	4·70 ± 0·06	6·08 ± 0·20	129 ± 5***

***$P < 0·001$, Student's *t*-test or Wilcoxon test.

pyridine 3-aldehyde and did not change significantly with D-glucuronate and DL-glyceraldehyde.

Discussion

The two forms of AR (high-K_m and low-K_m) are differentiated by their substrate specificity and sensitivity to inhibition by the anticonvulsant drug sodium valproate (DPA) (Turner & Whittle 1980; Whittle & Turner 1981). The low-K_m form appears to be responsible for the reduction of biogenic aldehydes and, on immunological and other criteria, to be identical with aldose reductase (Turner et al 1982). Previous results of experiments carried out with DPA suggest that the AR activity increase in the brain of old rats is the high-K_m form (Cao Danh et al 1984). Because aldehydes derived by deamination of endogenous amines are unstable and difficult to purify, D-glucuronate and D-xylose have been used as substrates for high-K_m and low-K_m forms, respectively (Von Wartburg & Wermuth 1980; Whittle & Turner 1981; Turner et al 1982).

The present results show that a significant increase in AR activity was found in several brain areas of old rats with D-glucuronate. These data confirm our previous results in whole brain (Cao Danh et al 1984), i.e. the increase of AR activity in the brain of old rats seems to be due preferentially to an increase of the high-K_m form. No significant change in AR activity in whole brain was found with D-glucose and preliminary experiments show that SDH activity increases in whole brain of old rats. Our results are in favour of the fact that sorbitol accumulation in the brain with age is improbable; this is different from what happens in the eye, where changes of enzymes of the sorbitol pathway with age are such as to favour sorbitol accumulation (Cao Danh et al 1985b). There is some evidence that brain ageing process is associated with a number of morphological (Scheibel & Scheibel 1975) and biochemical alterations (Dhopeshwarkar & Mead 1975). A reduction of neuron number compensated by gliosis has been reported (Vernadakis 1975; Brizzee et al 1976). Age-related increases in the activity of enzymes (MAO-B and glutamine synthetase) which seem to be markers for glial cells (Lewitt et al 1982; Martinez-Hernandez et al 1977; Norenberg & Martinez-Hernandez 1979; Norenberg 1979) have been reported (Strolin Benedetti & Keane 1980; Noda & McGeer 1982; Fowler et al 1980; Cao Danh et al 1983b, 1985a). Thus, the increase in SDH activity in brain might be attributable to a similar process. However, SDH localization in different brain cells is unknown and further investigation must be made to interpret these observations.

The present results also indicate that a significant age-related decrease, or tendency to decrease, is observed in kidney AR with all substrates used. Our previous and present results suggest that ageing exerts specific changes in AR activity of the various rat peripheral organs.

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Cyclic nucleotides fail to affect mucus glycoconjugate secretion from canine tracheal explants

EDWARD J. BARBIERI*, CARL BARSIGIAN†, *Department of Pharmacology, Hahnemann University School of Medicine, Philadelphia, Pennsylvania 19102, USA*

Canine tracheal explants, incubated overnight with [^{3}H]glucosamine, elicited an enhanced secretion of ethanol-precipitated [^{3}H]labelled glycoconjugate when challenged with methacholine, 10 μM. Neither the β-adrenoceptor agonist isoprenaline, 10 μM, nor the phosphodiesterase inhibitor theophylline, 10 mM, had any significant effect on glycoconjugate secretion. Dibutyryl cyclic AMP, 1 mM, and dibutyryl cyclic GMP, 1 mM, alone or in combination with theophylline, 10 mM, were devoid of activity on unstimulated or methacholine-stimulated tracheal explants. The calcium ionophore A23187, 10 μM, stimulated [^{3}H]glycoconjugate secretion from each of the tissues tested; however, the cyclic nucleotides failed to modify this response. These data indicate that the cyclic nucleotides play little, if any, role in mucus glycoconjugate secretion by the canine trachea.

It is currently believed that cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) serve as modulators of calcium-mediated secretion and that may even directly control secretion in certain tissues (for review, see Rubin 1982). Investigations into the role of cAMP in the secretion of respiratory mucus have focused principally on the secretion of electrolytes and water. β-Adrenoceptor agonists (Al-Bazzaz et al 1977; Marin & Zaremba 1978; Davis et al 1979), theophylline (Al-Bazzaz et al 1977), and dibutyryl cAMP (Al-Bazzaz 1981) have all been reported to increase the secretion of chloride ions towards the luminal mucosa of canine trachea in-vitro.

The role of cyclic nucleotides in the secretion of the macromolecular components of tracheal mucus is poorly characterized. Phipps et al (1980) showed that terbutaline enhanced the release of radiolabelled mucus from feline isolated trachea and Liedtke et al (1982) described the stimulant activity of β-adrenoceptor agonists, the phosphodiesterase inhibitor isobutylmethylxanthine, and 8-bromo-cAMP on mucus glycoprotein secretion from cat trachea in-vitro. Shelhamer et al (1980) showed that 8-bromo-cGMP was an effective mucus secretogogue in human bronchial tissues in-vitro, but that dibutyryl cAMP had no effect. The role of cyclic nucleotides as modulators of stimulated tracheal mucus secretion has not been reported, nor has their influence on basal mucus glycoconjugate secretion from canine tracheal explants.

* Correspondence.
† Present address: The Cardeza Foundation, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA.

Methods

The method used for the quantitation of canine tracheal mucus secretion was a modification of the organ culture technique of Coles & Reid (1981). Details of the procedure used have been described previously (Barsigian & Barbieri 1982; Barbieri et al 1984). Briefly, the tracheae of adult male mongrel dogs (9–11 kg), given a fatal intraperitoneal injection of pentobarbitone sodium solution, 50 mg kg^{-1}, were removed, sectioned, and tracheal explants were incubated overnight in serum-free Medium 199 with Earle's salts (Gibco Laboratories) containing D-[6-^{3}H]glucosamine hydrochloride (specific activity, 38 Ci mmol^{-1}), 3·6 μCi ml^{-1} (Amersham Corporation) under 95% O_2: 5% CO_2 at 37 °C in a metabolic shaker.

The following day the tissues were washed with fresh Medium 199 and incubated for two sequential periods (Harvesting Periods A and B) of either 10 or 30 min duration. Samples which served as controls were incubated in Medium 199 alone in both periods; drug-treated samples were incubated alone during