INHIBITION OF AMINO ACID UPTAKE IN ISOLATED HEPATOCYTES BY SELENITE

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1. Introduction

Alterations in peroxide metabolism and microsomal heme homeostasis connected with selenium deficiency tend to be counteracted by administration of < 1 μmol selenite/kg body wt [1]. The biological effects of doses $>$ 10-times higher might also be of some general interest. For example, 10 μmol selenite/kg has been demonstrated to affect the distribution and alleviate the toxicity of methylmercury [2]. In this dose range the effects of selenite can be expected to be both complex and drastic, as the LD_{50} in the rat is 3 mg/kg (11.4 μmol/kg) [3]. Furthermore, selenium compounds are designated as 'industrial hazards' [4] and the details of their toxicity are unknown [5].

For these reasons we have undertaken a study on the effects of toxic and nearly toxic concentrations of selenite on isolated hepatocytes. It was anticipated that this model system could be used to relate effects on liver cells to selenite metabolism, which has not yet been done [6–8]. This metabolism, as described [9], leads to the formation of methyl selenids via glutathione (GSH)-dependent activation. Here we report that selenite inhibits amino acid uptake in isolated hepatocytes and that this effect can be potentiated by low molecular weight thiols.

2. Material and methods

Selenite was purchased from Merck and GSH from Sigma and both used without further purification. α-Amino-[1-^{14}C]isobutyric acid (60 mCi/mmol), L-[^{35}S]cysteine (75 mCi/mmol), 2-[1-^{14}C]leucine (59 mCi/mmol) and L-[^{35}S]methionine (720 Ci/mmol) were purchased from The Radiochemical Centre, Amersham.

Collagenase perfusion was used to isolate hepatocytes from 200 g male Sprague-Dawley rats, which had free access to food and water [10]. The isolated cells were incubated ($1–2 \times 10^6$/ml) in rotating flasks with Krebs-Henseleit buffer and Hepes (25 mM) [10]. Cell membrane permeability was determined from the latency of lactate dehydrogenase, which monitors leakage of NADH [10]. The uptake of radioactively labelled amino acids was measured by sedimenting the cells at $20 \times g$ and washing them once in Krebs-Henseleit buffer. Radioactivity in the pelleted cells was measured in a scintillation counter. Under these conditions the leucine uptake was found to be linear for 5 min. About 1 nmol was accumulated per 10^6 control cells during this time period. Acid-soluble thiols were measured in cells, sedimented and washed, according to [11]; this has been found to be a good method to estimate GSH levels in untreated cells (unpublished observation).

3. Results

Sodium selenite added to a suspension of isolated hepatocytes decreased intracellular GSH levels to an extent that was approximately linear with dose during the first hour of incubation (fig.1). The effect of 25 μM selenite was very small, while 100 μM tended to deplete the intracellular GSH content. Addition of methionine (0.2–1.0 mM) to the medium caused an accumulation of thiols (and GSH; not documented) in selenite-treated cells at a rate similar to that seen with control cells [12] (fig. 1).

Selenite at 100 μM increased cellular permeability abruptly during the third hour (fig. 2) when GSH levels approached zero. Selenite at 50 μM also

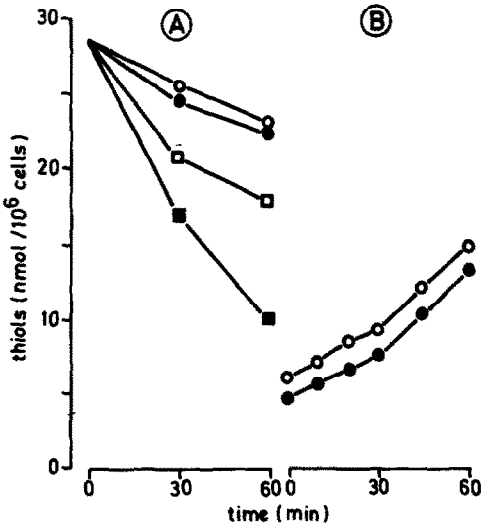

Fig.1. The effect of selenite on GSH levels (A) and on methionine-stimulated thiol formation (B) in isolated hepatocytes. Cells were incubated under control conditions (○——○) or with selenite in the medium at: (●——●) 25 μM; (□——□) 50 μM; (■——■) 100 μM; and the intracellular content of acid-soluble thiols was measured. In (A) selenite was added at zero time. In (B) the cells had been preincubated with diethylmaleate (0.03 μl/ml) for 2 h (to decrease intracellular levels of GSH) and with selenite for 10 min, while methionine (0.5 mM) was added at zero time.

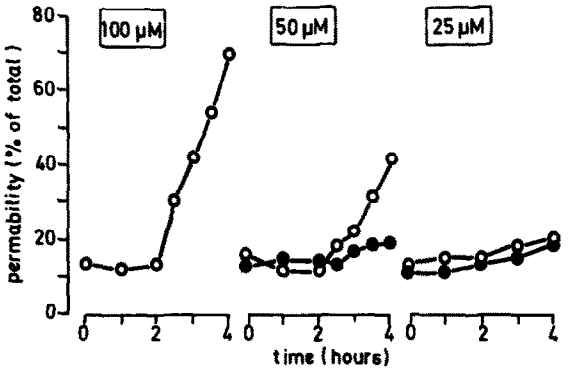

Fig.2. The effect of selenite and GSH on cellular permeability. Cells were incubated with various concentrations of selenite and with (●——●) or without (○——○) GSH (250 μM).

increased cellular permeability, but the effect was less pronounced and could be prevented by 250 μM GSH. Higher GSH concentrations potentiated selenite toxicity (not shown). Lower concentrations of selenite (25 μM), with or without GSH, had no significant effect on cellular permeability (fig. 2).

Amino acids are taken up and concentrated in isolated hepatocytes. The non-metabolizable amino acid α-aminoisobutyric acid (AIB) was concentrated 8–10-fold in control cells (fig. 3), assuming an intracellular water volume of 2.5 μl/10^6 cells [13]. Selenite at 100 μM decreased the cells' ability to concentrate AIB, while ⩽ 50 μM had no effect. Leucine was taken up more rapidly and the radioactivity was recovered almost completely in acid-precipitable compounds of the cells (fig. 3). The utilization of exogenous leucine for protein synthesis was decreased by 50 μM selenite ⩾ 1 h before increases in cellular permeability became evident (fig. 2,3). Selenite at 25 μM did not affect the utilization of exogenous leucine.

In combination with 250 μM GSH, a concentration which prevented rather than promoted cytolysis (fig. 2), selenite reduced the rate of leucine uptake. This was found to be the case after 10 min (table 1) or after 1 h exposure to selenite and GSH (not shown). Leucine has been reported to be taken up mainly by the Na^+-dependent ASC system, while this transport system has a lower affinity for AIB [14]. Only small and inconsistent changes in rates of AIB uptake could

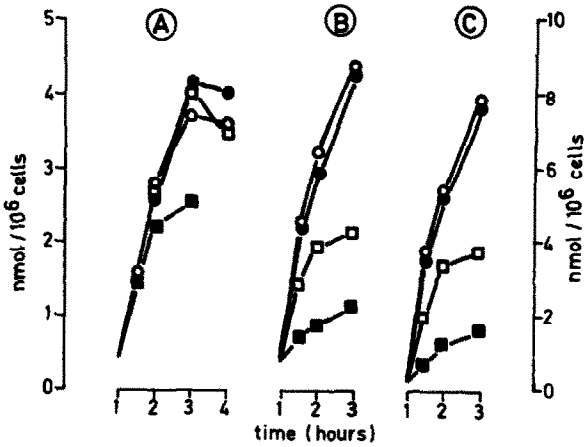

Fig.3. The effect of selenite on amino acid uptake in isolated hepatocytes. Cells were incubated with the same concentrations of selenite as in fig. 1. After 1 h amino acids (α-aminoisobutyric acid) in (A) and leucine in (B) and (C) were added (final conc. 0.2 mM). Amino acid uptake was measured in (A) and (B) and the amount of ^{14}C bound to acid-precipitable cellular compounds was determined in (C).

Table 1
Effect of selenite and GSH on amino acid uptake in isolated hepatocytes

Additions to the medium	Amino acid uptake (% of control)			
	α-amino isobutyric acid	Methionine	Cysteine	Leucine
None	100	100	100	100
GSH	95 (±10)	102 (± 3)	112 (±18)	105 (±12)
GSH + selenite	87 (± 7)	91 (±14)	73 (± 2)	59 (± 7)
Selenite	92 (±10)	102 (± 3)	53 (± 8)	102 (± 3)

Cells were incubated for 10 min in flasks with or without the addition of selenite (25 μM) and GSH (250 μM). Four aliquots (1 ml) were then transferred to test tubes containing the radiolabelled amino acid (final conc. 0.2 mM). The incubation was continued in the test tubes in a thermostated (37°C), oscillating water bath for 5 min. The figures (mean ± SD) are based on 4 expt. with separate batches of cells

be detected under the conditions documented in table 1. Similar results were obtained with methionine, which is also reported to be mainly taken up by other systems [14]. Cysteine is claimed to be taken up entirely by the ASC system [14] and, probably due to its thiol group, it was found in the presence of selenite to block its own uptake (table 1). Selenite plus cysteine had only minor effects on the rate of methionine uptake (not shown).

4. Discussion

The experiments presented here indicate that inhibition of amino acid uptake occurs as a consequence of selenite metabolism. In accordance with the conclusions [9] on GSH-dependent activation, it could be shown that thiols potentiate this inhibition by selenite. Of further interest is the observation that addition of thiols to the medium makes it possible to more clearly distinguish the inhibition of amino acid uptake from other effects of selenite in our model system. Thus, selenite in concentrations which markedly inhibited the uptake of extracellular cysteine, was found to have no effect on the cells' ability to concentrate AIB, to incorporate leucine into proteins*, or to transfer methionine sulphur to GSH. The rate of sulphur transfer from methionine is considered to be a sensitive indicator, since it has been shown that selenite (25 μmol/kg) inhibits methionine adenosyltransferase in mice [7].

The apparently selective effect of 25 μM selenite seems to be best explained by postulating an insignificant uptake of selenite into hepatocytes. The results suggest that selenite in this concentration range readily reacts with thiols outside (or on the surface of) the hepatocytes, but only to an insignificant extent with GSH inside the cells. Of interest in this respect is the fate of an intravenous dose of selenite. It has been shown that >50% is rapidly taken up by erythrocytes, but in <15 min is then recovered in (or on) the liver, after intermediate binding to plasma protein [15]. The uptake by erythrocytes is claimed to be GSH-dependent [15,16] and the compound leaving the blood cells is generally believed to be chemically-formed selenotrisulphide or one of its reduction products [9]. The apparent ability of erythrocytes to concentrate selenite and their high content of GSH make it possible to link our study with [15,16]. Our proposal is that the inhibition of amino acid uptake is the final step in a mechanism by which selenite in comparatively low doses can affect hepatic amino acid turnover. To what extent such a mechanism operates in vivo, or is blocked by plasma proteins, for example, remains to be elucidated.

The results of the amino acid uptake studies seem

* During the preparation of this manuscript, Vernie et al. (1979) Biochem. J. 180, 213–218, reported that GSSeSG inhibits leucine incorporation into proteins in fibroblasts; an effect which may be explained by inhibition of uptake

reasonably compatible with a selective blockage of the ASC system in hepatocytes [14], even though more complex effects cannot be ruled out at present. However, our data clearly show that the two sulphur-containing amino acids are taken up mainly by separate routes, thus confirming the observations in [14]. This means that hepatocytes are equipped with two, largely independent systems for taking up exogenous sulphur for GSH synthesis. We have reported [17] that the methionine-dependent pathway is preferably damaged by GSH-depleting xenobiotics, while the present results show that selenite inhibits the cysteine-dependent pathway.

Acknowledgement

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