Selenium (selenate) transport by human placental brush border membrane vesicles

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1. Selenate uptake by human placental brush-border-membrane vesicles was studied in order to establish whether this anion shares a pathway with sulphate.

2. Selenate uptake was found to be saturable with respect to medium selenate and was inhibited by the anion exchange inhibitor 4,4′-diisothiocyano-stilbene-2,2′-disulphonate (DIDS).

3. Anions which have a similar tetrahedral shape to selenate, e.g. chromate, molybdate, tungstate and sulphate, were effective inhibitors of selenate uptake when added to the incubation medium.

4. Sulphate inhibited selenate influx in a dose-dependent fashion; moreover sulphate was found to be a competitive inhibitor of selenate uptake.

5. It is concluded that selenate and sulphate share a pathway for transport in the human placental microvillus membrane.

Selenium is an essential trace element required by the fetus for normal growth, therefore knowledge on the placental transport of Se is important in the context of fetal nutrition. Recent work in this laboratory has shown that selenate is a competitive inhibitor of sulphate uptake by vesicles prepared from the brush-border membrane of the syncytiotrophoblast of the human term placenta (Shennan & Boyd, 1986). The brush-border membrane is the first barrier between the maternal and fetal circulations respectively. The interaction between selenate and sulphate strongly implies that they may share a pathway for transport in this membrane. Sulphate transport into human placental membrane vesicles is via a 4,4′-diisothiocyano-stilbene-2,2′-disulphonate (DIDS)-sensitive, sodium-independent system which may represent an anion exchange pathway (Cole, 1984; Boyd & Shennan, 1986*a*, *b*). The purpose of the present study was to investigate directly the transport of selenate by human placental microvillus membrane vesicles in order to establish whether this anion does indeed share a pathway with sulphate. Thus the effect of the anion exchange inhibitor DIDS and divalent anions (in particular sulphate) was tested on selenate uptake.

METHODS

Preparation of membrane vesicles

Brush-border-membrane vesicles were prepared from fresh, normal term human placentas using a calcium precipitation method based on the procedure of Smith *et al.* (1974) and Boyd & Lund (1981). This method of vesicle preparation enriches the brush-border marker enzyme, alkaline phosphatase, some 18–26 fold. In contrast the final pellet has no detectable Na^+, K^+-ATPase showing that there is little contamination by basal membranes (see Boyd & Lund, 1981). The placentas were placed on ice and the syncytiotrophoblast was scraped from the large blood vessels using a scalpel. The scraped tissue (100 g) was then washed with 200 ml ice-cold Krebs–Ringer bicarbonate solution. The placental tissue was then washed twice with an ice-cold solution (250 ml) containing 100 mM-calcium chloride. The washing was completed with a final wash with ice-cold Krebs-Ringer bicarbonate

solution. Following this the tissue was gently agitated for 60 min at 4°. The dense debris (including erythrocytes) was removed by centrifuging at 800 ***g*** for 10 min. The supernatant fraction was collected and centrifuged for 20 min at 10000 ***g***. The supernatant fraction was again collected and centrifuged for 40 min at 110000 ***g***. The pellets from the final spin were suspended in a solution containing 1 mM-sodium selenate, 160 mM-sucrose and 10 mM-potassium hydroxide–Hepes, pH 7·5, and homogenized by passing the solution twenty times through a fine (21 gauge) needle. Vesicles were stored at −70° before use. Recent work has shown that the transport activity is retained (for ^{36}Cl) following freezing for prolonged periods without appreciable loss of activity (Shennan *et al.* 1987).

Selenate uptake assay

$^{75}SeO_4^{2-}$ was used to assay selenate uptake by brush-border-membrane vesicles. All experiments were performed at room temperature (25±1°). The potassium ionophore valinomycin, at a concentration of 20 nmol/mg protein, was routinely used to clamp the electrical potential across the vesicle membrane. Since the intra- and extravesicular potassium concentrations were equal in all experiments the vesicle membrane potential would have been clamped to 0 mV. Selenate uptake was assayed according to the ion-exchange-column method of Gasko *et al.* (1976) as modified by Shennan *et al.* (1986). Disposable Pasteur pipettes were plugged with polymer filter wool and then filled with a strongly basic anion exchange resin (Chloride-form; Dowex). Each column was washed with 5 ml ice-cold solution containing 160 mM-sucrose and 10 mM-KOH–Hepes, pH 7·5. The assay was initiated by adding vesicles to incubation medium (giving a final protein concentration of approximately 0·75 mg/ml) which contained $^{75}SeO_4^{2-}$. At predetermined times, portions of reaction mixture were removed and applied to the ion-exchange columns. Vesicles were washed through the column with an ice-cold solution containing 160 mM-sucrose and 10 mM-KOH–Hepes, pH 7·5. The eluate was collected in vials and the radioactivity of the samples was counted using a Bertholt Mag 312 gamma counter. Selenate uptake was expressed as nmol/mg protein.

MATERIALS

$^{75}SeO_4^{2-}$ was obtained from Amersham International plc, Amersham, Bucks. Anion exchange resin (Cl-form Mesh 50–100, 8% cross linked; Dowex) was from Aldrich Chemical Co. (Gillingham, Kent). DIDS and valinomycin were purchased from Sigma, Poole, Dorset.

RESULTS

Fig. 1 shows the time-course of selenate uptake in the presence and absence of the stilbene derivative DIDS (2×10^{-4} M). It is evident that selenate uptake is time-dependent, reaching a point of equilibrium by 60 min. Uptake of a substrate by vesicles can represent transport into an intravesicular space or binding to the membrane surface, or both. Since vesicles loaded with isotopic selenate can subsequently be used to measure selenate efflux (Shennan, 1987), this would suggest that the majority of selenate accumulation by the vesicles represents real transport rather than binding. DIDS, which is a potent inhibitor of sulphate uptake by human placental microvillus membrane vesicles (Boyd & Shennan, 1986*a*), markedly inhibited the initial rate of selenate uptake. Moreover this drug also effectively inhibited selenate uptake even after 60 min of incubation at a point by which equilibrium had been attained under control conditions. This testifies to the potency of this inhibitor. Thus the pattern of DIDS inhibition of selenate uptake is very similar to that of sulphate uptake by human placental brush-border-membrane vesicles.

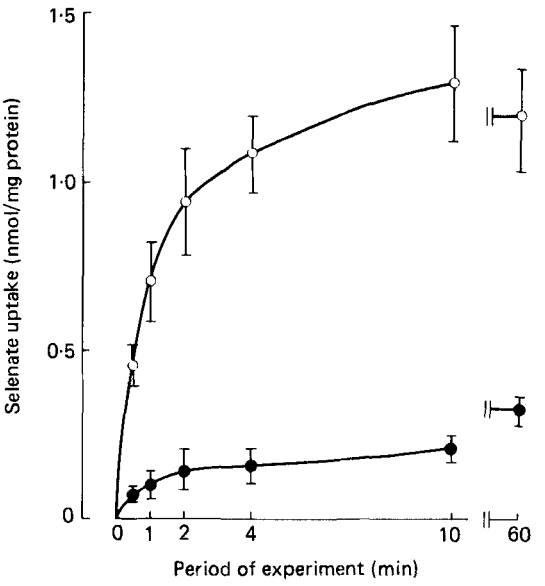

Fig. 1. Time-course of selenate uptake in the presence (●) and absence (○, control) of 4,4′-diisothiocyano-stilbene-2,2′-disulphonate (DIDS) by human placental brush-border-membrane vesicles. Vesicles and media contained 1 mM-sodium selenate, 160 mM-sucrose and 10 mM-potassium hydroxide–Hepes, pH 7·5. DIDS was used at a concentration of 2×10^{-4} M when required. Points are mean values, with their standard errors represented by vertical bars, for three experiments.

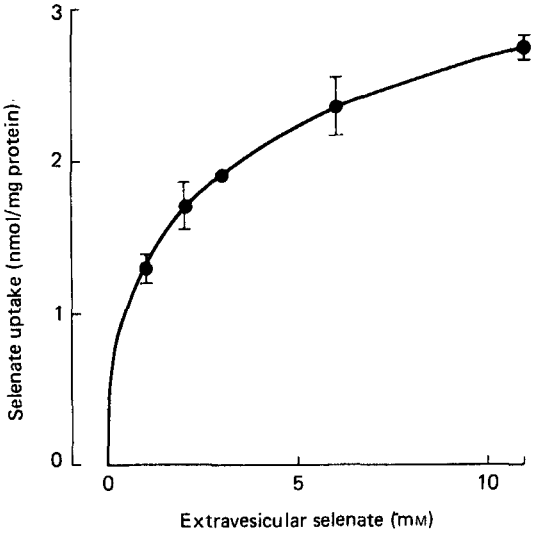

Fig. 2. The concentration dependence of selenate uptake by human placental brush-border-membrane vesicles. Vesicles contained 1 mM-sodium selenate, 160 mM-sucrose and 10 mM-potassium hydroxide–Hepes pH 7·5. Uptake was measured in media containing 1-11 mM-Na_2SeO_4, sucrose (concentration varied to maintain osmolarity), and 10 mM KOH–Hepes, pH 7·5. Uptake was assayed after 2 min incubation. Points are means, and standard deviations represented by vertical bars, of duplicate incubations.

Table 1. *The effect of divalent anions on selenate uptake by human placental brush-border-membrane vesicles*

(Vesicles and control incubation medium contained 1 mM-sodium selenate, 190 mM-sucrose and 10 mM-potassium hydroxide–Hepes, pH 7·5. When the anions were added the incubation medium contained 10 mM-Na_2X (where X represents anions tested), 1 mM-Na_2SeO_4, 160 mM-sucrose and 10 mM-KOH–Hepes, pH 7·5. Selenate uptake was assayed after 2 min incubation)

Anion (10 mM)	Percentage inhibiton of 1 mM-selenate uptake		
	Mean	SD	*n*
Sulphate	77·2	3·2	7
Sulphite	52·3	8·0	3
Selenite	47·8	2·9	4
Chromate	92·3	3·1	3
Molybdate	85·7	2·1	3
Tungstate	81·3	3·2	3
Phosphate	23·3	4·9	3

The next step was to test the effect of changing the extravesicular selenate concentration on selenate influx. Fig. 2 shows the effect of varying the medium selenate concentration over the range 1–11 mM. It is clear that selenate uptake is saturable with respect to the medium selenate concentration. It was established that the K_t of transport (i.e. the concentration giving half the maximal rate) was 1·81 (SE 0·19) mM (*n* 4).

Divalent anions that have a tetrahedral shape similar to sulphate have been shown to be the most effective inhibitors of sulphate uptake by human placental brush-border-membrane vesicles (Boyd & Shennan, 1986*b*). Therefore the effect of a wide range of divalent anions was tested on selenate influx in order to establish if there is a similar pattern of inhibition. The anions tested were chromate, molybdate, tungstate, sulphate, selenite, sulphite and phosphate. All anions were added as their Na salts to the incubation medium to give a final concentration of 10 mM. In the case of phosphate the concentration of the divalent form would have been approximately 8 mM. The results of these experiments are given in Table 1.

The most effective inhibitors of selenate uptake were chromate, molybdate, tungstate and sulphate. Less effective as inhibitors of selenate uptake were selenite and sulphite. Phosphate was the weakest inhibitor of all the anions tested. Therefore the anions with a shape similar to selenate (chromate, molybdate, tungstate and sulphate) were the most potent inhibitors of selenate influx. The inhibitory effect of the potent anions on influx appears to be specific since Shennan (1987) has shown that chromate is an effective inhibitor of selenate efflux from placental microvillus membrane vesicles whereas selenate itself is unaffective.

Selenate has been found to inhibit sulphate uptake in a dose-dependent fashion with a concentration of inhibition (K_I) of approximately 2·5 mM (Shennan & Boyd, 1986). Therefore it was decided to test the dose–response effect of sulphate on selenate uptake. The effect of varying the medium sulphate concentration (0·1–10 mM) on selenate uptake is shown in Fig. 3. It is evident that this anion inhibits selenate influx in a dose-dependent fashion. The K_I was calculated to be 2·76 (SE 0·12) mM (*n* 3). Thus it appears that sulphate inhibits selenate uptake with an affinity similar to the inhibition by selenate of sulphate uptake.

Selenate is a competitive inhibitor of sulphate influx into human placental microvillus membrane vesicles (Shennan & Boyd, 1986), therefore it was predicted that sulphate should

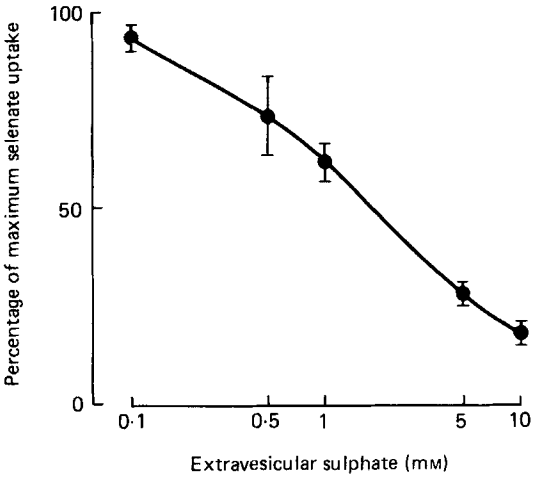

Fig. 3. Effect of medium sulphate concentration on selenate uptake by human placental brush-border-membrane vesicles. Vesicles contained 1 mM-sodium selenate, 160 mM-sucrose and 10 mM-potassium hydroxide–Hepes, pH 7·5. Uptake was measured from media containing 1 mM-Na_2SeO_4, 0·1–10 mM-Na_2SO_4, sucrose (concentration varied to maintain osmolarity) and 10 mM-KOH–Hepes, pH 7·5. Uptake was assayed after 2 min incubation. Points are mean values, and standard deviations represented by vertical bars, of duplicate incubations.

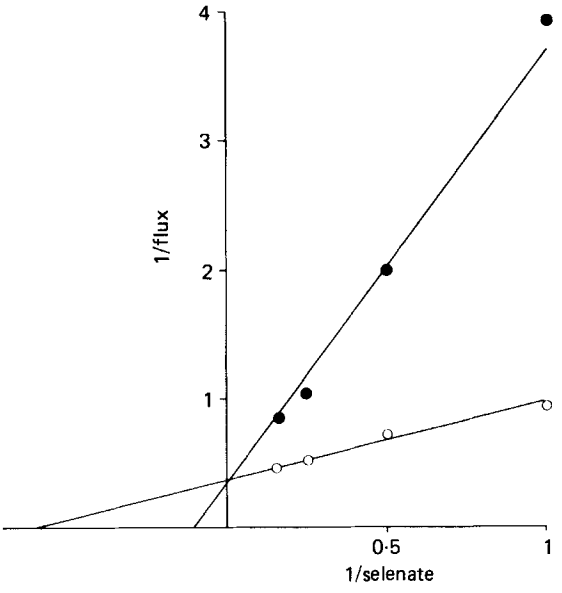

Fig. 4. Double-reciprocal plot of selenate uptake *v.* medium selenate concentration in the presence (●) and absence (○) of 10 mM-sulphate. Vesicles contained 1 mM-sodium selenate, 160 mM-sucrose, and 10 mM-potassium hydroxide–Hepes, pH 7·5. Uptake was measured from media containing 1–6 mM-Na_2SeO_4, sucrose (concentration varied to maintain osmolarity), 10 mM-KOH–Hepes, pH 7·5 and 10 mM-Na_2SO_4 when required. Uptake was assayed after 2 min incubation. Points are means of duplicate incubations.

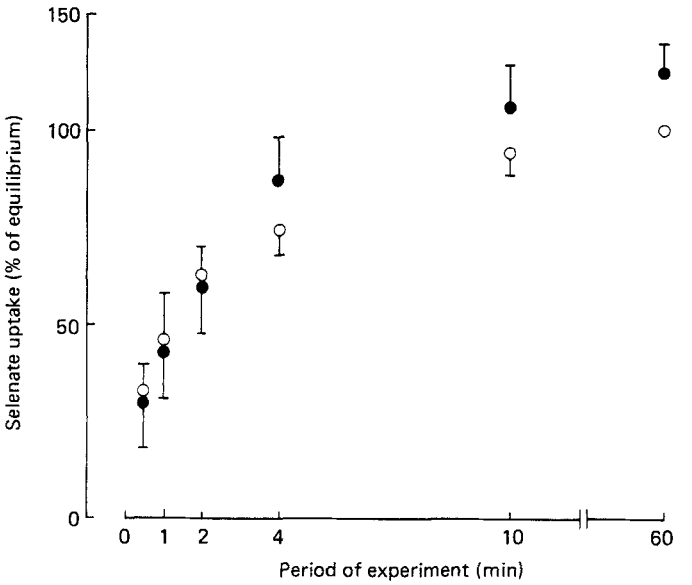

Fig. 5. The effect of an inwardly-directed sodium gradient on selenate uptake by human placental brush-border-membrane vesicles. Vesicles and control incubation medium (○) contained 1 mM-sodium selenate, 160 mM-sucrose and 10 mM-potassium hydroxide–Hepes, pH 7·5. The Na-rich medium (●) contained 1 mM-Na_2SeO_4, 80 mM-sodium gluconate and 10 mM-KOH–Hepes, pH 7·5. Points are mean values, with their standard errors represented by vertical bars, of three experiments (100% equilibrium corresponds to selenate uptake found with the control incubation medium after 60 min).

competitively inhibit selenate influx if these two anions share a transport pathway in this membrane. To test this prediction selenate uptake was measured from media containing 1, 2·5, 4 and 6 mM in the presence and absence of 10 mM-sulphate. It was found that increasing the medium selenate concentration reduced the proportion of selenate influx inhibited by sulphate. Fig. 4 shows a Lineweaver–Burk plot of one such experiment. It can be seen that the K_t of selenate uptake was increased by the addition of 10 mM-sulphate to the incubation medium, whereas the maximum initial velocity, V_{max}, was unaffected. Therefore, as predicted, it appears that sulphate is a competitive inhibitor of selenate uptake.

Both Cole (1984) and Boyd & Shennan (1986*b*) failed to find any evidence for Na–SO_4 co-transport in the human placental brush-border membrane. On this basis it was expected that selenate uptake should also be independent of Na. Fig. 5 shows that this is the case. Imposing an inwardly directed Na gradient did not alter the rate of selenate uptake compared with that found in a sucrose medium. Therefore it appears that selenate, like sulphate, is not transported in this membrane via a Na-linked co-transport system.

DISCUSSION

The experiments described in the present paper were performed to investigate the transport of selenate by human placental microvillus membrane vesicles in order to establish whether selenate shares a common transport pathway with sulphate. Sulphate crosses this membrane via a DIDS-sensitive, Na-independent route (Boyd & Shennan 1986*a*, *b*). It has also been shown that anions with a tetrahedral shape similar to that of sulphate interact strongly with sulphate transport. Anions which fall into this category include chromate, molybdate, tungstate and selenate (Boyd & Shennan, 1986*b*). The present findings that selenate uptake by human placental brush-border-membrane vesicles is inhibited by DIDS,

is Na-independent and is also inhibited by anions such as chromate, molybdate, tungstate and sulphate strongly suggest that selenate does utilize the sulphate transporter. The finding that sulphate competitively inhibits selenate transport further supports this notion. The kinetics of sulphate and selenate uptake are very similar. For example the K_t of selenate uptake is very similar to that for sulphate influx. In addition sulphate inhibits selenate uptake with a K_I similar to that found for the inhibition of sulphate uptake by selenate. From this it would be tempting to suggest that the transporter cannot readily distinguish between the two anionic species.

There are now several lines of evidence which suggest that sulphate and selenate share a common pathway(s) in the small intestine. For example, Cardin & Mason (1976) have shown that selenate inhibits the transport of sulphate by the small intestine in vitro. Moreover, Arduser *et al.* (1985) found that the intestinal absorption of selenate is inhibited by sulphate. Indeed a single transporter for sulphate, selenate and selenite has recently been identified in *Escherichia coli* K-12 (Lindblow-Kull *et al.* 1985).

If Se is present in maternal plasma in the form of selenate then the sulphate transporter may be an important pathway for Se transport from mother to fetus. A recent report by Kauppila *et al.* (1987) gives maternal plasma Se concentration as approximately 0·7 μM during the last trimester of pregnancy. This would suggest that the selenate (sulphate) transporter will never be saturated with respect to the maternal plasma selenate concentration given that the K_t of selenate uptake by human placental brush-border-membrane vesicles is 1·81 mM. Taken at face value this would suggest that the rate of selenate transport across the placental brush-border membrane will be a simple function of the plasma selenate concentration; however, it must be borne in mind that the plasma sulphate:selenate ratio will also be an important factor. The present results also suggest that selenate might be a good candidate if fetal Se supplementation is required.

The finding that both molybdate and chromate inhibit selenate transport as well as sulphate transport also implies that the sulphate transporter may be involved in the maternal–fetal transfer of the essential trace elements molybdenum and chromium, but since these two anions also inhibit sulphate efflux (Boyd & Shennan, 1986*b*) from placental microvillus membrane vesicles it can be predicted that they will be translocated at a rate slower than both sulphate and selenate.

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