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EFFECT OF SODIUM SELENITE ON GUINEA-PIG CARDIAC ISOLATED MITOCHONDRIA

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Abstract—The pre-incubation with sodium selenite reduces the respiratory index in guinea-pig cardiac mitochondria when α-ketoglutarate and glutamate are used as substrates. This decrease is dose-dependent. On the other hand, no modification in respiratory index is measured with malate or malate plus pyruvate. The effect on sodium selenite is prevented by CoA administration. Moreover, 25 μM sodium selenite reduces the K_m and V_{max} of α-ketoglutarate dehydrogenase measured at steady-state with variable amount of CoA.

Selenium is an essential element [1]; in fact there are numerous selenium-dependent enzymes [2–4]. This element was previously found to be of biological importance because of its toxic properties [5], but despite the extent of selenium poisoning in animals little information is available on the pathogenetic mechanism of toxicity. Our previous work *in vitro* and *in vivo* has in fact demonstrated that sodium selenite produces myocardial mitochondria damage in guinea pig, these lesions being characterized by swelling, disruption of cristae and marked cavitation of space [6–8]. Sodium selenite-induced myocardial mitochondria alterations are dose-dependent, rapid in onset and long-acting [8]; they are prevented *in vivo* and *in vitro* by pyruvate administration [6, 7]. It therefore seems interesting to evaluate the effect of sodium selenite on isolated mitochondria.

MATERIALS AND METHODS

Male guinea pigs were killed and the heart immediately removed, immersed in an ice-cold isolation medium (250 mM sucrose, 1 mM K_2 EDTA, 50 mM Tris, pH 7.4) and the blood removed. For each experiment ventricular myocardium obtained from two to six hearts was pooled to isolate mitochondria. The isolation was carried out at 4°, according to Sobel *et al.* [9]. Finally mitochondria were suspended in 0.25 ml of isolation buffer/g tissue and the concentration of protein was estimated by Lowry *et al.*'s method [10]. Oxidative and phosphorylation processes in the isolated myocardial mitochondria were measured using a polarographic method involving an oxygraph with a Clark electrode at 25°. Mitochondrial protein (0.81 mg) was incubated in 2 ml of medium containing 250 mM sucrose, 1 mM K_2 EDTA, 50 mM Tris, 7.5 mM potassium phosphate buffer and 1% of fatty acid-free bovine albumin previously dialyzed. The substrates were: glutamate (10 mM); α-ketoglutarate (5 mM); malate and malate plus pyruvate, 5 and 10 mM, respectively. Finally, ADP (300 μM) was added to the vessel.

The following parameters were calculated: (1) respiration of mitochondria (QO_2): in state S_3, the consumption of oxygen is expressed in n atoms O_2/mg protein/min, after addition of ADP to reaction vessel; likewise in state S_4 without ADP; (2) Respiratory Control Index (RCI) is the ratio of QO_2 in state S_3 to QO_2 of mitochondria in state S_4 and (3) coefficient of oxidative phosphorylation (ADP/O_2) is the ratio of nmoles ADP added to n atoms of oxygen consumed in state S_3.

When the effect of sodium selenite was tested, the mitochondria were pre-incubated with different concentrations of sodium selenite for 10 min before addition of substrate and ADP. All analyses were performed in parallel samples. Means and standard errors were calculated for each group. Differences in the means of control and experimental groups were assessed using Student *t*-test. A P value of 0.05 was accepted as statistically significant.

In order to measure α-ketoglutarate dehydrogenase activity (α-KGDH), mitochondria were prepared as previously described and were resuspended in potassium phosphate buffer (0.1 M), pH 7.2 and subjected to freezing and thawing twice. The thawed suspension was centrifuged at 18,000 *g* 30 min according to Garland [11]. The supernatant fluid was removed and assayed for α-KGDH activity. The overall activity α-KGDH complex was determined by spectrophotometrically monitoring NADH formation at 340 nm and 25°. The assay medium (1 ml) contained 0.107 mg protein, 50 mM NAD and CoA at different concentrations; after 5 min of pre-incubation the reaction was started by adding 2 mM α-ketoglutarate (control experiment). The effect of sodium selenite on the enzyme activity was tested after 5 min pre-incubation with sodium selenite 25 μM. The activity is expressed as nmoles/mg protein of NADH formed/min evaluated as initial

reaction rate. NAD, reduced glutathione and CoA were purchased from Boehringer (Mannheim, F.R.G.); α-ketoglutarate acid and sodium selenite were obtained from Merck (Darmstadt, F.R.G.); malic acid from BDH (Poole, U.K.); fatty acid-free bovine albumin, L-cystein and glutamic acid from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade.

RESULTS

The decrease in RCI induced by sodium selenite with α-ketoglutarate is dose-dependent (Fig. 1) and can be attributed to reduction in respiration during state S_3 (Table 1); ADP/O is not, however, modified. The effect of 10^{-4} M sodium selenite has been studied as a function of incubation time. The addition of sodium selenite immediately before or soon after the administration of substrate produces the same inhibitory effect on the rate of oxygen consumption as 5 min of pre-incubation. While 10 min of pre-incubation before the addition of α-ketoglutarate produces a 20% increase in the inhibitory effect of sodium selenite. The addition of sodium selenite during state S_3 does not modify the rate of oxygen consumption. Also in the presence of glutamate as substrate, sodium selenite pre-incubation dose-dependently reduces the RCI, becoming significant at 10^{-6} M of sodium selenite ($0.05 \geqslant P \geqslant 0.01$), but reaching $47.2 \pm 7\%$ with 10^{-4} M sodium selenite (data not shown).

It is known that α-ketoglutarate dehydrogenase utilizes NAD and CoA during the reaction [12], thus it seemed worthwhile to evaluate whether the addition of CoA and NAD is able to prevent the inhibitory effect of sodium selenite. Ten minutes of pre-incubation with 1 mM NAD does not alter the inhibitory effect of 10^{-4} M sodium selenite on RCI measured in isolated mitochondria. On the other hand, the addition of CoA, dose-dependently, prevents the decreases in RCI induced by 10^{-4} M sodium selenite, as shown in Table 2, the RCI value obtained with the highest doses of CoA in the presence of sodium selenite being over the control value in the absence of CoA. Other sulphydryl compounds, such as glutathione and cysteine have been tested in order to determine if the protection of CoA is specific or merely reflects a reaction of sodium selenite with SH

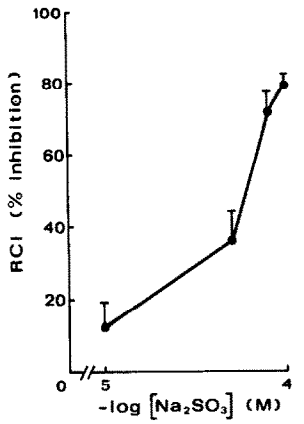

Fig. 1. Effect of sodium selenite on RCI. The experiments are performed in mitochondria isolated from guinea-pig heart in the presence of 5 mM α-ketoglutarate. Results are mean ± S.E. of four experiments.

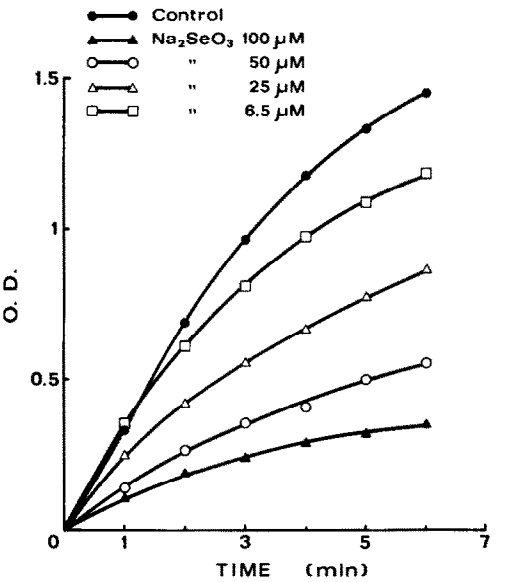

Fig. 2. The time dependent inactivation of α-ketoglutarate dehydrogenase complex. 0.2 mg/ml protein are pre-incubated for 5 min with different concentration of sodium selenite. NAD is 5 mM, α-ketoglutarate 2 mM and CoA 285 μM.

Table 1. Effect of sodium selenite 10^{-4} M on oxygen consumption of guinea-pig heart mitochondria in presence of various substrates

Substrate	*N*	$QO_2(4)$	$QO_2(3)$	RCI	ADP/O
Malate 10 mM + pyruvate 5 mM	5	85.5 ± 11.0	336.0 ± 38.8	4.1 ± 0.2	2.4 ± 0.1
Malate 10 mM + pyruvate 5 mM + Na_2ScO_3	5	80.6 ± 11.3	303.6 ± 42.9	3.8 ± 0.1	2.5 ± 0.1
Malate 5 mM	6	37.8 ± 4.4	125.7 ± 13.4	3.4 ± 0.2	4.0 ± 0.5
Malate 5 mM + Na_2SeO_3	6	42.7 ± 5.3	126.0 ± 17.9	2.9 ± 0.1	4.2 ± 0.5
Glutamate 10 mM	5	32.2 ± 4.9[a]	217.0 ± 18.5[b]	5.9 ± 0.3[a]	2.5 ± 0.3
Glutamate 10 mM + Na_2SeO_3	5	46.7 ± 4.8[a]	146.9 ± 26.2[b]	3.1 ± 0.5[a]	2.3 ± 0.2
α-Ketoglutarate 5 mM	4	40.0 ± 1.5	142.4 ± 9.3[c]	3.5 ± 0.3[c]	2.9 ± 0.1
α-Ketoglutarate 5 mM + Na_2SeO_3	4	34.5 ± 1.3	56.5 ± 5.5[c]	1.5 ± 0.1[c]	2.7 ± 0.1

* n atoms of oxygen/min/mg protein.
N = number of experiments.
Values are mean ± S.E. Statistical analysis: a, $P < 0.01$; b, $P < 0.05$; c, $P < 0.001$.

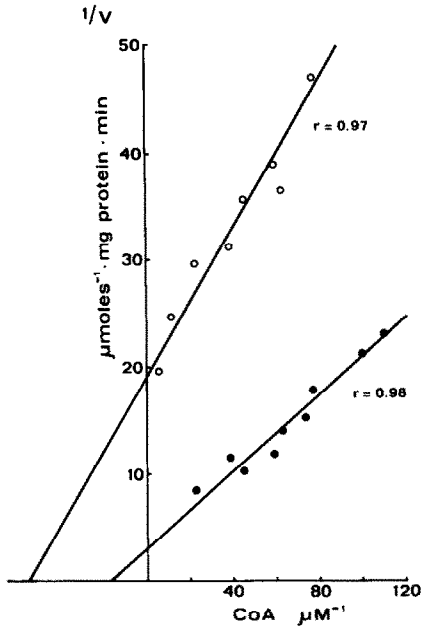

Fig. 3. The effect of 25 μM sodium selenite (°) on α-ketoglutarate dehydrogenase activity with different concentration of CoA. For details see Materials and Methods.

groups. 10^{-4} M Cysteine is ineffective while 10^{-4} M glutathione very partially reduces the effect of sodium selenite. Subsequently, the effect of sodium selenite on α-KGDH activity was investigated in relation to time.

As shown in Fig. 2, pre-incubation of the enzyme with different concentrations of sodium selenite (6.25–100 μM) in the presence of 285 μM of CoA measured enzymatically according to Garland [11] produces an inhibition of the initial reaction rate and reduces the final NADH formation, this effect being dose-dependent.

The effect of 25 μM sodium selenite on steady-state kinetics of α-KGDH was then investigated with variable amounts of CoA. The simple analysis of the Lineweaver–Burk plot shows that the K_m is 58.2 μM and 18.3 μM in the absence and in the presence of sodium selenite, respectively, while the V_{max} is 321 nmoles NADH/mg/min in control conditions, and 52 nmoles NADH/mg/min after sodium selenite incubation.

DISCUSSION

The mitochondrial function measured as RCI is reduced after preincubation of cardiac mitochondria with different concentrations of sodium selenite, but this reduction is dependent on the substrates tested, and in fact it is present with glutamate and α-ketoglutarate, while there is no modification with malate or malate plus pyruvate. On the other hand the ADP/O ratio is not modified by sodium selenite.

These differences among different substrates could be ascribed to alterations in the metabolism of the Krebs cycle; in fact, it is well known that glutamate must be metabolized in α-ketoglutarate in order to enter into the Krebs cycle [13]. It must be pointed out that malate and pyruvate can also be metabolized in a different pathway [14]. The decrease in RCI while the ADP/O ratio is unaffected could be due to a change in the rate of production of electrochemical gradients [15] rather than to the uncoupling activity of sodium selenite. In fact, the effect of sodium selenite is more marked on the rate of state S_3 of respiration and this rate tends to approximate to the rate of spontaneous dissipation of electrochemical gradient (state S_4) [16]. These results strongly suggest the possibility that α-KGDH could be inhibited by sodium selenite, and this has been demonstrated in Figs. 2 and 3.

α-KDGH is a complex of enzymes [17]. The reaction requires multiple steps as described in [17] and [18]. Therefore the steady-state kinetics studies performed in this paper are not sufficient to identify the step in which sodium selenite is involved but surely the complex is inhibited in a dose-dependent way and probably the sodium selenite interacts with a step that involves CoA utilization. In fact, preliminary experiments show that at the end of the reaction the addition of new CoA starts the reaction up again. CoA also prevents inhibition of the mitochondrial respiration, while other sulphydryl compounds such as glutathione and cysteine do not mimic the action of CoA. These results suggest the possibility that the effect of CoA is rather specific. Moreover the effect of sodium selenite is slightly influenced by incubation time. The K_m for CoA of enzyme obtained in our experimental conditions are consistent with those reported in literature [12]. This inhibition is of a mixed type for CoA, indicating a complex interaction among sodium selenite, CoA and α-KGDH. The inhibition of this enzyme, that plays a pivotal role in the regulation of the mitochondrial metabolism,

Table 2. Effect of CoA on oxygen consumption of guinea-pig heart mitochondria in presence of Na_2SeO_3 10^{-4} M with α-ketoglutarate 5 mM as substrate (mean ± S.E. of four samples)

	QO_2† (4)	QO_2† (3)	RCI	ADP/O
Control	27.6 ± 4.7	124.7 ± 25.8	3.4 ± 0.2	3.7 ± 0.2
CoA 5×10^{-4} M	35.7 ± 3.5	213.5 ± 19.2	5.0 ± 0.4**	4.0 ± 0.3
Na_2SeO_3 + CoA 5×10^{-5} M	24.0 ± 2.0	45.5 ± 2.5	1.7 ± 0.1*	3.4 ± 0.1
Na_2SeO_3 + CoA 10^{-4} M	26.5 ± 2.7	61.0 ± 4.5	2.0 ± 0.1*	3.7 ± 0.2
Na_2SeO_3 + CoA 5×10^{-4} M	35.2 ± 5.3	155.2 ± 9.3	4.0 ± 0.5	4.1 ± 0.3

Statistical difference from the control: *$P \leq 0.001$; **$P < 0.01$.
† n atoms of oxygen/min/mg protein.

could be the mechanism of mitochondrial toxicity of sodium selenite, although other interactions with other mitochondrial components cannot be ruled out.

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REFERENCES

1. T. C. Stadtman, *Nutr. Rev.* **35**, 161 (1977).
2. J. J. Rotruck, A. L. Pope, H. E. Ganther, D. G. Hafeman, A. B. Swanson and W. G. Hoekstra, *Science, Wash.* **179**, 588 (1973).
3. T. C. Stadtman, *Adv. Enzymol.* **48**, 1 (1979).
4. T. C. Stadtman, *A. Rev. Biochem.* **49**, 93 (1980).
5. I. Rosenfeld and O. A. Beath, in *Pathology of Selenium Poisoning Bull.* **275**, p. 1, University of Wyoming, Agricultural Experimental Station (1946).
6. F. Franconi, N. Manghi, A. Giotti, F. Martini and G. Dini, *Acta pharmac. tox.* **46**, 98 (1979).
7. G. Dini, F. Franconi and F. Martini, *Exp. Molec. Pathol.* **34**, 226 (1981).
8. G. Dini, F. Martini, I. Stendardi, F. Bennardini and F. Franconi, *Exp. Molec. Pathol.* **36**, 326 (1982).
9. B. Sobel, E. Jaquier, A. Sjoerdsma and W. Lovenberg, *Circulation Res.* **19**, 1050 (1966).
10. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. P. B. Garland, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 1981. Academic Press, New York (1974).
12. M. Hamada, K. Koike, Y. Nakaula, T. Hiraoka, M. Koike and T. Hashimoto, *Biochem. J.* **77**, 1047 (1975).
13. E. B. Cunningham, in *Biochemistry Mechanism of Metabolisms*, p. 611. McGraw Hill, New York (1978).
14. K. J. Peuhkurinem, *J. molec. cell. Cardiol.* **16**, 487 (1984).
15. P. Mitchell, *Biol. Rev. Camb. Phil. Soc.* **41**, 445 (1966).
16. D. G. Nicholls, in *Bioenergetics*. Academic Press, New York (1978).
17. C. L. McMinn and J. H. Ottaway, *Biochem. J.* **161**, 569 (1977).
18. C. M. Smith, J. Bryla and J. R. Williamson, *J. biol. Chem.* **249**, 1497 (1974).