Biochimica et Biophysica Acta, 602 (1980) 661–672
© Elsevier/North-Holland Biomedical Press

BBA 78988

EFFECTS OF THIOL-REACTIVE AGENTS ON AMINO ACID TRANSPORT BY SHEEP ERYTHROCYTES

JAMES D. YOUNG

ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (U.K.)

(Received April 8th, 1980)
(Revised manuscript received July 3rd, 1980)

Key words: Thiol-reactive agent; Facilitated diffusion; Amino acid transport; (Sheep erythrocyte)

Summary

(1) Sheep erythrocytes possess a facilitated diffusion transport system selective for small neutral amino acids of intermediate size (C-system). The effects of seven thiol-reactive agents on this system were investigated.

(2) L-Alanine influx by this route was inhibited by $HgCl_2$, *p*-chloromercuriphenylsulphonate (PCMBS), azodicarboxylic acid bisdimethylamide (diamide), *N*-ethylmaleimide and *t*-butylhydroperoxide. Iodoacetamide and 5,5′-dithiobis(2-nitrobenzoate) had no effect.

(3) Detailed analysis of these inhibitor effects suggested the presence of three distinct classes of cellular thiol groups essential for normal transport function.

(4) Class 1 thiols react with PCMBS and are located on the outer surface of the cell membrane in the region of the transport site. Class 2 thiols react with *N*-ethylmaleimide and diamide but are not affected by *t*-butylhydroperoxide. Class 3 thiols are oxidized during *t*-butylhydroperoxide treatment and are presumably also attacked by diamide and *N*-ethylmaleimide.

(5) Class 3 thiols are either intracellular GSH or reactive thiols which readily form mixed disulphides with GSSG. Any direct involvement of GSH in amino acid transport is not mediated by the γ-glutamyl cycle.

Introduction

Mammalian erythrocytes possess a number of distinct amino acid transport systems with differing substrate specificities and translocation capacities. Hu-

Abbreviations: PCMBS, *p*-chloromercuriphenylsulphonate; DTNB, 5,5′-dithiobis(2-nitrobenzoate).

man cells have four systems: one with a specificity for large neutral amino acids such as leucine, but excluding tryptophan; one selective for aromatic amino acids; another specific for dibasic amino acids and an Na-dependent system selective for neutral amino acids of intermediate size [1—11]. In contrast, sheep erythrocytes have a single Na-independent transport system (designated the C-system), for which the optimal substrates are cysteine, α-amino-*n*-butyrate and alanine [10,12—14]. This system has a lower, but still significant, affinity for a number of dibasic amino acids. An inherited transport defect associated with the functional absence of the C-system is found in the erythrocytes of some sheep [12,15]. Its absence results in a markedly diminished glutathione (GSH) concentration and the intracellular accumulation of a number of amino acids, notably ornithine and lysine [16,17]. Such transport-deficient cells have a decreased potential life-span [18] and an increased susceptibility to oxidative stress [19].

Despite this knowledge, there is, as yet, little information concerning the properties and identity of the membrane components responsible for carrier-mediated amino acid transport across the erythrocyte membrane. The present report is a detailed analysis of the effects of thiol-reactive agents on amino acid transport by the sheep C-system. The results suggest that there are three discrete classes of cellular thiol groups, of which the integrity is required for normal transport activity. One class reacts with *p*-chloromercuriphenylsulphonate (PCMBS) and is situated on the outer surface of the cell membrane in the region of the translocation site. A preliminary report of some of these findings has been published [20].

Materials and Methods

Blood samples were obtained from adult sheep by jugular venipuncture into heparinized evacuated tubes. All animals were maintained at Babraham under standard husbandry conditions. Sheep with an inherited erythrocyte deficiency of γ-glutamylcysteine synthetase [21] were selected as described previously [22].

Uniformly labelled L-[^{14}C]alanine was obtained from the Radiochemical Centre, Amersham, U.K. Bovine serum albumin, PCMBS, *N*-ethylmaleimide, azodicarboxylic acid bisdimethylamide (diamide), 5,5′-dithiobis(2-nitrobenzoate) (DTNB), dithioerythritol and iodoacetamide were purchased from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, U.K. *t*-Butylhydroperoxide was obtained from Koch-Light Laboratories Ltd., Colnbrook, U.K.

Inhibition and flux experiments

Erythrocytes were washed three times with 20 vols. of a medium containing 140 mM NaCl, 5 mM KCl, 20 mM Tris-HCl (pH 7.9 at 20°C), 2 mM $MgCl_2$ and 0.2 mM EDTA. The buffy coat was discarded.

Cells were treated with thiol-reactive agents at 1°C by incubating 0.25 ml aliquots of pre-cooled washed erythrocytes (haematocrit approx. 40%) with 0.5 ml of pre-cooled medium containing the appropriate inhibitor. At predetermined time intervals, incubations were stopped by rapidly washing the cells four times with 1 ml portions of ice-cold medium using an Eppendorf 3200

microcentrifuge (10 s, 15 000 × *g*). The cells were resuspended in medium and kept at 1°C until required. In some inhibition experiments, the incubation medium contained high concentrations of D- and L-alanine. Isoosmolarity at various amino acid concentrations was maintained by adjusting the concentration of NaCl in the medium. The pH of these solutions at 1°C was adjusted so as to be the same as that of the original medium.

The amino acid transport activity of normal and treated cells was measured at 37°C by estimating the initial rate of L-[^{14}C]alanine uptake as previously described [11]. Unless otherwise stated, the L-alanine concentration was 0.2 mM (10 min flux). At this concentration, approx. 98% of the total L-alanine uptake is mediated by the C-system [12].

Erythrocyte GSH estimations

Erythrocyte GSH concentrations were measured using the non-specific thiol reagent, DTNB [23]. Previous experiments have demonstrated that more than 92% of the DTNB-reactive thiol in sheep erythrocytes is GSH [24]. In one series of experiments, GSH was also measured by an alternative method using alloxan as chromogen [25]. This method is specific for GSH. In particular, alloxan distinguishes between GSH and its thiol precursors and degradation products (cysteine, γ-glutamylcysteine and cysteinylglycine) [26,27].

Results and Discussion

Preliminary inhibition experiments

The effects of seven thiol-reactive agents (1 mM) on L-alanine uptake by normal sheep erythrocytes were investigated. $HgCl_2$, PCMBS, *N*-ethylmaleimide and diamide all produced considerable inhibition of L-alanine uptake when preincubated with cells for 1 h at 1°C (79, 94, 60 and 54% inhibition, respectively (means of triplicate estimates)). *t*-Butylhydroperoxide produced a small but significant inhibition of transport (28%) whereas iodoacetamide and DTNB were without effect. DTNB (1 mM) did not inhibit transport even when preincubated with cells for 1 h at 37°C. Under these conditions, iodoacetamide caused a 29% reduction in uptake rate. In subsequent experiments, the inhibitory effects of PCMBS, diamide, *N*-ethylmaleimide and *t*-butylhydroperoxide were investigated in detail.

PCMBS inhibition

The time-course and concentration dependence (0.1—5 mM) of PCMBS inhibition of L-alanine uptake were investigated. The degree of transport inhibition was dependent on both the incubation time and PCMBS concentration. The highest concentration of PCMBS gave essentially complete inhibition of uptake within 30 min whereas incubation of cells with 0.1 mM PCMBS for 30 min resulted in only 27% inhibition of transport activity. L-Alanine efflux from preloaded cells (10 min flux, initial intracellular concentration 5.3 mmol/l cells) was inhibited 80% by preincubation of cells with 1 mM PCMBS for 40 min at 1°C. PCMBS inhibition of L-alanine influx and efflux occurred in the absence of detectable entry of organomercurial into the cell, as judged by the absence of any decrease in intracellular GSH concentration (less than 2%) when

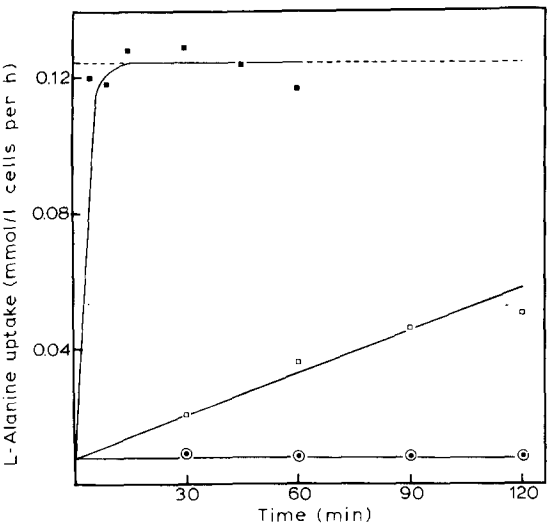

Fig. 1. Effects of thiols on PCMBS inhibition of L-alanine uptake. Cells were incubated with 1 mM PCMBS for 1 h at 1°C as described in Materials and Methods, washed free of excess inhibitor and incubated at 37°C in the presence of 10 mM dithioerythritol (■), cysteine (□), GSH (●) or with no addition (o). At appropriate time intervals, erythrocytes were washed free of thiol, and L-alanine transport activity determined (0.2 mM, 10 min flux at 37°C). The control L-alanine transport rate of this cell preparation is indicated by the broken line.

cells were incubated with 1 mM PCMBS for 1 h at 1°C. For this experiment, GSH was assayed using DTNB as chromogen. Appropriate controls confirmed that the PCMBS-GSH complex was unreactive towards DTNB under the experimental conditions used in the GSH assay.

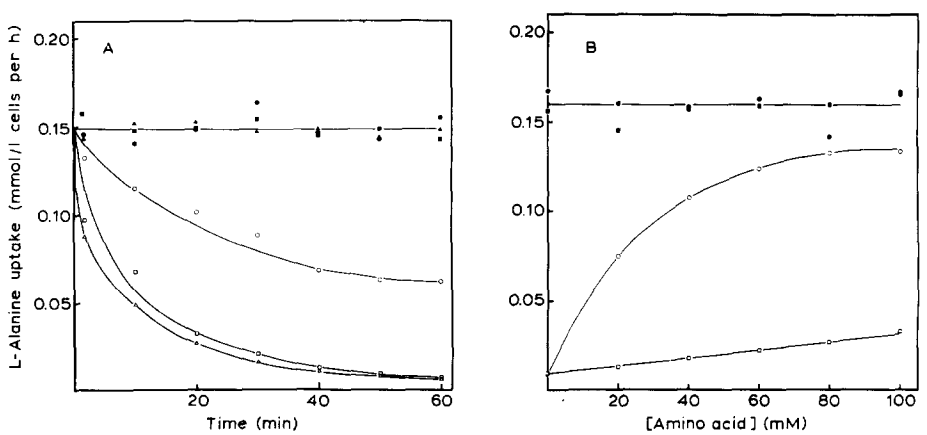

Fig. 2. Effects of extracellular D- and L-alanine on PCMBS inhibition of L-alanine uptake. (A) Cells were incubated ±1 mM PCMBS at 1°C in the presence of 20 mM D- and L-alanine. Cells were washed free of excess inhibitor and amino acid, and the initial rate of L-alanine uptake (0.2 mM, 10 min flux) measured at 37°C. PCMBS incubations: △, no amino acid; □, D-alanine; ○, L-alanine. Control incubations in the absence of PCMBS: ▲, no amino acid; ■, D-alanine; ●, L-alanine. (B) Cells were incubated ±1 mM PCMBS at 1°C for 1 h in the presence of varying concentrations of D- and L-alanine. Excess inhibitor and amino acid were removed by washing. PCMBS incubations: □, D-alanine; o, L-alanine. Control incubations in the absence of PCMBS: ■, D-alanine; ●, L-alanine. See text for other experimental details.

Incubation of PCMBS-treated cells at 37°C with dithioerythritol (10 mM) resulted in rapid and complete recovery of transport activity (Fig. 1). Cysteine (a substrate for the transport system) produced a slow reversal of transport inhibition. In contrast, GSH had no effect. In other experiments, it was found that bovine serum albumin (1 mM) was also unable to regenerate transport activity. Control experiments established that none of these thiols affected L-alanine transport in untreated cells.

Fig. 2A shows the time-course of PCMBS (1 mM) inhibition in the presence of 20 mM extracellular D- and L-alanine. L-Alanine but not D-alanine gave considerable protection against inhibition. The concentration dependence of this protective effect is presented in Fig. 2B. At high L-alanine concentrations, PCMBS was almost completely ineffective. Half-maximal protection was given at an L-alanine concentration of 19 mM. In contrast, D-alanine was relatively ineffective even when present at high concentrations. Since the V value for L-alanine uptake at 1°C is extremely low (less than 0.25 mmol/l cells per h [12]), the protective effect of L-alanine occurred under conditions where there was little transport of amino acid into the cell. It is therefore likely that the PCMBS-sensitive thiol groups are located on the outer surface of the cell membrane within the amino acid binding site of the transport system. Schaeffer et al. [28] reported a similar effect of PCMBS on Na-dependent neutral amino acid transport by rabbit ileum. Preliminary [^{203}Hg]PCMBS binding studies estimate that there are up to 10^4 L-alanine-protected PCMBS binding sites per erythrocyte.

The ability of dithioerythritol and cysteine, but not GSH or bovine serum albumin, to restore transport activity may simply reflect the relative reactivities of these thiols [27], but steric hindrance in the region of the transport site may also have been a contributory factor since thiol-containing proteins have been reported to readily reverse the effects of organomercurials on erythrocyte glucose and cation transport [29,30].

Diamide and N-ethylmaleimide inhibition

Initially, the time-courses of diamide and *N*-ethylamleimide (both 1 mM) inhibition of L-alanine influx were followed. For both reagents the onset of inhibition was relatively rapid, maximum inhibition occurring within 20 min (data not shown). Fig. 3A illustrates the concentration dependence of diamide inhibition of L-alanine uptake. In contrast to PCMBS, diamide only caused partial inhibition of uptake (60%) even at high reagent concentrations. Fig. 3A further demonstrates that the decrease in transport activity was associated with the oxidation of intracellular GSH. The relationship between transport activity and GSH concentration is shown in the insert in Fig. 3A. The curve is distinctly biphasic. As the GSH concentration drops to 20% of its control value, there is a progressive but gradual decrease in transport activity (approx. 30%). At lower GSH concentrations the relative drop in transport activity is much more marked. The inhibitory effect of diamide was completely reversed by incubation of treated cells with either dithioerythritol or glucose. Reappearance of transport activity in the presence of glucose was associated with the regeneration of intracellular GSH.

The concentration dependence of *N*-ethylmaleimide inhibition of L-alanine

uptake is shown in Fig. 3B. Like diamide, *N*-ethylmaleimide caused only partial (70%) inhibition of transport. This inhibition was associated with the depletion of intracellular GSH, and the relationship between GSH concentration and transport activity was biphasic.

As expected, *N*-ethylmaleimide inhibition of L-alanine uptake was not affected by dithioerythritol. The ability of dithioerythritol to reverse PCMBS and diamide but not *N*-ethylmaleimide inhibition of amino acid transport was exploited in experiments to test whether the three inhibitors acted at common sites. Table I shows the effect of pretreating cells with diamide before addition

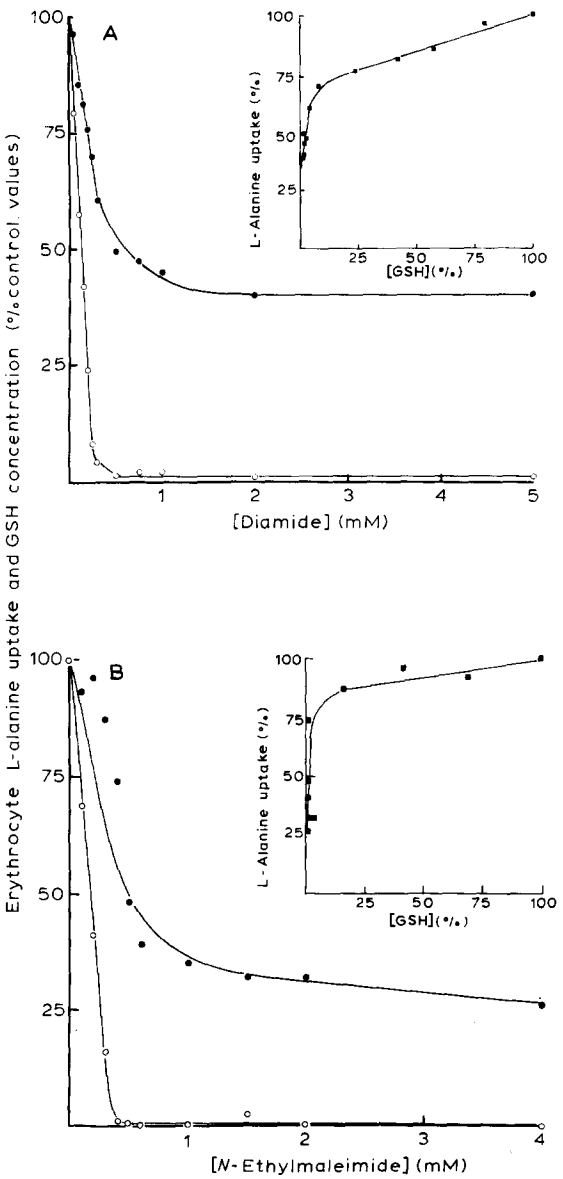

Fig. 3. A and B.

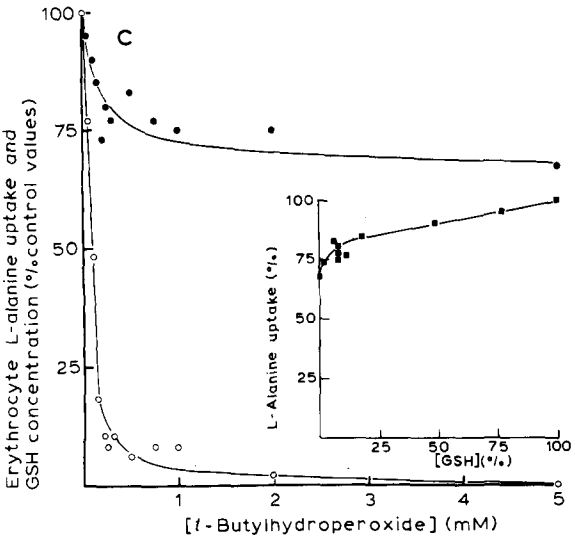

Fig. 3. Concentration dependence of diamide, *N*-ethylmaleimide and *t*-butylhydroperoxide inhibition of L-alanine uptake. Incubations in the presence of diamide (A), *N*-ethylmaleimide (B) and *t*-butylhydroperoxide (C) were for 1 h at 1°C. GSH was estimated using DTNB as chromogen. •, L-alanine uptake (0.2 mM, 10 min flux at 37°C); o, intracellular GSH concentration. Other experimental details are given in Materials and Methods.

TABLE I

N-ETHYLMALEIMIDE INHIBITION OF L-ALANINE UPTAKE: EFFECT OF PRETREATMENT WITH DIAMIDE, PCMBS AND *t*-BUTYLHYDROPEROXIDE

Cells were subjected to three consecutive 1 h incubations after which amino acid uptake was measured (0.2 mM, 10 min flux at 37°C). Erythrocytes were washed between incubations to remove excess inhibitor and dithioerythritol. Inhibitors were present at 1 mM and dithioerythritol at 10 mM. —, denotes incubations with no additions. Values are means of duplicate estimates. Other experimental details are given in Materials and Methods.

	Incubation			L-alanine uptake (% inhibition)
	A (1°C)	B (1°C)	C (37°C)	
Expt. 1	diamide	—	—	64
		—	dithioerythritol	0
		N-ethylmaleimide	—	63
		N-ethylmaleimide	dithioerythritol	7
	N-ethyl-maleimide	—	—	71
		—	dithioerythritol	75
		diamide	—	73
		diamide	dithioerythritol	74
Expt. 2	PCMBS	—	—	90
		—	dithioerythritol	1
		N-ethylmaleimide	—	97
		N-ethylmaleimide	dithioerythritol	68
	N-ethyl-maleimide	—	—	67
		—	dithioerythritol	66
		PCMBS	—	97
		PCMBS	dithioerythritol	68
Expt. 3	*t*-butyl-hydroperoxide	—	—	37
		—	dithioerythritol	3
		N-ethylmaleimide	—	64
		N-ethylmaleimide	dithioerythritol	60
	N-ethyl-maleimide	—	—	67
		—	dithioerythritol	66
		t-butyl-hydroperoxide	—	68
			dithioerythritol	68

of *N*-ethylmaleimide and vice versa. Addition of *N*-ethylmaleimide to diamide-treated cells caused no further inhibition of transport, and the inhibition could still be reversed by dithioerythritol. Similarly, addition of diamide to *N*-ethylmaleimide-treated erythrocytes resulted in no additional inhibition of transport, and dithioerythritol had no effect. Table I also shows the results of an experiment in which the effect of pretreating cells with PCMBS before *N*-ethylmaleimide addition was investigated. In contrast to the previous experiment, pretreatment of erythrocytes with PCMBS did not protect the cell against the effects of *N*-ethylmaleimide as shown by the inability of dithioerythritol to restore normal transport activity. Furthermore, addition of PCMBS to *N*-ethylmaleimide-treated cells resulted in further inhibition of L-alanine uptake. Other experiments (not shown) established that pretreatment of erythrocytes with PCMBS did not interfere with the ability of diamide to protect against the effects of *N*-ethylmaleimide. Incubation of diamide-treated cells with PCMBS resulted in complete inhibition of transport. Diamide had no observable effect on L-alanine transport by PCMBS-inhibited erythrocytes.

The results presented in Table I suggest that *N*-ethylmaleimide and diamide act at a common site(s) which differs from that attacked by PCMBS. An alternative possibility is that PCMBS, diamide and *N*-ethylmaleimide act at the same site(s) but that diamide and *N*-ethylmaleimide displace PCMBS from the thiol groups. Such reactions can occur. For example, DTNB and diamide are both capable of displacing GSH-bound mercurial at pH 7 (Jocelyn, P.C., personal communication). However, it is unlikely that displacement occurs to any significant extent in the present experiments because addition of *N*-ethylmaleimide or diamide to erythrocytes fully inhibited by PCMBS did not result in the predicted partial recovery of transport activity which would be expected to accompany such an exchange. The observation that PCMBS inhibited the residual transport activity remaining after diamide and *N*-ethylmaleimide treatment is further evidence that PCMBS acts at a different site from the other two inhibitors. Since both diamide and *N*-ethylmaleimide rapidly penetrate the erythrocyte membrane, even at 0°C, it is possible that the effects of these two agents on L-alanine transport were the result of reaction with thiol groups on the inner surface of the cell membrane or within the cytoplasm.

t-Butylhydroperoxide inhibition

t-Butylhydroperoxide is a stable organic peroxide and exerts most of its effects on cells by acting as a glutathione peroxidase substrate. Incubation of erythrocytes for 1 h at 1°C with varying concentrations of *t*-butylhydroperoxide (0.05—5 mM) resulted in progressive depletion of intracellular GSH and a parallel loss of transport activity (Fig. 3C). However, the maximum inhibition of L-alanine uptake (33%) was approximately half that induced by either diamide or *N*-ethylmaleimide. A plot of transport activity vs. intracellular GSH concentration (insert in Fig. 3C) resembles the initial component of the corresponding *N*-ethylmaleimide and diamide curves. Control experiments established that the time-course of *t*-butylhydroperoxide inhibition of L-alanine influx was rapid, and similar to those of diamide and *N*-ethylmaleimide. *t*-Butylhydroperoxide inhibition was reversed by incubation of cells with either dithioerythritol or glucose.

Addition of *t*-butylhydroperoxide to *N*-ethylmaleimide-treated erythrocytes resulted in no additional inhibition of transport, and incubation of *t*-butylhydroperoxide-treated cells with *N*-ethylmaleimide gave a total inhibition equivalent to that found with *N*-ethylmaleimide alone (Table I). Pretreatment of erythrocytes with organic peroxide did not protect the cell against *N*-ethylmaleimide inhibition.

In view of the supposed specificity of *t*-butylhydroperoxide action [31], and the finding that the transport inhibition was reversed by both dithioerythritol and glucose, it is likely that the *t*-butylhydroperoxide-sensitive thiols are intracellular GSH itself of highly reactive thiols capable of being rapidly oxidised by thiol-disulphide exchange with GSSG. Diamide oxidation of intracellular GSH would be expected to have similar effects. The results presented in this and the preceding section therefore suggest that diamide and *N*-ethylmaleimide act at a minimum of two sites, only one of which is susceptible to organic peroxide treatment.

Kinetic analysis of diamide, PCMBS and t-butylhydroperoxide inhibition

Fig. 4 shows double-reciprocal plots ($1/v$ vs. $1/s$) of L-alanine uptake (5—50 mM) by normal and diamide-treated erythrocytes. Inhibition of uptake was associated with a decrease in V with no effect on apparent K_m (18 mM). Parallel experiments with *t*-butylhydroperoxide and PCMBS-treated cells (23 and 59% inhibition of uptake, respectively) gave similar non-competitive inhibition patterns.

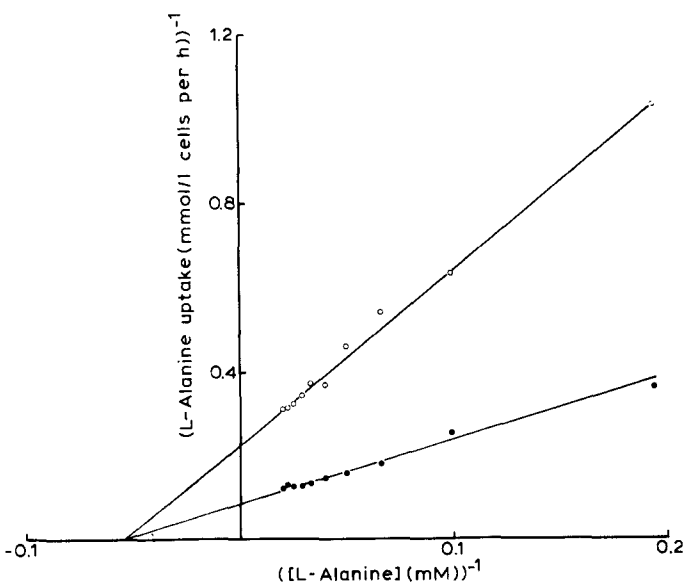

Fig. 4. Effect of diamide on the apparent K_m and V of L-alanine uptake. o, diamide-treated cells (1 h incubation at 1°C with 1 mM diamide); •, control. L-Alanine uptake (5—50 mM, 10 min influx) was measured at 37°C. See text for other experimental details.

GSH and amino acid transport

The diamide, *N*-ethylmaleimide and *t*-butylhydroperoxide inhibition data suggest a link between intracellular GSH and amino acid transport. This relationship was investigated further in two final experiments. First, the possible participation of the γ-glutamyl cycle in erythrocyte amino acid transport [32—34] was tested by monitoring the GSH content of cells during large net transport of L-alanine (7 mmol/l cells), achieved by incubating cells with 25 mM amino acid for 2 h at 37°C. No decrease in GSH concentration was observed with alloxan as chromogen, demonstrating that no GSH degradation had occurred during the incubation period. Additional evidence against the participation of the γ-glutamyl cycle in erythrocyte amino acid transport has been recently reviewed [35]. Second, L-alanine transport in control and γ-glutamylcysteine synthetase-deficient sheep erythrocytes was compared. Despite a 64% reduction in GSH concentration, γ-glutamylcysteine synthetase-deficient cells showed normal transport activity (alanine transport (0.2 mM) 0.241 ± 0.017 (11) and 0.257 ± 0.025 (10) mmol/l cells per h for normal and enzyme-deficient cells, respectively (mean ±S.E. (*n*)), GSH concentration 2.61 ± 0.10 and 0.95 ± 0.14 mmol/l cells, respectively). It is therefore unlikely that GSH participates directly in erythrocyte amino acid transport.

Conclusions

The present series of experiments suggest that there are at least three distinct classes of cellular thiol groups essential for normal amino acid transport. Fig. 5 shows a schematic representation of the most straightforward interpretation of the experiment results. For simplicity, the *N*-ethylmaleimide, diamide and *t*-butylhydroperoxide inhibitory sites are depicted on the transport system

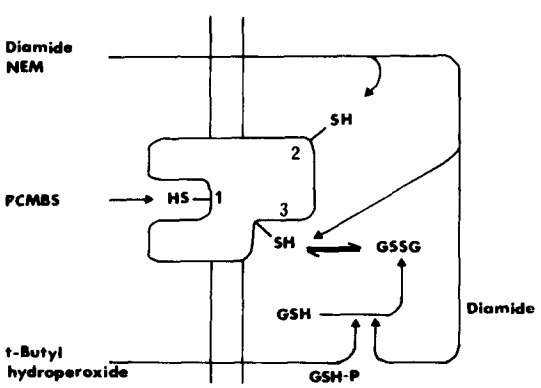

Fig. 5. Postulated interactions between the C-system and thiol-reactive agents. Class 1 thiols react selectively with PCMBS and are located on the outer surface of the cell membrane in the region of the transport site. Reaction with PCMBS results in almost complete loss of transport activity. Class 2 thiols react with *N*-ethylmaleimide (NEM) and diamide but not with PCMBS or *t*-butylhydroperoxide. Oxidation or alkylation of these thiol groups results in a 60% inhibition of transport. Class 3 thiols are located on the inner surface of the cell membrane or within the cytoplasm and are oxidised during *t*-butylhydroperoxide treatment. They presumably also react with diamide and *N*-ethylmaleimide. Reaction of these thiols results in a 30% inhibition of transport which is not additive to the inhibition resulting from reaction of Class 2 thiols. GSH-P, glutathione peroxidase.

itself although it is possible that they act on other cell components intimately involved in transport function.

Previous studies have shown that sheep erythrocytes with an inherited deficiency of the C-system accumulate amino acids probably derived from protein degradation during reticulocyte maturation [17,35,36]. This finding together with the present results offers an explanation for the previously puzzling observation that a case of human GSH synthetase deficiency was associated with abnormally high erythrocyte amino acid concentration as well as the expected severe GSH deficiency [37]. If amino acid transport in human erythrocytes is also sensitive to the redox state of cellular thiol groups, and if, as seems likely, GSH-deficient human erythrocytes cannot always support these thiols in the reduced state, then perturbed amino acid transport and hence amino acid accumulation could result.

Acknowledgements

I thank Mrs. S. Mansfield and Mr. D.A. Fincham for skilled and enthusiastic assistance.

References

1 Winter, C.G. and Christensen, H.N. (1964) J. Biol. Chem. 239, 872—878
2 Gardner, J.D. and Levy, A.G. (1972) Metab. Clin. Exp. 21, 413—431
3 Hoare, D.G. (1972) J. Physiol. 221, 311—329
4 Hoare, D.G. (1972) J. Physiol. 221, 331—348
5 Young, J.D. and Ellory, J.C. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 301—325, Academic Press, London
6 Ellory, J.C. and Young, J.D. (1978) J. Physiol. 285, 51P—52P
7 Rosenberg, R. (1979) J. Neural. Trans. Suppl. 15, 153—160
8 Young, J.D. and Ellory, J.C. (1979) J. Neural Trans. Suppl. 15, 139—151
9 Young, J.D., Wolowyk, M.W., Jones, S.E.M. and Ellory, J.C. (1979) Nature 279, 800—802
10 Rosenberg, R., Young, J.D. and Ellory, J.C. (1980) Biochim. Biophys. Acta 598, 375—384
11 Young, J.D., Jones, S.E.M. and Ellory, J.C. (1980) Proc. R. Soc. B., in the press
12 Young, J.D., Tucker, E.M. and Ellory, J.C. (1976) Biochem. J. 154, 43—48
13 Ellory, J.C. and Young, J.D. (1977) J. Physiol. 272, 43P—44P
14 Young, J.D. and Ellory, J.C. (1977) Biochem. J. 162, 33—38
15 Young, J.D., Tucker, E.M. and Ellory, J.C. (1975) Nature 254, 156—157
16 Tucker, E.M. and Kilgour, L. (1970) Experientia 26, 203—204
17 Ellory, J.C., Tucker, E.M. and Deverson, E.V. (1972) Biochim. Biophys. Acta 279, 481—483
18 Tucker, E.M. (1974) Res. Vet. Sci. 16, 19—22
19 Tucker, E.M. and Kilgour, L. (1973) Res. Vet. Sci. 14, 306—311
20 Young, J.D. (1979) Biochem. Soc. Trans. 7, 683—685
21 Young, J.D. and Nimmo, I.A. (1975) Biochim. Biophys. Acta 404, 132—141
22 Tucker, E.M., Kilgour, L. and Young, J.D. (1976) J. Agric. Sci. 87, 315—323
23 Beutler, E., Duron, O. and Kelly, B.M. (1963) J. Lab. Clin. Med. 61, 882—888
24 Young, J.D., Nimmo, I.A. and Hall, J.G. (1975) Biochim. Biophys. Acta 404, 124—131
25 Patterson, J.W. and Lazarow, A. (1955) Methods Biochem. Anal. 2. 259—278
26 Patterson, J.W. and Lazarow, A. (1954) in Glutathione (Colowick, S., Lazarow, A., Racker, E., Schwarz, D.R., Stadtman, E. and Waelsch, H., eds.), pp. 63—75, Academic Press, London
27 Jocelyn, P.C. (1972) Biochemistry of the SH Group, Academic Press, London
28 Schaeffer, J.F., Preston, R.L. and Curran, P.F. (1973) J. Gen. Physiol. 62, 131—146
29 Van Steveninck, J., Weed, R.I. and Rothstein, A. (1965) J. Gen. Physiol. 48, 617—632
30 Sutherland, R.M., Rothstein, A. and Weed, R.I. (1967) J. Cell. Physiol. 69, 185—198
31 Srivastava, S.K., Awasthi, Y.C. and Beutler, E. (1974) Biochem. J. 139, 289—295
32 Meister, A. (1973) Science 180, 33—39
33 Agar, N.S., Gruca, M. and Harley, J.D. (1974) Anim. Blood Groups Biochem. Genet. 5, 63—64

34 Palekar, A.G., Tate, S.S. and Meister, A. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 293—297
35 Young, J.D. and Jarvis, S.M. (1980) in Transport in Inherited Disease (Toothill, C., ed.), MTP Press, Lancaster, in the press
36 Tucker, E.M., Wright, P.C. and Young, J.D. (1977) J. Physiol. 271, 47P—48P
37 Marstein, S., Jellum, E., Halpern, B., Eldjarn, L. and Perry, T.L. (1976) New Engl. J. Med. 295, 406—412