Brain Glutathione and the Anti-Hypoxic Effect of Glutathione Depletors in Mice

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Abstract—The anti-hypoxic effect (assessed by a standard hypoxia survival test) 3 hr after 2-cyclohexene-1-one (CHX) was completely abolished by injections of L-cysteine after CHX. Under these conditions, brain GSH was depleted to about 50% of the control level in CHX-treated mice and recovered to about 80% of control level following L-cysteine post-treatment. However, D-cysteine could not cause such effects. Intracerebroventricular injection of CHX, which caused a selective depletion of brain GSH, produced an anti-hypoxic effect. Thus, the anti-hypoxic effect may be related to the decrease of brain GSH levels.

In our previous report in which an anti-hypoxic effect was assessed using a hypoxia survival test (1), CHX and phorone, which depleted cerebral GSH, possessed a marked anti-hypoxic effect, but buthionine sulfoximine caused neither an anti-hypoxic effect nor the depletion of brain GSH. With regard to a possible mechanism for the anti-hypoxic effect caused by the GSH depletors, we suggested that the elevation of blood glucose may be involved in the anti-hypoxic effect in an early stage after injection of the GSH depletors (1). However, the anti-hypoxic effect could not be explained by the elevated blood glucose alone, since the effect persisted to a later stage (3 hr after treatment) when recovery of blood glucose and maximum depletion of cerebral GSH occurred. These findings led us to assess a possible relationship between the depletion of cerebral GSH and the anti-hypoxic effect of the GSH depletors.

The animals used were male mice (5–6 weeks old) of the ICR strain weighing 25–30 g. Intracerebroventricular administration of CHX was done according to the method of Haley and McCormick (2). A hypoxia survival test by exposure of normobaric hypoxic gas (96% N₂, 4% O₂) and assay of nonprotein sulfhydryls were carried out according to the previously reported method (1). Regarding a

separative assay of GSH and cysteine, GSH and cysteine, derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate, were separated on a HPLC column and detected fluorometrically according to the method of Toyooka and Imai (3).

When mice were exposed to hypoxic gas 3 hr after CHX (100 mg/kg, i.p.), the survival time was markedly prolonged (Table 1). The anti-hypoxic effect of CHX was completely abolished by two injections of L-cysteine (500 mg/kg, s.c.) after CHX. However, the treatment of D-cysteine (500 mg/kg, s.c.) did not affect the anti-hypoxic effect of CHX. L- and D-Cysteine themselves (500 mg/kg, s.c.) did not possess an anti-hypoxic effect (T. Masukawa et al., unpublished data). Under the same conditions, when mice received L-cysteine after treatment of CHX, cerebral GSH depleted to about 50% of the control values in CHX-treated mice recovered to about 80% of the control. However, D-cysteine which is not utilized as a precursor for GSH synthesis did not exhibit such an effect. Similar changes were found in the hepatic GSH of mice receiving L- or D-cysteine with CHX. Additionally, while cysteine could be barely detected in mice treated with L- or D-cysteine, cysteine was observed to be accumulated in considerable amounts in the brain in comparison with the liver. These results are likely

Table 1. Influence of cysteine treatment on the anti-hypoxic effect and the decrease of GSH induced by CHX in mice

Treatment	Survival time ^a (min)	Brain		Liver	
		GSH ^b (μ mol/g tissue)	Cysteine ^b	GSH ^b (μ mol/g tissue)	Cysteine ^b
Vehicle (saline)	6.18 $\pm$ 0.35	2.12 $\pm$ 0.06	N.D.	8.72 $\pm$ 0.59	N.D.
CHX	7.79 $\pm$ 0.66*	1.17 $\pm$ 0.03**	N.D.	7.64 $\pm$ 0.62	N.D.
+ L-Cysteine	5.15 $\pm$ 0.35**	1.66 $\pm$ 0.08***	0.52 $\pm$ 0.04	8.18 $\pm$ 0.30	0.19 $\pm$ 0.05
+ D-Cysteine	10.09 $\pm$ 1.23**	1.12 $\pm$ 0.02**	0.76 $\pm$ 0.01	5.84 $\pm$ 0.32***	0.52 $\pm$ 0.05

To assay the anti-hypoxic effect, mice were exposed to hypoxia 3 hr after CHX (100 mg/kg, i.p.). L- or D-Cysteine (500 mg/kg, s.c.) was injected 1 and 2 hr after CHX. For the assay (HPLC method) of GSH and cysteine, mice were killed 3 hr after CHX. ^amean $\pm$ S.E.M. of 10 mice, ^bmean $\pm$ S.E.M. of 5 mice. N.D.: Not detectable. Significantly different from the vehicle (* P <0.05, ** P <0.01), significantly different from CHX (* P <0.05, *** P <0.01).

Table 2. Brain GSH levels and anti-hypoxic effect in mice with intracerebroventricular injection of CHX

Treatment (μ g)	Brain GSH ^a (μ mol/g tissue)	Survival time ^b (min)
Vehicle (saline)	2.44 $\pm$ 0.07	6.23 $\pm$ 0.51
CHX 125	2.03 $\pm$ 0.06**	7.28 $\pm$ 0.83
250	1.92 $\pm$ 0.08**	7.70 $\pm$ 0.75
500	1.80 $\pm$ 0.09**	9.51 $\pm$ 1.14*
1000	1.61 $\pm$ 0.07**	10.42 $\pm$ 1.67*

Mice were killed 1 hr after treatment and GSH was determined (Ellman method). For the test of anti-hypoxic effect, mice were exposed to hypoxia 1 hr after treatment. ^amean $\pm$ S.E.M. of 5 mice, ^bmean $\pm$ S.E.M. of 10 mice. Significantly different from the vehicle (* P <0.05, ** P <0.01).

to reflect the lower turnover rate of GSH in the brain as compared to that in the liver.

Since selective GSH depletion in the brain could not be achieved by systemic administration of each GSH depletor tested (4), unknown actions occurring via the depletion of GSH in peripheral tissues can not be excluded as one of the mechanisms for the anti-hypoxic effect of the GSH depletors. So, we attempted an intracerebroventricular application of CHX which is soluble in water (4). As shown in Table 2, the intracerebroventricular injection of CHX at doses of 500 and 1000 μ g (in a volume of 10 μ l) caused an anti-hypoxic effect at 1 hr after dosing when maximum depletion of brain GSH was found (4). At higher doses (500 and 1000 μ g), clonic seizure was observed immediately after injection, but disappeared completely within 1 min. The slight sensitivity to touch was gradually increased until 0.5 hr after

dosing (1000 μ g) and later disappeared. Under the same conditions, the depletion of cerebral GSH was produced dose-dependently, but hepatic GSH was not altered at all.

The present findings suggest that the depletion of brain GSH rather than CHX itself may be related to the anti-hypoxic effect. We reported that the elevated blood glucose by CHX may be involved in one of the mechanisms in the early stage of the anti-hypoxic effect (1). Hyperglycemia is expected to result in the increased supply of glucose into the brain, enhancing energy reserves in the brain. In fact, CHX increased brain glucose and ATP levels and decreased lactate production and the lactate pyruvate ratio at 0.5 hr under normal conditions, the latter changes suggesting a decrease of the glycolytic rate (T. Masukawa et al., unpublished data). In this respect, the injection of glucose increased the brain glucose level in mice, whereas the

energy metabolites except for glucose were not affected under normal conditions (5). Additionally, Gilbert (6) reported that glycolysis is regulated in response to a changing GSH/GSSG ratio, since phosphofructokinase, a key enzyme in glycolysis, is rapidly inactivated with GSSG. Considering that the maximum depletion of brain GSH was found at 1 hr and 3 hr after intracerebroventricular and intraperitoneal injection of CHX, respectively, alteration of the brain GSH/GSSG ratio via GSH depletion may be thought to affect glycolysis in the brain. Thereafter, to ascertain whether the depletion of brain GSH may lead to the development of the anti-hypoxic effect through the alteration of brain energy metabolism, it is necessary to investigate the relationship between energy metabolism and GSH depletion in the brain.

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