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MERCAPTOPYRIDOXINES, HYPOXIA AND PLASMA ENZYMES

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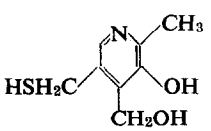
Abstract—5-Mercaptopyridoxin a radioprotective thiol induces in the rat a large and rapid increase in the level of five enzymes normally concentrated inside the cell: lactic dehydrogenase, malic dehydrogenase, glutamic dehydrogenase, glutamo-oxaloacetic transaminase and β-glucuronidase. 4-Mercaptopyridoxin, a non protective thiol, does not alter the level of these enzymes in the plasma.

Exposure for 18 min to severe hypoxia (95% N_2 + 5% O_2) also increases the level of these enzymes in the plasma. The increase is rapid but not long lasting. These data show that both chemical protectors and hypoxia induce rapid but reparable cellular lesions which may have some importance for the mechanism of increased resistance to ionizing radiation.

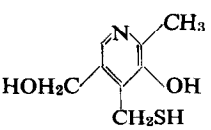
CYSTEAMINE (β-mercaptoethylamine MEA) a well-known radioprotective substance induces in a few minutes important metabolic changes[1–3] and lesions of the mitochondria and endoplasmic reticulum easily recognised in the electron microscope.[4,5] In the rat it increases the concentration in the plasma of all intracellular enzymes tested: lactic, malic and glutamic dehydrogenases, oxaloacetic transaminase and β-glucuronidase.[6] β-Mercaptoethanol which does not protect the mammal against ionizing radiation, does not induce these subcellular lesions[5] nor change the level in the plasma of the above mentioned enzymes.[7] Our working hypothesis[1, 8] is that these metabolic troubles (biochemical shock) play a dominant role in the radioprotection.

In order to confirm that the correlation between radioprotection, subcellular lesions and liberation of cellular enzymes in the plasma is not fortuitous, we have used a pair of isomeric thiol substances one of which (5-mercaptopyridoxin) is a protective agent inducing mitochondrial lesions (according to the late R. Koch and H. Braun) while the other (4-mercaptopyridoxin) does not protect nor induce subcellular lesions.

We have also, for the sake of comparison, investigated what happens to the plasma enzymes after exposure to severe anoxia (5% 0_2 + 95% N_2) during 18 min.



5-Mercaptopyridoxin



4-Mercaptopyridoxin

MATERIALS AND METHODS

Male Wistar rats (180–200 g) were grouped in batches of 20 or 30 and killed at different times (from 3 to 120 min) after intraperitoneal injection of 250 mg/kg 4- or 5-MP* (Merck) or after exposure for 18 min to severe anoxia (95% N_2 + 5% O_2). The solutions of the mercaptopyridoxines were freshly prepared at pH 6·5 in NaCl 0·9%. The activity of the five enzymes tested has been measured according to the techniques previously described.[6] Student's *t*-test has been used for the statistical analysis of the results.

RESULTS

Results are shown in Fig. 1 and Table 1. Invariably 5-MP induces rapid and considerable increase in plasma enzymes activity while 4-MP is strictly devoid of action. When the effects of 5-MP are compared to those cysteamine[6] there is no question that the action of 5-MP is more rapid and less permanent than that of MEA. For instance

TABLE 1. CHANGES IN ACTIVITIES OF SOME PLASMA ENZYMES IN THE RAT AFTER EXPOSURE TO SEVERE HYPOXIA (5% P_2 + 95% N_2 DURING 18 min)

Time (min)	Enzymes mIU				
	LDH	MDH	GlDH	GOT	β-Gl
0	242·00 ± 94·00	424·00 ± 130·00	0·92 ± 0·58	102·00 ± 60·00	0·80 ± 0·41
3	820·00† ± 110·00	1020·00† ± 241·00	0·94 ± 0·39	154·00 ± 62·00	1·20 ± 0·44
10	940·00† ± 130·00	9575·00† ± 281·00	0·87 ± 0·31	190·00 ± 45·00	0·94 ± 0·37
25	725·00† ± 110·00	1124·00† ± 187·00	1·92* ± 0·30	240·00* ± 61·00	1·68* ± 0·42
45	495·00* ± 105·00	840·00* ± 149·00	0·94 ± 0·40	110·00 ± 49·00	0·88 ± 0·67
60	249·00 ± 124·00	620·00 ± 210·00	0·89 ± 0·32	140·00 ± 60·00	0·79 ± 0·44
120	360·00 ± 120·00	480·00 ± 180·00	1·00 ± 0·42	162·00 ± 92·00	0·84 ± 0·74

* $P < 0·05$.
† $P < 0·01$.

with 5-MP the maximum in MDH increase is reached in 25 min and only in 2 hr with MEA. As far as LDH, GlDH, GOT and β-Gl are concerned the maximum after 5-MP is reached in 45 min while with MEA one must wait 2 hr to obtain it.

The return to normal plasma concentrations is also much more rapid after 5-MP than after MEA at least in the case of MDH, GOT and β-Gl.

After 18 min of hypoxia the level of GOT, MDH and LDH rises after a further 3 min and the maximal concentrations of MDH and LDH is reached within 10 min. GlDH, GOT and β-Gl rise more slowly and reach a maximum in 25 min. The return to normal is faster than after injection of 5-MP.

DISCUSSION

The correlation between radioprotection, mitochondrial lesion and leakage in the plasma of intracellular enzymes is confirmed. There is certainly no better control for the radioprotective 5-mercaptopyridoxin than the inactive 4-mercapto derivative.

*Abbreviations used: 5-MP 5-Mercaptopyridoxin, 4-MP 4-Mercaptopyridoxin, LDH Lactic dehydrogenase, MDH Malic dehydrogenase, GlDH Glutamic dehydrogenase, GOT Glutamo-oxaloacetic transaminase, β-Gl β-Glucuronidase.

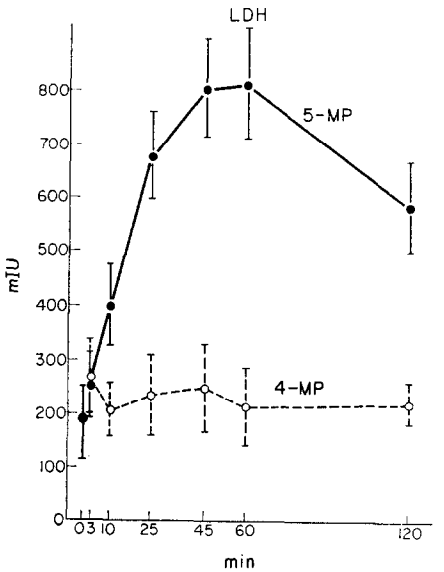


FIG. 1A

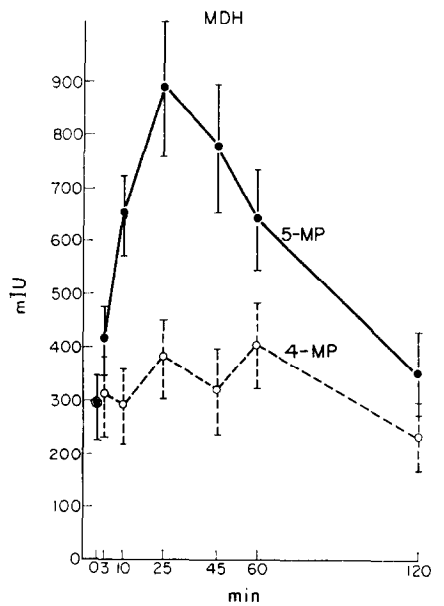


FIG. 1B

FIG. 1. Plasmatic activities of some enzymes, LDH (a), MDH (b), G1DH (c), GOT (d) and β-G1 (e), after i.p. injection of 5- or 4-Mercaptopyridoxines. Ordinate: value in mIU. Abcissa: time after the intraperitoneal injection in minutes. At 0 min values for controls injected with NaCl 0·9%. For the animals injected with mercaptopyridoxines the standard deviations refers to group of animals sacrificed at the same time after injection. For control animals (0 min) the standard deviation refers to animals sacrificed from 3 to 120 min after NaCl injection.

Figure 1 continued overleaf

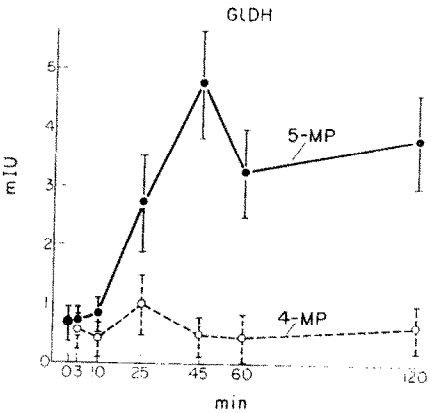


FIG. 1C.

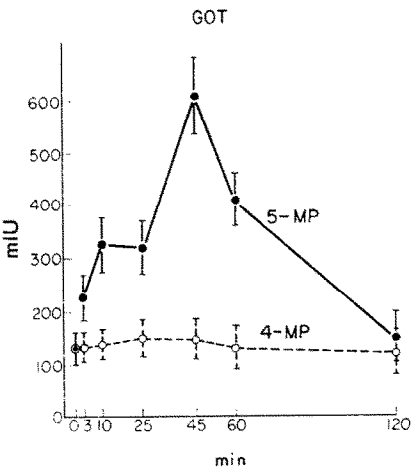


FIG. 1D.

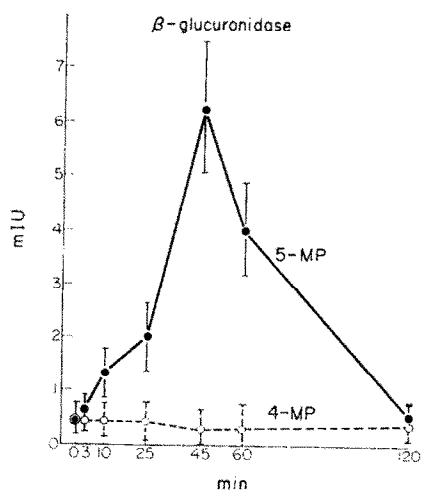


FIG. 1E.

The leakage in the plasma concerns enzymes located in different positions inside the cell: cell sap (supernatant), mitochondria or lysosomes. One has the impression that if we had chosen any other intracellular enzymes we would have obtained the same results. In fact, it has already been observed that aldolase and cathepsines behave like the five enzymes mentioned above after cystamine[10] injection.

We know that in both the rat and mouse the radioprotection is not maximal before 10 min after the intraperitoneal injection of cysteamine.[11] The results shown above seem to indicate that 5-MP might induce radioprotection more rapidly but no experiments have been done so far to investigate whether this is so: it seems that in Koch's experiments 5-MP was always injected 5 min before irradiation.[12]

The type of hypoxia which we have used is classical in radiobiological research, but one should not forget that it is a severe trauma and that much more happens in the body of a hypoxic mammal than simply a decreased O_2 pressure within the tissues. Our findings indicate that one cannot neglect the damage inflicted to the cells in the interpretation of the effects of hypoxia, since this trouble has common features with the shock induced by radioprotectors, one might speculate that the bodily changes in the hypoxic animal may contribute with the decreased O_2 pressure to the well-known increased in radioresistance (see ref. 1).

It would not be logical to expect an exact time correlation between the intensity of the protection against ionizing radiation and the changes in the plasma level of enzymes. This irruption in the blood of cell constituents is merely a secondary sign, a consequence of the primary intracellular damage which in our sense is the important factor for reaction to ionizing radiation.

Unfortunately it is difficult, if not impossible to separate cell damage from increased plasma enzymatic activities in order to estimate the contribution to the biochemical shock of the high level of cellular enzymes circulating in the blood. How could one imitate by intravenous injection of concentrated enzymes what happens in the blood of a rat injected with a radioprotector or exposed to hypoxia? One would be obliged to make an artificial choice of enzymes and many trials would be needed to reproduce the shape of the changes observed in the plasma.

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