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Induction of cystine transport via system x_c^- and maintenance of intracellular glutathione levels in pancreatic acinar and islet cell lines

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Abstract

The relationship between L-cystine transport and intracellular glutathione (GSH) levels was investigated in cultured pancreatic AR42J acinar and βTC3 islet cells exposed to diethylmaleate, an electrophilic agent known to activate cellular antioxidant responses. Cystine transport was mediated predominantly by the Na^+-independent anionic amino acid transport system x_c^-, with influx inhibited potently by glutamate and homocysteate but unaffected by cationic or neutral amino acids. Saturable cystine transport was 10-fold higher in AR42J (531 pmol (mg protein)$^{-1}$ min^{-1}) than in βTC3 (49 pmol (mg protein)$^{-1}$ min^{-1}) cells, and GSH levels were higher in AR42J cells. Treatment with 2-mercaptoethanol increased GSH levels in βTC3 cells from 7.5 to 36 nmol (mg protein)$^{-1}$, whilst the GSH content in AR42J cells (64 nmol (mg protein)$^{-1}$) was not altered significantly. Incubation of AR42J or βTC3 cells with homocysteate (2.5 mM, 0–48 h), a competitive inhibitor of cystine transport via system x_c^-, reduced intracellular GSH levels and resulted in a time-dependent (6–24 h) induction of system x_c^- transport activity. Treatment of AR42J cells with diethylmaleate (100 μM, 0–48 h) resulted in a time- (5–10 h) and protein synthesis-dependent induction of cystine transport, with intracellular GSH levels initially decreasing and then increasing 2-fold above control levels after 24 h. Diethylmaleate also depressed GSH levels in βTC3 cells, but cystine transport was not elevated significantly. In both AR42J and βTC3 cells, inhibition of γ-glutamyl cysteine synthetase by buthionine sulphoximine (100 μM, 24 h) reduced GSH levels but had no effect on cystine transport. The present findings establish that induction of system x_c^- leads to changes in GSH levels in pancreatic AR42J acinar and βTC3 islet cells, with changes in the intracellular redox state stimulating transporter expression. Induction of activity of system x_c^-, together with adaptive increases in GSH synthesis in response to oxidative stress, may contribute to cellular antioxidant defences in pancreatic disease.

Keywords: L-Cystine transport; Glutathione; Pancreatic acinar cell; Islet cell; Oxidative stress; Diethylmaleate

1. Introduction

Acute inflammatory pancreatitis is characterized by severe acinar cell damage, extensive interstitial oedema, haemorrhage and migration of neutrophils into the damaged pancreatic gland [1]. Accumulating evidence suggests that reactive oxygen metabolites play an important step in the initiation and progression of acute pancreatitis [2–7]. Although pancreatic levels of glutathione are rapidly depleted in experi-

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mental models of acute pancreatitis [7], there is only limited information on the mechanisms regulating glutathione synthesis and turnover in pancreatic acinar and islet cells [8].

As the key intracellular antioxidant, glutathione (GSH) reacts with electrophilic compounds and serves as a reductant for eliminating hydrogen peroxide and lipid hydroperoxides [9]. Supply of cyst(e)ine is rate-limiting for GSH biosynthesis in many cell types and, as cysteine is rapidly oxidized to cystine in extracellular fluid, cystine predominates in plasma and culture media. Transport of cystine is usually mediated by a Na^+-independent anionic amino acid transport system x_c^-, which exchanges cystine for intracellular glutamate [9–12]. Within cells cystine is rapidly reduced to cysteine, which is used for GSH and protein synthesis or released from the cell via the Na^+-dependent and Na^+-independent neutral amino acid transport systems ASC and asc, respectively.

We have previously reported that the electrophilic agent diethylmaleate induced adaptive increases in cystine transport and intracellular GSH levels in the human pancreatic duct cell line PaTu8902 [13]. In the present study we have characterized cystine transport in rat acinar AR42J [14] and murine islet βTC3 [15] cell lines and investigated whether induction of cystine transport activity in response to substrate deprivation or diethylmaleate regulates intracellular GSH levels. A preliminary account of this work has been presented in abstract form [16].

2. Methods

2.1. Culture of pancreatic AR42J acinar and βTC3 islet cell lines

The rat pancreatic acinar cell line AR42J [14] was purchased from the American Type Culture Collection (Rockville, MD, USA) and the murine islet cell line βTC3 [15] was a kind gift from Dr. Elaine Baileys from Addenbrooks Hospital (University of Cambridge, UK). Both pancreatic cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) containing 5.6 mM D-glucose, 10% fetal calf serum, 2 mM glutamine, 50 U ml^{-1} penicillin, and 50 mg ml^{-1} streptomycin at 37°C in a 5% CO_2 atmosphere [17]. Cells were subcultured every 2–3 days with a seeding ratio of 1:5–1:10 for AR42J cells or every 4–5 days with a seeding ratio of 1:2–1:3 for βTC3 cells. AR42J and βTC3 cells were trypsinized, and 2×10^5 and 2×10^6 cells were plated into 35 mm diameter plastic culture dishes, respectively. Two days later confluent cell monolayers were exposed for up to 48 h to different extracellular amino acids (2.5 mM), including L-serine, L-leucine, L-arginine, L-aspartate, L-glutamate or L-homocysteate, or the electrophilic agent diethylmaleate (DEM, 100 μM). Initial rates of L-cystine transport (2 min) and total intracellular glutathione levels were then measured after specified time intervals.

2.2. Measurement of L-cystine transport

Initial rates of L-cystine transport were determined as described previously [10]. Cells in 35 mm culture dishes were rinsed three times with warmed phosphate buffered saline (PBS) (mmol/l): Na_2PO_4, 80, $NaHPO_4$, 20, NaCl, 137, KCl, 3, $CaCl_2$, 1, $MgCl_2$, 0.5, glucose, 5.6, pH 7.4). Cells were then incubated at 37°C in 0.5 ml of fresh PBS, containing L-[^{14}C]cystine (0.1 μCi/0.5 ml). Transport of L-cystine was linear for up to 5 min (data not shown), and hence all subsequent experiments were performed over a 2 min incubation interval to maximize radioactivity in cell extracts and unidirectional flux measurements. In experiments where L-cystine transport was determined under nominally sodium-free conditions, sodium containing compounds in the buffer were replaced by their choline equivalents. Kinetics of L-cystine transport in AR42J and βTC3 cells were examined at L-cystine concentrations ranging from 0.01 to 0.5 mM. Data for AR42J cells were best fitted by a single Michaelis-Menten hyperbola, with mean influx values weighted for their reciprocal standard error. Kinetics of L-cystine transport in βTC3 cells were best fitted by a Michaelis-Menten equation with a linear non-saturable component. Data were analysed using FigP 60 software (Biosoft).

Transport was terminated by rapid removal of transport buffer from the cells followed by three washes with ice-cold PBS. After addition of 0.5 M NaOH to each well, total cellular protein concentrations were determined using the BCA protein assay. Disintegrations per minute (dpm) in each sample were determined by liquid scintillation and rates of

amino acid transport were expressed as pmol (mg protein)$^{-1}$ min^{-1} [18].

2.3. Determination of intracellular glutathione levels

AR42J and βTC3 cell monolayers in 35 mm dishes were rinsed three times with ice-cold PBS, and total glutathione (comprised of reduced (GSH) and oxidized (GSSG) glutathione) was extracted with 1 ml of 5% trichloroacetic acid. To remove trichloroacetic acid, cell extracts were treated four times with 2 ml of 0.01 M HCl-saturated diethyl ether. The glutathione content (defined as total GSH) in the solution was measured using an enzymatic method described previously [12], which is based on the catalytic action of glutathione in the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) by glutathione reductase [19].

2.4. Materials

DMEM, fetal calf serum, glutathione reductase (type III), buthionine sulphoximine, diethylmaleate, 2-mercaptoethanol and all other chemicals were purchased from Sigma (Poole, UK). The BCA protein assay kit was obtained from Pierce (Chester, UK) and L-[U-^{14}C]cystine (291.3 mCi mmol^{-1}) from New England Nuclear (Dreieich, Germany).

2.5. Statistics

Data are expressed as mean ± S.D. of measurements in n experiments conducted on different days. Statistical significance was assessed using a Student's unpaired t-test, with $P < 0.05$ considered significant.

3. Results

3.1. Characteristics of cystine transport in AR42J cells and βTC3 cells

Fig. 1A shows the kinetics of cystine transport in cultured pancreatic AR42J acinar cells. Influx of cystine was saturable, with K_m and V_{max} values similar to those reported for system x_c^- in other cell types (see Table 1) [9]. To characterize the specificity of

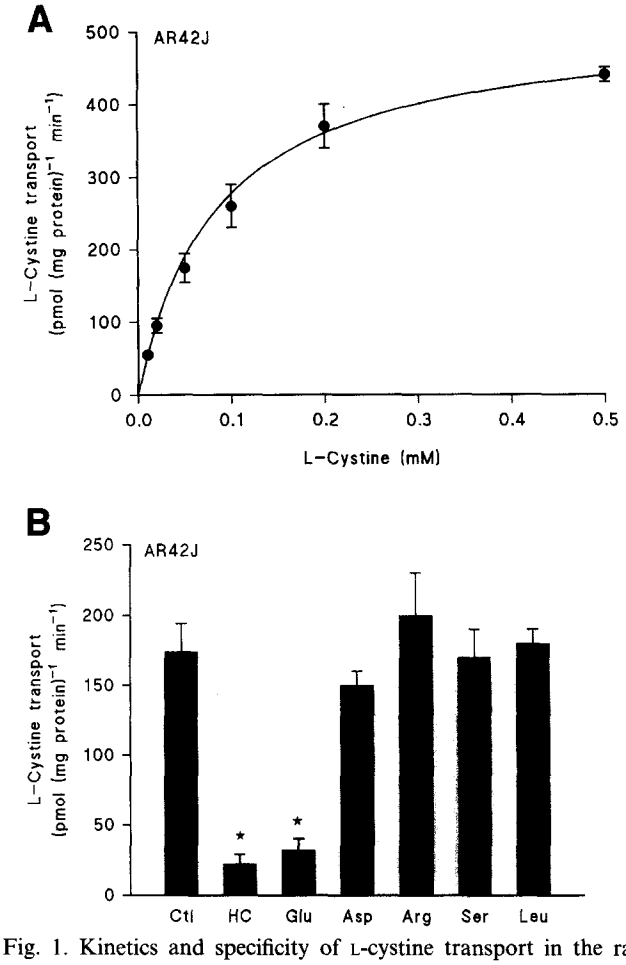

Fig. 1. Kinetics and specificity of L-cystine transport in the rat pancreatic acinar cell line AR42J. (A) Cystine transport was measured over 2 min in confluent monolayers incubated with the specified substrate concentrations (0.01–0.5 mM). Influx data were fitted by a single Michaelis-Menten entry site analysis weighted for the reciprocal standard deviation of each mean influx value (see Section 2). (B) Initial rates of L-cystine transport (0.05 mM, 2 min) were measured in cells incubated in Na^+ containing transport buffer in the absence (Ctl) or presence of a specified putative inhibitor amino acid (2.5 mM). HC, L-homocysteate; Glu, L-glutamic acid; Asp, L-aspartic acid; Arg, L-arginine; Ser, L-serine; Leu, L-leucine. Values denote the means ± S.D. of four to six experiments. *$P < 0.05$ relative to control.

cystine transport, influx was measured in the presence of a 50-fold excess concentration of putative inhibitor amino acids. As shown in Fig. 1B, transport of cystine in AR42J cells was inhibited significantly by L-glutamate and L-homocysteate (substrates for system x_c^-). The lack of inhibition of L-cystine transport by L-arginine, L-serine, L-leucine

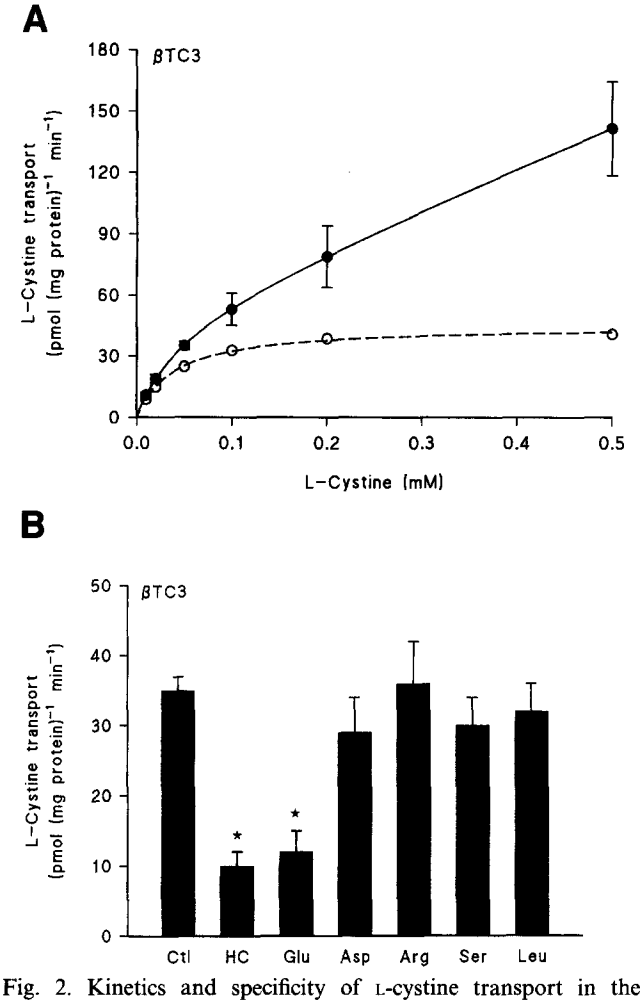

Fig. 2. Kinetics and specificity of L-cystine transport in the murine pancreatic islet cell line βTC3. (A) Cystine transport was measured over 2 min (0.01–0.5 mM), and influx values weighted for the reciprocal standard deviation were fitted by a Michaelis-Menten equation including a non-saturable component. Overall (●) and saturable (○) rates of cystine transport are shown. (B) Initial rates of L-cystine transport (0.05 mM, 2 min) were measured in cells incubated in PBS in the absence (Ctl) or presence of a specified putative inhibitor amino acid (2.5 mM). HC, L-homocysteate; Glu, L-glutamic acid; Asp, L-aspartic acid; Arg, L-arginine; Ser, L-serine; Leu, L-leucine. Values denote the means ± S.D. of four to six different experiments. *$P < 0.05$ relative to control.

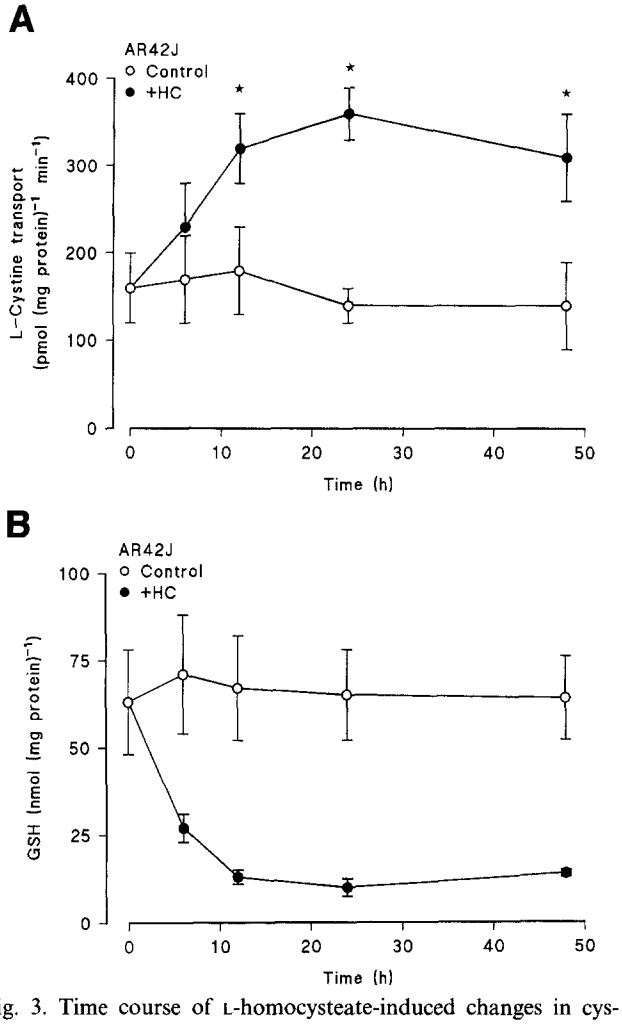

Fig. 3. Time course of L-homocysteate-induced changes in cystine transport and intracellular GSH levels in AR42J cells. Cell monolayers were cultured for 0–48 h in the absence (○) or presence of 2.5 mM L-homocysteate (●). Initial rates of L-cystine transport (A, 0.05 mM, 2 min) and total intracellular GSH levels (B) were then measured at the specified time intervals. Values denote the means ± S.D. of four experiments. *$P < 0.05$ relative to control rates of cystine transport at time zero. GSH levels after treatment with homocysteate were all significantly decreased relative to control GSH levels.

Table 1
Summary of kinetic parameters for cystine transport in pancreatic AR42J acinar and βTC3 islet cell lines

	K_m (μM)	V_{max} (pmol (mg protein)$^{-1}$ min^{-1})	K_d (pmol (mg protein)$^{-1}$ min^{-1})
AR42J cells	98 ± 10	531 ± 16	–
βTC3 cells	45 ± 10	49 ± 4	193 ± 6

Pancreatic cells were cultured for 24 h and then the kinetics of cystine transport were measured over a 50-fold range of substrate concentrations (0.01–0.5 mM). Kinetic parameters were weighted for the reciprocal error at each mean and fitted by Michaelis-Menten equations as described in Section 2. Values denote the mean ± S.D. of four experiments.

and L-aspartate demonstrates that cystine enters these cells via system x_c^- and not by systems y^+, L, ASC, $b^{0,+}$, $B^{0,+}$, or X_{AG}^- (see review [20]).

In pancreatic βTC3 islet cells, transport of cystine was comprised of saturable and non-saturable components (Fig. 2A), with the V_{max} value for saturable transport 10-fold lower than that measured in AR42J acinar cells (Table 1). Although an apparent non-saturable component exists, further characterization of this component could not be performed due to the low solubility of cystine. As in AR42J acinar cells, the total transport of cystine in βTC3 cells was only inhibited by L-glutamate and L-homocysteate (Fig. 2B). Cystine transport in both AR42J and βTC3 cells was Na^+-independent (data not shown), and the selective inhibition of cystine transport by glutamate and homocysteate together with the Na^+ independence of influx are consistent with transport being mediated by system x_c^-.

3.2. Modulation of GSH levels and cystine transport in AR42J and βTC3 cells

Table 2 compares total intracellular GSH levels in AR42J acinar and βTC3 islet cells cultured for 24 h in the absence or presence of elevated concentrations of different amino acids. Pretreatment of AR42J and βTC3 cells with 2.5 mM L-glutamate or L-homocysteate significantly decreased intracellular GSH levels,

Table 2
Effects of extracellular amino acids on intracellular GSH levels in AR42J acinar and βTC3 islet cells

Extracellular amino acid	Total intracellular GSH (nmol (mg protein)$^{-1}$)	
	AR42J cells	βTC3 cells
Control	71 ± 8	8.0 ± 0.8
L-Homocysteate	22 ± 10*	1.2 ± 0.4*
L-Glutamate	31 ± 11*	2.3 ± 0.6*
L-Aspartate	64 ± 7	6.3 ± 0.6
L-Arginine	76 ± 9	8.3 ± 0.6
L-Serine	61 ± 4	6.9 ± 0.3
L-Leucine	65 ± 3	7.3 ± 0.6

Cells were incubated for 24 h in complete DMEM containing a given amino acid (2.5 mM). Total intracellular glutathione levels were then determined in washed cell extracts as described in Section 2. Values denote the means ± S.D. of four experiments. *$P < 0.05$ relative to control GSH content in AR42J and βTC3 cells, respectively.

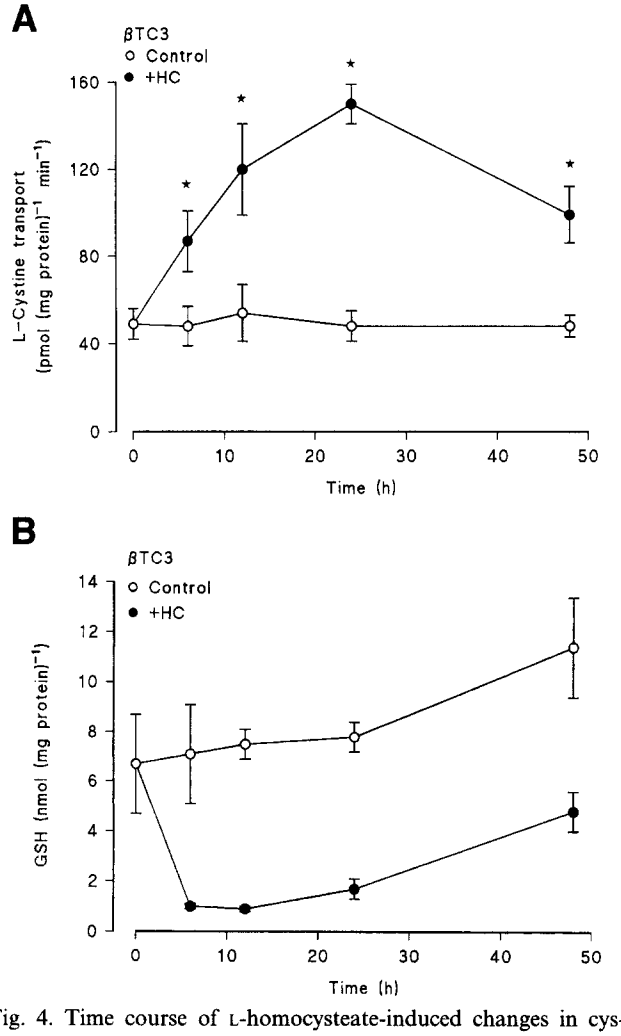

Fig. 4. Time course of L-homocysteate-induced changes in cystine transport and intracellular GSH levels in βTC3 cells. Cell monolayers were cultured for 0–48 h in the absence (○) or presence of 2.5 mM L-homocysteate (●). Initial rates of L-cystine transport (A, 0.05 mM, 2 min) and total intracellular GSH levels (B) were then measured at the specified time intervals. Values denote the means ± S.D. of seven to nine experiments. *$P < 0.05$ relative to control cystine transport rates at time zero. GSH levels after treatment of cells with homocysteate were all significantly decreased relative to control GSH levels.

whereas L-aspartate, L-arginine, L-serine or L-leucine had no such effect. The selective inhibition of cystine transport caused by glutamate and homocysteate (see Fig. 1B and Fig. 2B) most likely explains the sustained decrease in GSH levels observed in both pancreatic cell lines.

In subsequent experiments, we examined the time course (0–48 h) of homocysteate-induced changes in cystine transport and total intracellular GSH levels.

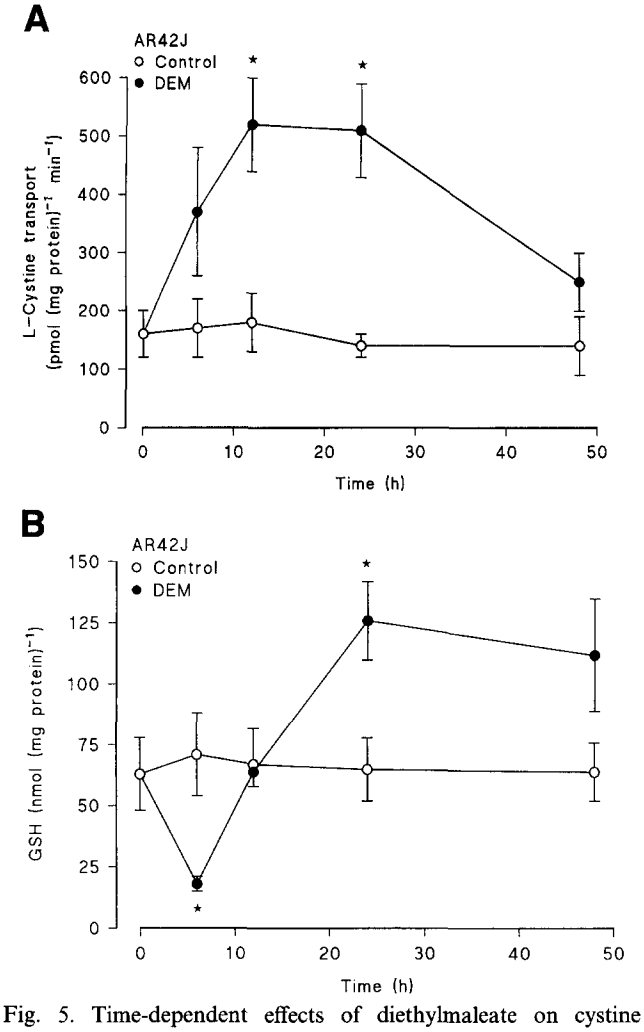

Fig. 5. Time-dependent effects of diethylmaleate on cystine transport and intracellular GSH levels in AR42J cells. Confluent cell monolayers were cultured for 0–48 h in the absence (○) or presence (●) of 100 μM diethylmaleate. Monolayers were then rinsed and initial rates of L-cystine transport (A, 0.05 mM, 2 min) and total intracellular GSH levels (B) measured at the specified time intervals. Values denote the means ± S.D. of seven to nine experiments. $^*P < 0.05$ relative to control values at time zero.

In the absence of homocysteate, initial rates of cystine transport and GSH levels in AR42J (Fig. 3) and βTC3 (Fig. 4) cells remained constant over a 48 h incubation period. Exposure of both cell types to 2.5 mM homocysteate resulted in a time-dependent increase in cystine transport, which was detectable within 5 h and reached a maximum after 25 h treatment. Although cystine transport rates were elevated after 5 h exposure to homocysteate, intracellular GSH levels decreased significantly and remained depressed below control levels throughout the 48 h incubation period (Fig. 3BFig. 4B).

3.3. *Effects of diethylmaleate on cystine transport and GSH content*

Cystine transport and intracellular GSH levels were also examined in AR42J (Fig. 5A) and βTC3 (Fig. 6A) cells treated with diethylmaleate, a sulfhydryl reactive agent proposed to activate electro-

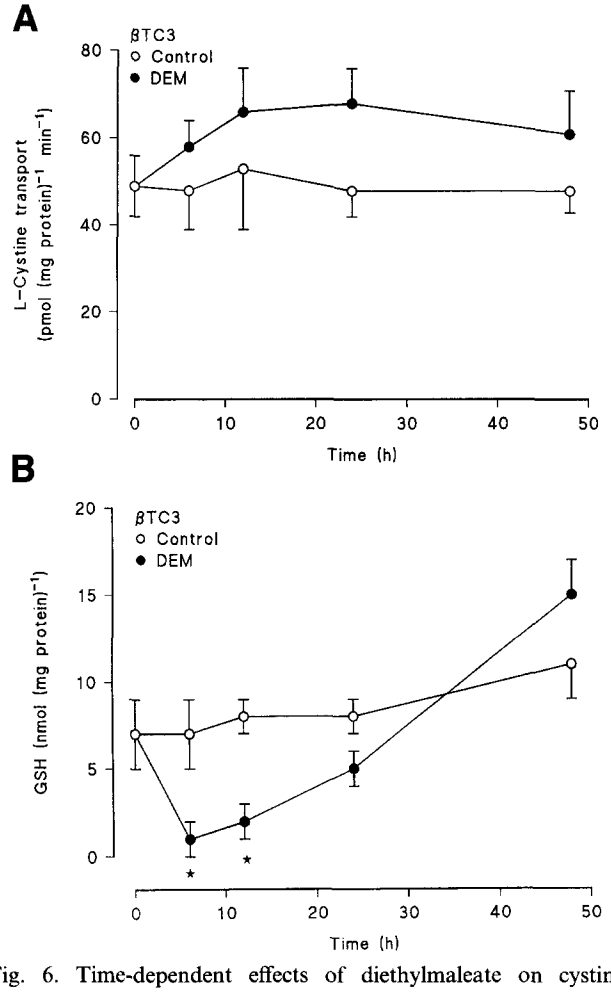

Fig. 6. Time-dependent effects of diethylmaleate on cystine transport and intracellular GSH levels in βTC3 cells. Confluent cell monolayers were cultured for 0–48 h in the absence (○) or presence (●) of 100 μM diethylmaleate, and initial rates of L-cystine transport (A, 0.05 mM, 2 min) and total intracellular GSH levels (B) measured at the specified time intervals. Values denote the means ± S.D. of seven to nine experiments. $^*P < 0.05$ relative to control values at time zero.

phile-responsive/antioxidant-responsive elements in genes encoding the transporter for system x_c^- and the haem degrading enzyme haem oxygenase-1 [21,22]. In the absence of diethylmaleate, initial rates of cystine transport in both pancreatic cell lines remained constant over a 48 h incubation period. Treatment of AR42J cells with diethylmaleate (100 μM) increased cystine transport activity from 150 to 500 pmol (mg protein)$^{-1}$ min^{-1} within 10–20 h, and thereafter transport rates decreased to control values within 48 h. Transport of L-cystine in βTC3 cells exposed to diethylmaleate was not significantly elevated, with rates of cystine transport only increasing from 50 to 65 pmol (mg protein)$^{-1}$ min^{-1} after 25 h. Diethylmaleate-induced increases in cystine transport activity in AR42J cells lines were abolished following inhibition of protein synthesis with cycloheximide (CLX, 1 μM); control: 120 ± 20; DEM: 550 ± 140; DEM+CLX: 110 ± 20 pmol (mg protein)$^{-1}$ min^{-1} (n = 4–6). Actinomycin D (0.1 μg ml^{-1}) also inhibited the induction of cystine transport activity by diethylmaleate (data not shown). Thus, the significant increase in cystine transport activity in AR42J cells in response to diethylmaleate suggests that this sulfhydryl reagent induced a time-dependent synthesis of x_c^- transporter protein.

Diethylmaleate (100 μM) reduced total intracellular GSH levels in AR42J cells from 60 to 23 nmol mg protein^{-1} within 6 h (Fig. 5B). GSH levels were restored by 12 h, subsequently increased to 125 nmol (mg protein)$^{-1}$ and remained elevated for up to 48 h. GSH levels were also decreased in βTC3 cells after 6 h exposure to diethylmaleate (Fig. 6B), although the subsequent recovery of GSH levels was delayed compared to AR42J cells.

3.4. *Effects of buthionine sulphoximine and 2-mercaptoethanol on GSH levels and cystine transport*

To investigate whether inhibition of GSH synthesis influenced cystine transport, AR42J acinar and βTC3 islet cells were exposed for 24 h to buthionine sulphoximine (100 μM), an inhibitor of γ-glutamyl-cysteine synthetase. Buthionine sulphoximine decreased intracellular GSH levels significantly in both pancreatic cell lines without altering initial rates of cystine transport (Table 3), suggesting that inhibition of GSH synthesis alone does not induce system x_c^- transport activity. It is worth noting that basal GSH levels in βTC3 cells were significantly lower than in AR42J cells. As extracellular thiol supplementation increases intracellular GSH levels in cells deficient in cystine transport activity [8,23], we examined the effects of 2-mercaptoethanol on cystine transport and GSH levels. Treatment with 2-mercaptoethanol (50 μM) for 24 h significantly elevated intracellular GSH levels in βTC3 cells, whilst changes were less marked in AR42J cells expressing an 8-fold higher basal content of GSH (Table 3).

4. Discussion

The present study provides the first data on the specificity and kinetics of cystine transport in pancreatic AR42J acinar and βTC3 islet cell lines. The Na^+ independence and marked inhibition of cystine transport by homocysteate and glutamate suggests that both pancreatic cell types express the Na^+-independent anionic amino acid transport system x_c^-. Pro-

Table 3
Effect of buthionine sulphoximine and 2-mercaptoethanol on cystine transport and total intracellular GSH levels in AR42J acinar and βTC3 islet cells

	AR42J cells		βTC3 cells	
	Cystine transport (pmol (mg protein)$^{-1}$ min^{-1})	Total GSH (nmol (mg protein)$^{-1}$)	Cystine transport (pmol (mg protein)$^{-1}$ min^{-1})	Total GSH (nmol (mg protein)$^{-1}$)
Control	148 ± 33	64 ± 12	47 ± 17	7.5 ± 1.2
2-Mercaptoethanol	149 ± 21	90 ± 16	30 ± 5	36 ± 7*
BSO	160 ± 19	8 ± 3*	62 ± 20	1.2 ± 1.1*

Cells were cultured for 24 h in the absence (control) or presence of 100 μM buthionine sulphoximine (BSO) or 50 μM 2-mercaptoethanol, and cystine transport (50 μM) and total intracellular GSH levels were then measured. Values denote the means ± S.D. of three to four experiments. *$P < 0.05$ relative to the respective control values.

longed inhibition of cystine influx by homocysteate resulted in a sustained decrease in total intracellular GSH levels and a time- and protein synthesis-dependent induction of system x_c^- transport. The sulfhydryl reactive agent diethylmaleate, known to activate electrophile-responsive elements in stress response genes [24], may have induced the x_c^- transporter protein itself and/or a putative activator of system x_c^-. Induced x_c^- transport activity was accompanied by an adaptive increase in intracellular GSH levels. The importance of system x_c^- in maintaining GSH levels in these pancreatic acinar and islet cell lines confirms previous findings in other cell types [11,12,25,26], and provides the first such evidence in exocrine and endocrine cells involved in inflammatory responses in pancreatic diseases [7].

We have reported previously that human pancreatic PaTu8902 duct cells transport cystine via system x_c^- and a γ-glutamyl-dipeptide transporter [18], which transports γ-glutamyl amino acids formed on or near the cell membrane by the action of membrane-bound γ-glutamyl transpeptidase [27]. Although γ-glutamyl transpeptidase is expressed in the apical membrane of human pancreatic duct cells [28], this cycle plays no role in amino acid transport across the basolateral membrane of the exocrine pancreatic epithelium [29]. The specific inhibition of cystine transport in AR42J acinar and βTC3 islet cells (Fig. 1B and Fig. 2B) by glutamate and homocysteate suggests further that system x_c^-, rather than the less selective amino transport systems $b^{0,+}$ and $B^{0,+}$ [30,31], mediates entry of cystine in these cells.

Islet cells are known to have low levels of antioxidant enzyme gene expression [32], which may explain their sensitivity to the cytotoxic actions of nitric oxide generated by inducible nitric oxide synthase during acute and chronic inflammation of the pancreas [33–35]. As GSH contributes significantly to cellular defences against oxidative stress, including nitric oxide-induced cytotoxicity [36], our findings in βTC3 islet cells suggest that the sensitivity of pancreatic β-cells to oxidative stress in vivo may be attributable to their low expression of system x_c^- compared to AR42J acinar cells.

Glutathione and cysteine contents are decreased markedly in cultured mouse lymphoma L1210 cells, which are deficient in cystine transport activity [23]. Treatment of βTC3 islet cells with 2-mercaptoethanol significantly elevated intracellular GSH levels, confirming earlier reports in L1210 lymphoma and islet cell lines [8,23]. The reaction of 2-mercaptoethanol with cystine produces a mixed disulphide and cysteine which are taken up into cells. The mixed disulphide is then reduced to cysteine and 2-mercaptoethanol and efflux of 2-mercaptoethanol and its rapid reaction again with cystine in the culture medium enables cells to utilize cystine more efficiently [23]. Specific cells in the central nervous system are also deficient in cystine transport activity [26]. Glial cells express a high activity of system x_c^- whilst neurones are only able to maintain intracellular GSH levels by taking up cysteine released from glial cells. As the GSH content in islets and islet cell lines [8] (see Tables 2 and 3) is extremely low in the absence of extracellular thiols, it is possible that pancreatic β-cells in vivo maintain their GSH content by taking up cysteine released from surrounding pancreatic acinar and ductal epithelial cells.

On the basis of the results presented in Figs. 3–6, one would initially conclude that a decrease in pancreatic GSH is associated with an induction of system x_c^- transport activity. However, as shown in Table 3, depletion of GSH following inhibition of GSH synthesis with buthionine sulphoximine (BSO) does not seem to be a sufficient signal to induce system x_c^- in AR42J acinar or βTC3 islet cells. Treatment of these pancreatic cell lines with BSO decreased intracellular GSH levels without altering cystine transport rates significantly. Our findings with BSO are consistent with similar studies in human umbilical vein endothelial cells [37] but contrast with the reported stimulation of cystine and glutamate transport in bovine pulmonary artery endothelial cells [11]. During inhibition of GSH synthesis by BSO, cysteine accumulates within cells. In contrast, prolonged exposure to elevated extracellular concentrations of glutamate or homocysteate inhibits cystine transport, resulting in low intracellular concentrations of cysteine and GSH. Under these conditions, induction of system x_c^- transport activity appears to be mediated by the total depletion of non-protein SH groups rather than changes in GSH alone.

In caerulein-induced experimental pancreatitis, total GSH levels in pancreatic homogenates decrease substantially within 2–4 h after caerulein treatment and are restored to normal within 24 h of discontin-

uing caerulein treatment [3,4,7]. Reactive oxygen radicals generated in acute pancreatitis [2,38–40] are probably responsible for the initial depletion of pancreatic GSH. As system x_c^- is induced by reactive oxygen radicals and sulfhydryl reactive agents [9,11,13], one could speculate that induction of this transporter in pancreatitis would increase the supply of cystine required to restore depleted pancreatic GSH levels. Such an adaptive increase in system x_c^- transport activity may play an important role in attenuating oxidative stress induced by acute pancreatitis and diabetes in vivo.

Although the signalling mechanisms underlying induction of the gene encoding the transporter for system x_c^- remain to be elucidated, we hypothesize that alterations in the intracellular redox state of pancreatic cells activates a transcription factor(s) associated with electrophile-responsive/antioxidant-responsive elements in stress response genes [21,22,24]. Intracellular GSH levels in these pancreatic cell types is dependent on the activity of system x_c^-, and induction of cystine transport by oxidative stress and electrophilic agents ensures enhanced supply of cyst(e)ine for GSH synthesis. Further studies at a molecular level are necessary to identify which transcription factor(s) are involved in the induction of system x_c^- transporter in response to inflammation in pancreatic disease.

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