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# LABILIZATION OF CEREBRAL LYSOSOMES BY AUTOLOGOUS MICROSOMES AND PROTECTION BY CYTOSOLIC FRACTION AND A SUPEROXIDE SCAVENGER IN VITRO

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SUMMARY

Incubation of cerebral lysosomes with autologous microsomes resulted in labilization of the lysosomal complex as assayed through determination of lysosomal acid proteinase activity in the incubate supernatant. Addition of cytosolic fraction with significant glutathione peroxidase activity largely prevented the labilization whereas an artificial scavanger of active oxygen (benzoic acid) was not as effective. Frozen and thawed lysosomes served as controls. The labilization of lysosomes by microsomal oxygen activation mechanism in the absence of exogenous substrate may explain neuronal degeneration in cases of induction of the oxidative activity without generation of reactive metabolites from the exogenous substrate.

Microsomal mechanisms for the activation of molecular oxygen produce superoxide anions in liver even in the absence of exogenous substrates (1). This production increases in cases of induction of the microsomal monooxygenase, and addition of substrates for the oxygenases diminish the release of activated oxygen species in the incubation medium (1). Activation of molecular oxygen takes place also in brain cells although they contain 10-20 times less the specific oxidative capacity than the liver cells (2). Therefore, the cerebral microsomes can oxidize various substrates which can eventually form reactive metabolites of possible toxicological significance (3). Furthermore, cerebral oxidative metabolism can be enhanced by powerful inducers of the microsomal monooxygenase (4). The activated species of molecular oxygen are reactive and they

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may participate in lipoperoxidation when they escape from the original oxidation reaction (for review, see ref. 5). Most cells contain therefore superoxide dismutase (E.C. 1.15.1.1) which reduces superoxide anion by one electron in a dismutation reaction to form hydrogen peroxide anion. It is reduced further to water by catalase or glutathione peroxidase (E.C. 1.11.1.9). Cerebral glial cells contain superoxide dismutase and glutathione peroxidase activities (6) as would be expected because two enzymes should be needed to provide protection against the reactive oxygen molecules (7). To test this hypothesis cerebral lysosomes have been incubated in vitro with autologous microsomes together with added glutathione peroxidase activity or with an artificial superoxide scavenger.

## MATERIALS AND METHODS

Lysosome-enriched fraction was isolated from the brain of 25 male Wistar rats (8). Cerebral microsomes (9) and cytosolic fractions were also obtained from the same animals. Lysosomal acid proteinase (E.C. 3.4.23.5, ref. 10) activity increased in the enriched fraction 10-fold, and a superoxide dismutase (11) activity of 30.5 ± 2.4 nmol/min x mg protein (N = 25, ± S.D.) could be detected in the microsomal fraction. The glutathione peroxidase (12) activity in the soluble fraction was 45.2 ± 4.6 nmol/min x mg protein (N = 25, ± S.D.). The lysosomal fractions (0.8 mg protein) were incubated in vitro for 20 min at 37°C with 55.6 µM NADPH (final concentration), or with NADPH and autologous microsomes (1 mg microsomal protein), or with NADPH, microsomes, glutathione (48.9 µM, final concentration) and 1 ml of the cytosolic fraction (2 mg protein). Incubation with 44.4 µM benzoic acid (final conc.) and microsomes plus NADPH and incubation of frozen and thawed lysosomes served as controls. The incubations were terminated by ultracentrifugation at 105,000 g and 4°C for 30 min. The supernatants were retained and analyzed for the acid proteinase activity. Statistical analyses were made with the Student's t-test.

## RESULTS AND DISCUSSION

Incubation with microsomes plus NADPH caused increasing acid proteinase activity in the medium according to the incubation time. The increase in the enzyme activity appeared linear up to

30 min in these assay conditions (coefficient 8.4 nmol x $min^{-2}$ x $mg^{-1}$ protein). The enzyme activity released during incubation of the frozen and thawed lysosomes can be thought to represent the total enzyme activity, and it would agree with the linear coefficient. The lysosomal fraction was not entirely pure as incubation with NADPH alone caused some release of proteinase activity (Table 1). The addition of glutathione and glutathione peroxidase-containing fraction reduced very significantly the release of the proteinase whereas incubation with benzoic acid was not as effective (Table 1).

The present data indicate that the cerebral microsomes can labilize lysosomes also in the absence of exogenous substrates for the oxidative reaction. The labilizing molecules can be related to activated molecular oxygen as the effect can be reduced by a superoxide scavenger. Superoxide dismutase activity alone cannot prevent the lysis. The protection provided by the glutathione peroxidase-containing fraction is in keeping with the necessity of inactivation of hydrogen peroxide in order to prevent secondary generation of singlet oxygen or hydroxyl radical from superoxide and hydrogen peroxide anions (7).

The present findings can also provide a tempting explanation for the toxicity of strong inducers of the oxidative metabolism which do not form reactive metabolites (4). Increased generation of active oxygen could be thus directly harmful, e.g. in the nerve cells,without the participation of reactive compounds from the oxidative metabolism. This could explain e.g. neuronal degeneration in the later stages of 2,3,7,8-tetrachlorodibenzo-p-dioxin intoxication (13). The reduction of superoxide dismutase activity in cells of old animals (14, 15) assumes

Table 1

Labilization of Cerebral Lysosomes by Autologous Microsomes in vitro

| | Treatment | | | | |
|---|---|---|---|---|---|
| | Microsomes NADPH | Microsomes NADPH Cytosolic fraction Glutathione | Microsomes NADPH Benzoic acid | NADPH | Freezing and thawing |
| Acid proteinase in the supernatant (nmol/min x mg protein) | 173 ± 10[a] | 12 ± 0.5[b] | 53 ± 8.6[c] | 37 ± 3.7 | 308 ± 46[a] |

Isolated lysosomes were incubated with microsomes plus NADPH for 20 min at 37°C and the medium was assayed for acid proteinase activity. The results were compared to similar incubations with added cytosolic fraction or benzoic acid. Frozen and thawed lysosomes and incubation with NADPH alone served as controls. Each figure is the mean of five experiments ± S.D.

[a]Differs from NADPH-treated at $P < 0.001$

[b]Differs from NADPH-treated at $P < 0.001$ and from Microsome plus NADPH-treated at $P < 0.001$

[c]Differs from NADPH-treated at $P < 0.01$ and from Microsome plus NADPH-treated at $P < 0.001$

also a very important role in view of the present results as it would allow increased peroxidative damage.

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