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Age-related changes in glutathione synthesis in the eye lens

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Eye lenses from young rats or mice synthesize GSH from methionine or *N*-acetylcysteine. However, lenses from old animals do not synthesize GSH from methionine. This is due to the absence of cystathionase activity in old lenses. GSH monoethyl ester, but not free GSH, increases GSH content and protects the lens against experimental oxidative stress. The importance of these results in the prevention of cataractogenesis is discussed.

INTRODUCTION

Understanding the mechanisms of the development of senile cataract is a problem of scientific and social interest because cataract is one of the major causes of reversible blindness. It is estimated that over 50 million people worldwide suffer from cataracts (Taylor & Davies, 1987). Oxidative stress is a major common cause of the many changes in the development of senile cataracts (Chylack, 1984). The glutathione system plays a key role in the protection against oxidative stress (Sies, 1986), and a depletion of GSH is found in many cataractous lenses (Chand *et al.*, 1982; Pau *et al.*, 1990). Under conditions of oxidative stress GSH will be oxidized and partially released from the lens (Beutler & Srivastava, 1974). Thus to maintain normal GSH contents an active synthesis is required. In the present paper we report changes that occur in the characteristics of GSH synthesis in the lens during aging. In young mice or rats there is an active synthesis of GSH from methionine. However, in old animals methionine is not a precursor of GSH in the lens. We found that this is due to the absence of detectable cystathionase activity in lenses of old animals. In addition, Sethna *et al.* (1983) observed a loss in α-glutamylcysteine synthetase activity in old human lenses. Therefore it is important to find means of increasing GSH contents in lenses in a way that is independent of the activity of cystathionase or of α-glutamylcysteine synthetase. More recently, Anderson *et al.* (1985) observed that, although GSH is unable to enter the cells of most tissues (Hahn *et al.*, 1978), GSH monoethyl ester enters many cell types. We found that GSH monoethyl ester served as an efficient GSH precursor in lenses from both young and old animals. Furthermore, this ester prevented both the GSH depletion and the malondialdehyde formation that occurred in lenses incubated with t-butyl hydroperoxide.

MATERIALS AND METHODS

Animals

Eye lenses were obtained from Wistar rats or Swiss mice of different ages. Rats that were 3–6 months old were considered as young rats, and 22–26-month-old ones were considered as old rats. Mice that were 3–6 months old were considered as young mice, and 20–22-month-old mice were considered as old mice. In all cases animals had free access to food and water and were maintained on a 12 h-light–12 h-dark cycle.

Incubation of eye lenses and metabolite analysis

Lenses were incubated in Krebs–Henseleit saline solution (Krebs & Henseleit, 1932) containing 10 mM-glucose for 15 or 30 min.

GSH depletion was achieved by incubating lenses in Krebs–Henseleit saline with 0.5 mM-diethyl maleate for 8 min. This was effective in significantly depleting the GSH content. After incubation with diethyl maleate, the lenses were washed twice with fresh Krebs–Henseleit saline and were then incubated in the presence of suitable precursors. Diethyl maleate concentration was measured by using its ability to conjugate with GSH catalysed by glutathione *S*-transferase; by this test it was not detectable in the medium after washing out the lenses. The rate of GSH synthesis was calculated from the GSH contents obtained at different incubation periods. We found that, under our incubatin conditions, GSH synthesis proceeded linearly for at least 30 min. Incubations were terminated by the addition of $HClO_4$ (final concn. 3%, v/v). GSH was measured as described by Brigelius *et al.* (1983). ATP was determined by the method of Lamprecht & Trautschold (1963), and malondialdehyde was measured as thiobarbituric-reacting material (Buege & Aust, 1978). GSH monoethyl ester was synthesized as described by Anderson *et al.* (1985) and analysed by the method of Fariss & Reed (1987).

RESULTS

Effect of age on GSH and ATP contents of rat lenses

The GSH content of lenses freshly obtained from young rats was 2.8 ± 0.6 μmol/g ($n = 4$). This value fell significantly ($P \leqslant 0.05$) in old rats to 1.8 ± 0.5 μmol/g ($n = 9$). To test the metabolic competence of the lenses, we measured their ATP contents: lenses from young rats contained 0.9 ± 0.2 μmol of ATP/g ($n = 6$) and those from old rats contained 1.2 ± 0.3 μmol of ATP/g ($n = 9$) ($P \leqslant 0.05$).

GSH synthesis in lenses from young and old animals

Fig. 1 shows the rate of GSH synthesis in lenses of young and old rats and mice. The rates of GSH synthesis were calculated from GSH concentration in lenses previously depleted of GSH (see the Materials and methods section) upon incubation with precursors for different periods of time. Lenses from young rats or mice readily synthesize GSH from methionine (1 mM) and serine (1 mM). The rates of GSH synthesis observed were 32±

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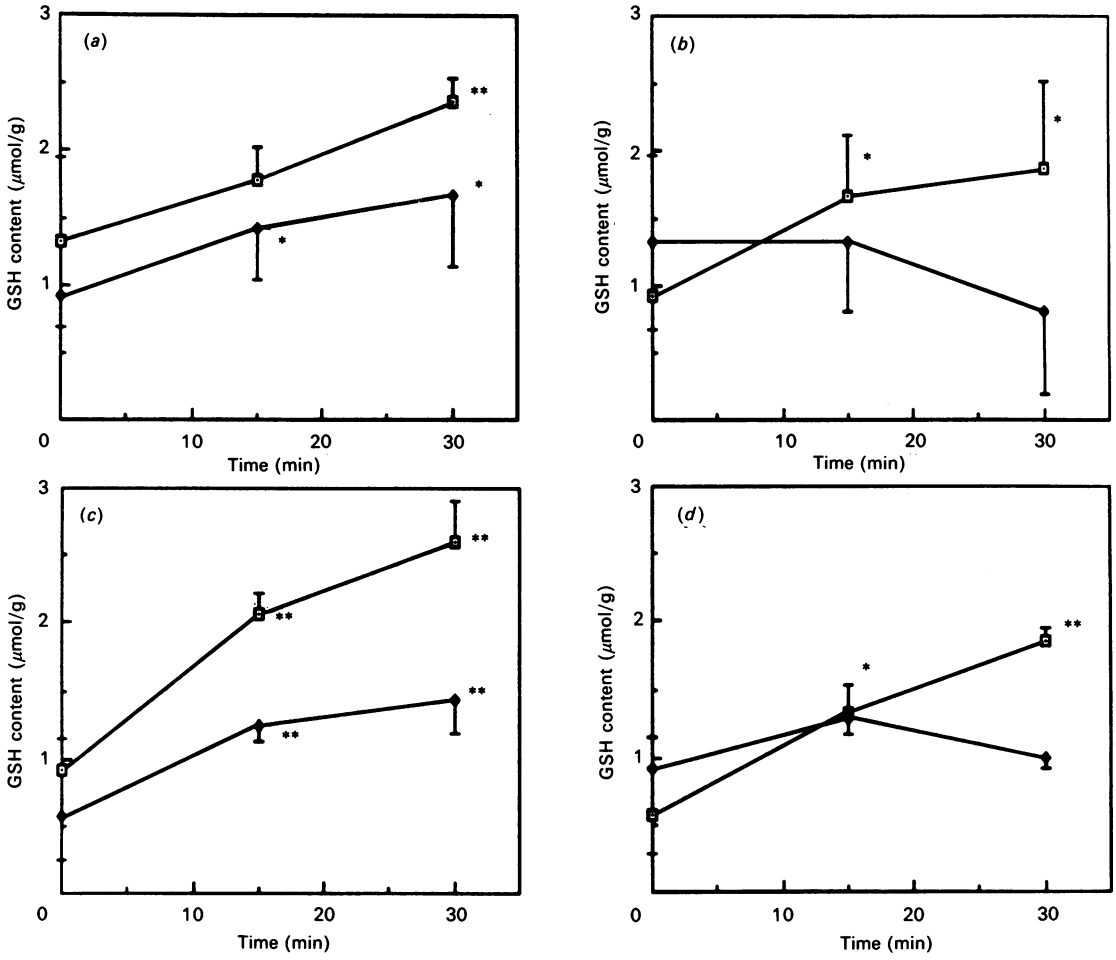

Fig. 1. GSH synthesis in lenses of young and old rats [(*a*) and (*b*)] and mice [(*c*) and (*d*)]

Lenses were incubated in Krebs–Henseleit saline for 15 or 30 min with either glutamine (1 mM), glycine (1 mM), methionine (1 mM) and serine (1 mM) [(*b*) and (*d*): ⊡, young animals; ◆, old animals] or glutamine (1 mM), glycine (1 mM) and *N*-acetylcysteine (1 mM) [(*a*) and (*c*): ◆, young animals; ⊡, old animals] as GSH precursors. Each value represents the means ± S.D. for four to twelve lenses. Significance of differences from the initial value was tested by Student's *t* test: $*P < 0.05$; $**P < 0.01$.

18 μmol/min per g ($n = 3$) and 42 ± 8 μmol/min per g ($n = 3$) for young rats and mice respectively (Fig. 1). However, in old animals GSH synthesis was completely abolished. [The rates of GSH synthesis in lenses from old rats and mice were −17 ± 24 μmol/min per g ($n = 3$) and 5 ± 6 μmol/min per g ($n = 3$) respectively.]

Fig. 1 also shows the rate of GSH synthesis from *N*-acetylcysteine. In contrast with methionine, *N*-acetylcysteine promoted GSH synthesis in both young and old animals. Furthermore, the rate of synthesis was higher in old than in young animals. The rate of GSH synthesis in lenses from young rats was 25 ± 16 nmol/min per g ($n = 3$), and in lenses from old rats it was 35 ± 16 nmol/min per g ($n = 3$). In a similar fashion lenses from young mice synthesized GSH at a rate of 28 ± 10 nmol/min per g ($n = 3$), and those from old mice synthesized 56 ± 9 nmol of GSH/min per g ($n = 3$).

We used *N*-acetylcysteine rather than cysteine because cysteine is highly unstable and can undergo a rapid autoxidation to yield cystine. When added to suspensions of isolated hepatocytes, cysteine is cytotoxic, promoting GSH and ATP depletion and other signs of cell damage (Viña *et al.*, 1978, 1983). We observed that cysteine toxicity is due to its autoxidation (Viña *et al.*, 1983), and Saez *et al.* (1982) found that autoxidizing cysteine yields free radicals, which are responsible for its toxicity.

Cystathionase activity in lenses from young and old rats

To explain the failure of lenses from old animals to synthesize GSH from methionine, we measured cystathionase activity in lenses from young and old rats and found that this activity is not detectable in lenses from old animals. The cystathionase activity found in young rats was 5.9 ± 2.9 nmol/min per g ($n = 3$) and in old rats it was not detectable (less than 0.5 nmol/min per g).

Effects of GSH and of GSH monoethyl ester on GSH contents of lenses from young and old rats

Table 1 shows the effect of GSH monoethyl ester on GSH contents in rat lenses pretreated with diethyl maleate (see the Materials and methods section). Incubation with GSH did not promote an increase in GSH contents in the lens, in agreement with previous findings in other organs, e.g. the liver (Hahn *et al.*, 1978). GSH contents in young rat lenses pretreated with diethyl maleate were 0.9 ± 0.3 μmol/g wet wt. ($n = 12$), and after 30 min of incubation with GSH they were 1.3 ± 0.6 μmol/g wet wt. ($n = 4$) (i.e. not significant).

In contrast, we have found that incubation of lenses from rats of different ages with GSH monoethyl ester increases the GSH contents in both young and old rats (see Table 1). The rates of GSH synthesis obtained were 31 and 33 nmol/min per g

Table 1. Effect of GSH monoethyl ester on GSH contents in lenses of young and old rats

Lenses of young and old rats were incubated in Krebs–Henseleit saline with or without 1 mM-GSH monoethyl ester. Results are expressed as means ± S.D. for the numbers of experiments in parentheses. Significance of differences from the initial value was tested by Student's t test: $*P < 0.05$; $**P < 0.01$.

	Control	GSH monoethyl ester	
Time (min)	Young	Young	Old
0	0.91 ± 0.25 (12)	0.91 ± 0.25 (12)	1.04 ± 0.62 (5)
15	1.15 ± 0.30 (3)	1.14 ± 0.36 (4)	2.03 ± 0.60* (4)
30	1.13 ± 0.19 (5)	1.85 ± 0.37** (4)	2.05 ± 0.55* (5)

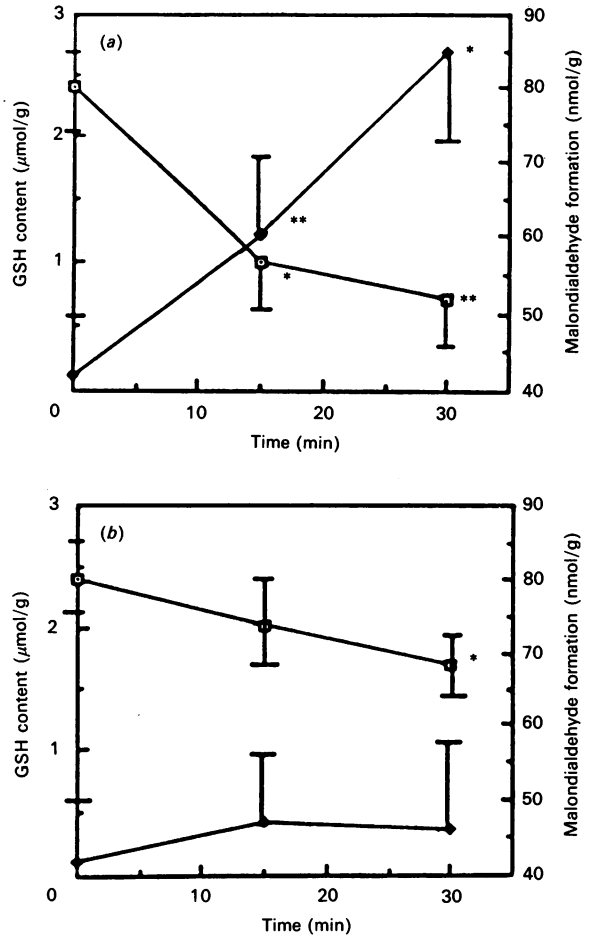

Fig. 2. Protective effect of GSH monoethyl ester on oxidative damage in lenses caused by t-butyl hydroperoxide

Lenses were incubated in Krebs–Henseleit saline (*a*) or in Krebs–Henseleit saline containing 1 mM-GSH monoethyl ester (*b*) for 15 or 30 min. Each value represents the means ± S.D. for four to twelve lenses for GSH content (□) or malondialdehyde formation (◆). Significance of differences from the initial value was tested by Student's t test: $*P < 0.05$; $**P < 0.01$.

respectively. GSH monoethyl ester was not detectable in lenses after incubation with this ester for 30 min (results not shown).

GSH monoethyl ester protects against oxidative damage caused by t-butyl hydroperoxide

Since GSH monoethyl ester served as an excellent GSH precursor in lenses from old and young animals, we tested its ability to protect the lens against oxidative damage caused by hydroperoxides. When lenses from young rats were incubated with t-butyl hydroperoxide, we observed a fall in GSH content and an increase in malondialdehyde formation by the lens (Fig. 2*a*). However, when 1 mM-GSH monoethyl ester was present in the incubation medium, t-butyl hydroperoxide did not promote a decrease in GSH or an increase in malondialdehyde formation.

DISCUSSION

Oxidative stress is a common denominator in the development of cataracts (Chylack, 1984). It causes lipid peroxidation and oxidation of crystallins, which leads to lens opacity (Bhuyan *et al.*, 1986; Taylor & Davies, 1987; Babizhayev *et al.*, 1988). The glutathione system plays a major role in the protection against oxidative stress (Sies, 1986). The lens contains high GSH contents, and in fact GSH was the first component of the lens whose synthesis was quantitatively studied (Kinsey & Merriam, 1950).

Low GSH contents occur in most, if not in all, cases of irreversible cataracts. Furthermore, GSH depletion caused by treatment with buthionine sulphoximine, a specific inhibitor of γ-glutamylcysteine synthetase (Griffith & Meister, 1979), induces the formation of cataracts in mice (Calvin *et al.*, 1986). More recently, Rikans & Moore (1988) have found that the GSH content in lens is significantly lower in old rats than in younger rats. Our results confirm that the GSH content in lens decreases with age. This may be an important factor in age-related cataractogenesis.

Methionine is an excellent, and physiologically important, precursor of GSH in organs such as the liver (Reed & Orrenius, 1977). We have found that, whereas GSH synthesis from methionine occurs at a high rate in lenses from young rats or mice, it does not take place in lenses from old animals. This may be responsible, at least in part, for the low GSH contents observed in lenses of old rats. Unlike the situation with methionine, the rate of GSH synthesis from *N*-acetylcysteine did not fall with age. Thus the decrease in GSH synthesis from methionine in rodents was due, not to an impaired synthesis of GSH from its constituent amino acids, but to an impaired trans-sulphuration pathway. We studied the activity of cystathionase in lenses from young and old animals and found that it is not detectable in lenses of old animals. This change may be important in determining the low rate of GSH synthesis under physiological conditions *in vivo*.

Rathbun and his associates, in a remarkable series of papers, studied the changes in activities of GSH-related enzymes that occur with age. These changes depend on the species studied. In mice, γ-glutamylcysteine synthetase and glutathione synthetase activities do not fall with age. This is in agreement with our finding that GSH synthesis from *N*-acetylcysteine does not fall in

old mice or rats. However, they also found that in lenses of old humans γ-glutamylcysteine synthetase activity falls to one-sixteenth of the value found in young ones (Sethna *et al.*, 1983). The low γ-glutamylcysteine synthetase activity in human lens is highly significant (Sethna *et al.*, 1983).

Thus a means to increase GSH contents in lenses in a way that is not dependent on the activity of cystathionase or that of γ-glutamylcysteine synthetase was investigated. Hockwin *et al.* (1985) observed that GSH, when added to the incubation medium in sufficient amounts, is able to penetrate bovine lenses. However, in our experiments addition of GSH to the incubation medium did not serve to increase the GSH content of the lens. This is in agreement with the fact that GSH does not enter cells from other tissues (Hahn *et al.*, 1978). The reason for these species differences remains to be investigated.

More recently, Anderson *et al.* (1985) observed that GSH monoethyl ester enters many cell types. Inside the cells the ester bond is hydrolysed, yielding free GSH intracellularly. While this work was in progress, Meister and co-workers (Martensson *et al.*, 1989) reported that intraperitoneal administration of GSH ester prevents buthionine sulphoximine-induced cataracts in newborn rodents. We have found that GSH monoethyl ester is an excellent precursor of GSH in lenses from young and old animals. Moreover, in lenses from old rats the recovery of GSH contents upon incubation with GSH monoethyl ester is faster than in lenses from young animals (see Table 1). This may be due to an increased permeability in lenses from old animals (Taura *et al.*, 1986).

t-Butyl hydroperoxide was used to induce oxidative stress in the lens by Taura *et al.* (1986). We found that incubation of lenses with t-butyl hydroperoxide induced a fall in GSH content and an increase in malondialdehyde formation in lenses. GSH monoethyl ester protected the lens against these deleterious effects. Moreover, GSH synthesis from GSH monoethyl ester does not require the involvement of γ-glutamylcysteine synthetase, the activity of which falls in lenses of old humans (Sethna *et al.*, 1983). These results suggest that GSH monoethyl ester may prevent cataractogenesis. Therefore the possible prevention of cataracts by administration of this ester may be of practical importance.

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