Biochimica et Biophysica Acta, 467 (1977) 238–250

BBA 77711

MODULATION OF DRUG PERMEABILITY IN CHINESE HAMSTER OVARY CELLS

POSSIBLE ROLE FOR PHOSPHORYLATION OF SURFACE GLYCOPROTEINS

SVEIN A. CARLSEN, JAMES E. TILL and VICTOR LING

Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, M4X 1K9 (Canada)

(Received October 7th, 1976)

Summary

We have previously reported that the uptake of colchicine and other drugs in Chinese hamster ovary (CHO) cells can be greatly enhanced by the addition of metabolic inhibitors such as cyanide (See, Y.P., Carlsen, S.A., Till, J.E. and Ling, V. (1974) Biochim. Biophys. Acta 373, 242–252). This has led us to postulate the presence of an active drug permeability barrier in these cells. In this paper we provide evidence for the dependence of this permeability barrier on intracellular ATP levels. Colchicine-resistant mutants of CHO cells exhibiting a reduced drug permeability, however, can maintain this drug permeability barrier at much lower ATP levels, suggesting that they possess an altered active drug permeability barrier. We have also observed a membrane-associated protein kinase-phosphoprotein phosphatase system in the isolated membranes of mutant and wild-type cells. Differences in the intrinsic protein phosphorylation patterns between the membranes of these cells have led us to conclude that the control of the drug permeability barrier may be mediated via the phosphorylation of at least two high molecular weight surface glycoproteins.

Introduction

The maintenance of a selective permeability barrier is one of the major functions of the plasma membrane. At present little is known regarding the mechanisms regulating drug permeability in mammalian cells. In our study of this process we have utilized drug-resistant mutants isolated from mammalian

Abbreviations: CHO, Chinese hamster ovary; EGTA, (ethylenebis(oxyethylenenitrilo))tetraacetic acid.

cells in culture. Mutants of Chinese hamster ovary (CHO) cells selected for resistance to the antimitotic drug colchicine have been found to possess a decreased membrane permeability to the drug [1]. Moreover, the development of resistance to colchicine leads to a concomitant resistance to a number of other structurally different drugs [1,2]. Using these mutants we have made a number of interesting observations regarding drug permeability in these cells. For example we have shown that: (1) Colchicine enters these cells via an unmediated mechanism [3]. (2) Membrane-active agents such as local anesthetics and non-ionic detergents greatly enhance drug permeability [3]. (3) Metabolic inhibitors also stimulate drug uptake in mutant and parental cells to a point where the rate of uptake becomes equal for both cell lines [4].

The enhancement of colchicine uptake by metabolic inhibitors such as cyanide occurs rapidly and can be completely reversed by the addition of glucose [4]. Differences in the kinetics of this cyanide-induced potentiation have also been observed between colchicine-resistant mutants and the parental cell line [4]. These results suggest that certain metabolic process(es) of CHO cells may be important for maintaining a membrane permeability barrier against colchicine and other drugs, and that this energy-dependent barrier may be altered in drug-resistant mutants.

The purpose of the present study is to test the hypothesis that the drug permeability barrier in CHO cells is dependent on the intracellular ATP level [4]. In this context, we show that in cells treated with cyanide, the rates of fluctuation in ATP level correlate with rapid changes in drug uptake rates. The mutants are able to maintain their permeability barriers at much lower ATP levels than the parental cell line. In addition, we observed the presence of a membrane-associated protein kinase-phosphoprotein phosphatase system in these cells, which may be involved in the maintenance of this active drug permeability barrier.

Materials and Methods

Cells. The cell lines CH^RC4 and CH^RC5 used in this study were selected for resistance to colchicine in two dependent three-step selections by Ling and Thompson [1]. The parent of these mutants (AUXB1) is a Chinese hamster ovary (CHO) cell line auxotrophic for glycine, adenosine and thymidine [5]. Revertant I10-1 was isolated from CH^RC5 in a single step; details of revertant isolation will be published elsewhere (Ling, V., in preparation). Procedures used for maintaining mutant lines were as described by Thompson and Baker [6]. All cells used in the experiments described in this paper were grown in suspension culture at 37°C in α-minimum essential medium [7] supplemented with antibiotics and 10% fetal calf serum (Flow Laboratories).

ATP measurements. Cell supensions at $3 \cdot 10^6$ cells/ml in phosphate-buffered saline were added to 0.5 ml of ice-cold 1.2 M $HClO_4$, the samples were mixed and immedicately placed on ice. The precipitate formed was removed by centrifugation and duplicate 50-μl samples of the supernatant used for determination of ATP concentrations by the method of Stanley and Williams [8] using a Mark I Liquid Scintillation counter (Nuclear Chicago).

Generation of cells containing various levels of ATP. Cell suspensions at

$3 \cdot 10^6$ cells/ml in phosphate-buffered saline were incubated at 37°C with concentrations of rotenone from 0.1 to 100 ng/ml as described by Johnstone [9]. The ATP level steadily decreased for the first 20 min after the addition of rotenone but then remained stable for the next 20 min during which time the rate of colchicine uptake was measured as previously described [4]. The ATP levels in the cells were measured as described above immediately before and after the measurement of colchicine uptake rates to monitor any change in the ATP level during the course of the uptake measurement.

Isolation of membranes. Membranes were isolated by the method of Brunette and Till [10]. Membrane protein concentrations were determined by the method of Lowry et al. [11] using bovine serum albumin as a standard.

Measurement of protein kinase activity. The reaction mixture consisted of 100 μg of membrane protein in a final volume of 200 μl containing 50 mM acetate buffer (sodium salts), pH 6.0, 10 mM magnesium acetate, 0.3 mM (ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) and 10 μCi of [γ-^{32}P]-ATP. Reactions were carried out for 5 min at room temperature during which time the rate of incorporation of labelled phosphorus into acid-precipitable material was linear with time. The reactions were terminated by one of two methods. If the sample was to be used for acrylamide gel electrophoresis, 25 μl of a solution of 10% sodium dodecyl sulfate, 5 mM ethylenediaminetetraacetic acid (EDTA) and 6 mM 2-mercaptoethanol was added. In all other samples the reaction was terminated by the addition of 5 ml of 20% trichloroacetic acid. The precipitate was collected on 0.45 μm pore size Millipore filters and washed four times with 10 ml of Bray's solution [12] and counted in a liquid scintillation counter.

Phosphoprotein phosphatase assay. Phosphoprotein phosphatase activity was assayed using [^{32}P]protamine as described by Maeno and Greengard [13].

Polyacrylamide gel electrophoresis. Samples of phosphorylated membranes prepared as described above were heated in a boiling water bath for 2 min, glycerol was added to a final concentration of 10% and the samples layered onto 10 cm cylindrical 7% polyacrylamide gels prepared according to Laemmli [14]. Gels were electrophoresed at 3 mA per gel until the tracking dye was 1 cm from the bottom of the gel. Gels were sliced and then digested in NCS solubilizer (Amersham Searle)/water (9 : 1, v/v) overnight at 37°C. 10 ml of toluene scintillation fluid was added and radioactivity determined using a liquid scintillation counter.

Chemicals and reagents. [γ-^{32}P]ATP purchased from Amersham Searle (PB.132) was resuspended in water to an activity of 1 mCi/ml. [^{3}H]colchicine (18 Ci/mmol) was obtained from New England Nuclear and [^{3}H]glucosamine (19 Ci/mmol) was purchased from Amersham Searle. Rotenone, bovine serum albumin and desiccated firefly lanterns were obtained from Sigma Chemical Co. All other reagents were of analytical grade.

Results

Kinetics of ATP breakdown and synthesis

Previous work [4] implicated the involvement of cellular metabolism in the modulation of colchicine uptake in CHO cells. To investigate the possibility that

ATP was involved in this modulation, we determined whether the fluctuations in the rate of colchicine uptake correlated with changes in intracellular ATP levels. The kinetics of ATP breakdown after addition of cyanide and its rate of synthesis after addition of glucose were measured for both the mutant and parental cell lines. As seen in Fig. 1, the ATP level decreased after the addition of cyanide with a half-time of about 2 min with the rate of ATP breakdown being the same for both the mutant and parental cell lines. The addition of glucose to cyanide-treated cells caused an immediate increase in ATP levels (Fig. 1). Thus, in general, the rapid decrease in ATP level upon addition of cyanide and subsequent rapid increase in ATP on the addition of glucose correlated with the rapid changes in colchicine flux (increased and decreased uptake, respectively) seen under the same conditions [4]. These results are consistent with the hypothesis that modulation of colchicine uptake is mediated via fluctuations in the cellular ATP level.

Differences in the cyanide-induced potentiation of colchicine uptake between the parental and mutant cell lines allowed this hypothesis to be tested more quantitatively. We have previously shown [4] that: (1) the mutant CH^RC4 exhibited a 5 min lag in the cyanide-induced potentiation of colchicine uptake while in the parental cell line the stimulation was virtually immediate and (2) the mutant CH^RC4 line required lower concentrations of glucose to reverse the cyanide-induced potentiation of colchicine uptake than the parental cell line [4] (0.3 mM compared to 0.7 mM, respectively, to reduce the maximally stimulated rate by 50% (from ref. 4)).

We investigated whether or not quantitative differences existed between the ATP levels of mutant and parental cells which could account for these differences in response to cyanide and glucose. As shown in Fig. 1, the lag in the stimulation of colchicine uptake seen in the mutant after the addition of

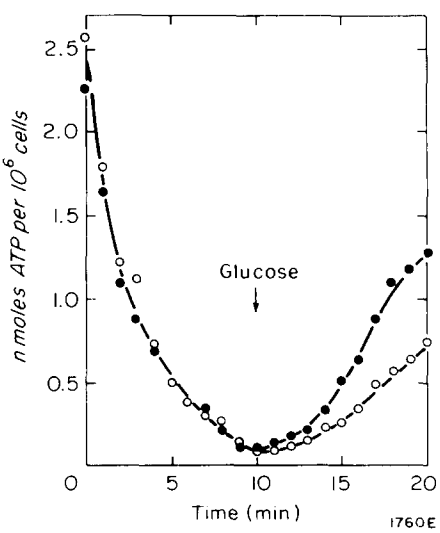

Fig. 1. Time course of ATP breakdown after inhibition by cyanide and its reversal by glucose. ATP levels in AUXB1 (●——●) and CH^RC4 (○——○) were measured as described in Materials and Methods at various times of incubation in 2 mM KCN at 37°C. After 10 min glucose was added to a final concentration of 15 mM.

cyanide [4], is not reflected in a concomitant lag in the rate of ATP breakdown; both mutant and parental cells had similar rates of ATP decay.

The previously observed ability of mutant cells to utilize lower concentrations of glucose to reverse the cyanide-induced potentiation of colchicine uptake [4] suggested that the mutant might be able to metabolize glucose more efficiently to produce ATP than the parental cell line; however, the data presented in Fig. 1 shows that the rate of ATP synthesis after the addition of glucose was slower in the CH^RC4 cell line than in the parental cell line. To investigate this latter point further, the ATP level in cells exposed to 2 mM cyanide plus various concentrations of glucose was measured. It is seen in Fig. 2 that the ATP level started to increase in both the CH^RC4 and AUXB1 cell lines at glucose concentrations greater than 0.2 mM; however, above 0.2 mM glucose, the ATP level was always greater in the parental cell line than in the mutant CH^RC4 cell line. This is in agreement with the date presented in Fig. 1 showing that in the presence of cyanide the parental cell line can utilize glucose to generate a higher intracellular ATP level than in the mutant cell line.

These data indicate that the rates of ATP breakdown on cyanide treatment and ATP synthesis on subsequent addition of glucose in mutant and parental cells (Fig. 1) do not quantitatively correlate with the previously observed differences in colchicine uptake rates in these two lines [4] when treated with these reagents under similar conditions.

Effect of ATP level on colchicine uptake

Since we had shown previously that the cell membrane of the mutants was different from that of the parental cells, having an increased amount of a high molecular weight glycoprotein [15,16], it was possible that the response of the membrane of the mutant cells to intracellular ATP was different from that of the parental cells. To test this possibility we measured the rates of colchicine

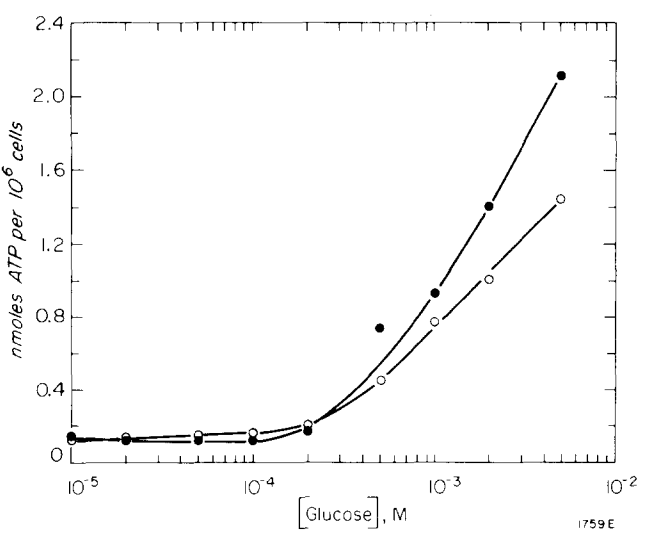

Fig. 2. Utilization of various concentrations of glucose in the presence of cyanide. AUXB1 (●——●) and CH^RC4 (○——○) cells were incubated in 2 mM KCN plus various concentrations of glucose for 10 min at 37° C. ATP levels were measured as described in Materials and Methods.

uptake as a function of ATP level. Cells containing various levels of ATP were generated by the method of Johnstone [9]. This method consists of incubating the cells in nanomolar concentrations of the metabolic inhibitor rotenone which caused the ATP concentration to drop to an intermediate level that remained relatively constant for 15—20 min during which time the rate of colchicine uptake was measured. The result of this experiment is shown in Fig. 3. In the parental cell line AUXB1 the rate of colchicine uptake started to increase as the ATP level dropped below 2.0 nmol/10^6 cells. However, in the mutant cell line CH^RC4 stimulation of colchicine uptake did not occur before the ATP level had dropped below 0.5 nmol/10^6 cells. These results show that there is an alteration in this energy-dependent drug permeability barrier in the colchicine-resistant mutant resulting in the mutant being able to maintain a decreased drug permeability at lower ATP levels.

These results also explain the 5 min lag seen in the cyanide-induced potentiation of colchicine uptake in the mutant cell line [4]. In Fig. 1 we see that it takes less than 1 min after the addition of cyanide for the ATP level to drop below 2.0 nmol per 10^6 cells at which point stimulation of colchicine uptake is seen in AUXB1 (Fig. 3); however, it takes 5 min after the addition of cyanide for the ATP level to drop below 0.5 nmol per 10^6 cells at which point stimulation is seen in CH^RC4 (Fig. 3). Thus, these results are in accord with the hypothesis that changes in the rate of colchicine uptake are mediated through changes in cellular ATP levels. However, an alternate view, that the rate of colchicine uptake is related to some other product of glucose metabolism whose level varied in a manner similar to ATP in these experiments, cannot be ruled out.

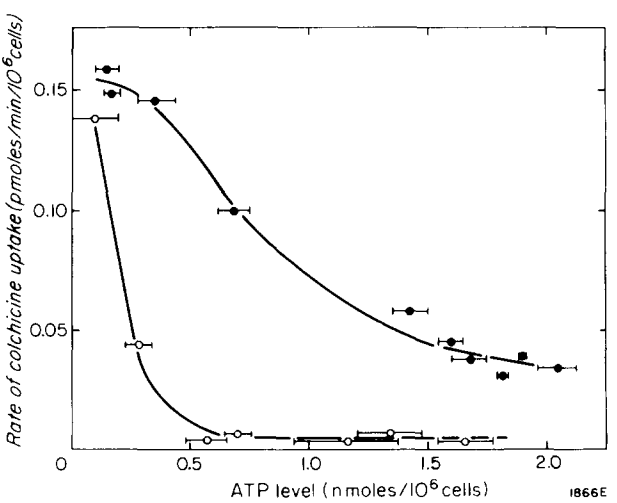

Fig. 3. Effect of cellular ATP level on colchicine uptake. AUXB1 (●———●) and CH^RC4 (○———○) cells containing various levels of ATP were generated as described in Materials and Methods. Rates of colchicine uptake were determined from the linear portions of uptake vs. time curves as previously described [4]. The error bars represent the ATP levels at the beginning and end of the uptake measurement.

Membrane-associated protein kinase

If it is assumed that ATP might cause an alteration in membrane function, one possible mechanism is through the phosphorylation of specific membrane components. To test for the involvement of membrane phosphorylation in the maintenance of the active drug permeability barrier, we first assayed for the presence of a membrane-associated protein kinase activity in isolated membranes. Table I shows that isolated membranes of both the parental cell line AUXB1 and the highly resistant mutant CH^RC5 were able to utilize [γ-^{32}P]ATP to phosphorylate endogenous membrane proteins as well as exogenous acceptors such as casein and protamine. In addition, this activity could not be removed by washing the membranes with high concentrations of NaCl or KCl (data not shown). These data support the idea that the phosphorylation is mediated via a protein kinase(s) and that this kinase is an integral part of the membrane.

We next investigated the phosphorylation of various membrane components by polyacrylamide gel electrophoresis. In particular, we were interested in whether or not the 165 000 molecular weight surface glycoprotein associated with reduced permeability in these cells [15,16] was phosphorylated. As can be seen in Fig. 4, the two independently selected highly resistant mutants CH^RC4 and CH^RC5 both have enhanced phosphorylation of a 165 000 and 200 000 molecular weight band compared to the parental cell line AUXB1. The label in both of these bands was stable to hydroxylamine hydrolysis but was susceptible to treatment with alkali [30] (data not shown). These results are consistent with the phosphorylation being through a phosphate ester linkage rather than an acyl phosphate linkage. The surface glycoprotein associated with reduced drug permeability, the "P" glycoprotein, is readily labeled by metabolic incorporation of [^{3}H]glucosamine [16] and, as shown in Fig. 4, co-electrophoresed with the 165 000 molecular weight phosphorylated protein(s). I10-1, a revertant isolated from CH^RC5, lacks the enhanced phosphorylation of these proteins. These data support the hypothesis that the "P" glycoprotein is phosphorylated by the membrane-associated kinase and that this phosphorylation may be important in the maintenance of reduced permeability in the mutants.

To investigate the nature of this membrane kinase further, we measured the effect of cyclic AMP on the phosphorylation of membrane proteins. As can be seen in Table II, cyclic AMP at concentrations from 10^{-8} to 10^{-4} M did not

TABLE I

DEMONSTRATION OF MEMBRANE-ASSOCIATED KINASE ACTIVITY

Phosphorylation of membranes (100 μg membrane protein) was performed as described in Materials and Methods.

Additions	Relative incorporation (cpm)	
	AUXB1	CH^RC5
Membranes	2519	2125
Heat-denaturated membranes	91	114
Membranes + 100 μg casein	3598	3097
Membranes + 100 μg protamine	4046	5499

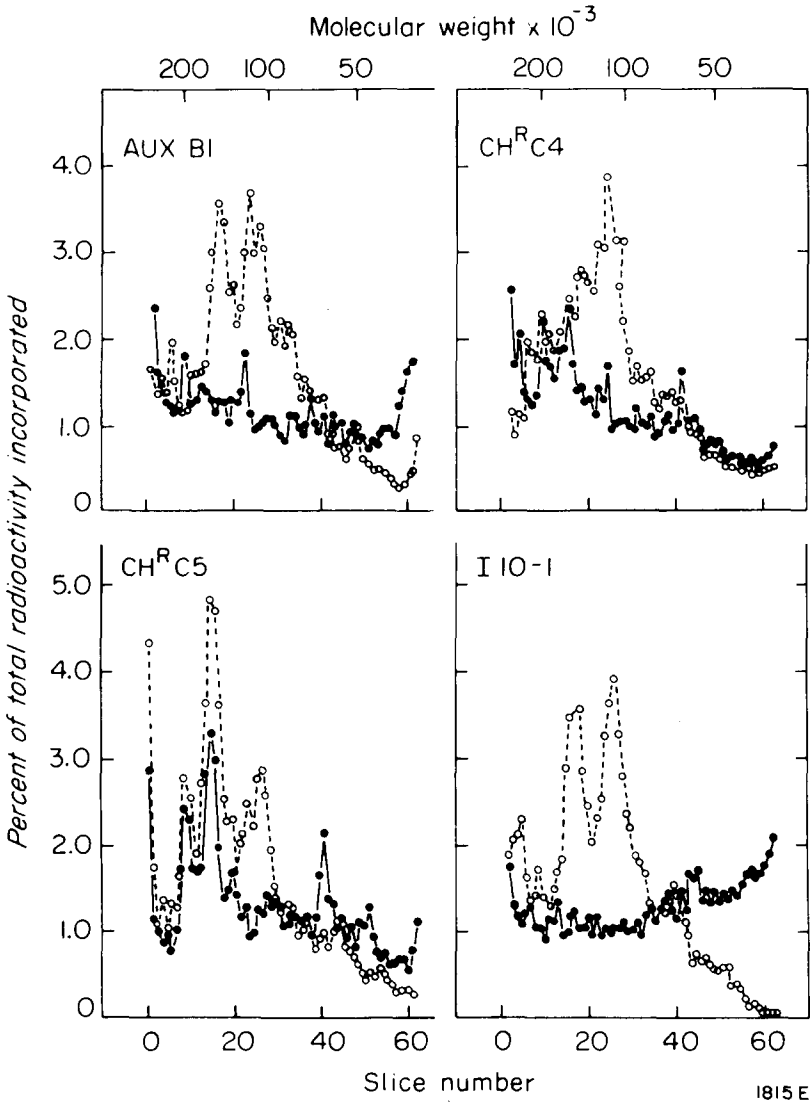

Fig. 4. Analysis of membrane phosphorylation by polyacrylamide gel electrophoresis. Membranes were isolated from cells grown for two generations in the presence of 1 μCi/ml of [^{3}H]glucosamine (19 Ci/mmol) and were phosphorylated and electrophoresed as described in Materials and Methods. Each gel contained 100 μg of membrane protein. Profiles of ^{32}P (●——●) and ^{3}H (○——○) incorporation were determined.

significantly affect the phosphorylation of membranes from either AUXB1 or CH^{R}C4.

To determine if there was a change in the pattern of phosphorylation in the presence of cyclic AMP, polyacrylamide gel electrophoresis of membranes phosphorylated in the presence and absence of cyclic AMP was performed. As shown in Fig. 5, the presence of either 10^{-7} or 10^{-5} M cyclic AMP did not cause any significant alteration in the labeling pattern in either AUXB1 or CH^{R}C5. Thus the membrane-associated protein kinase in these cells does not

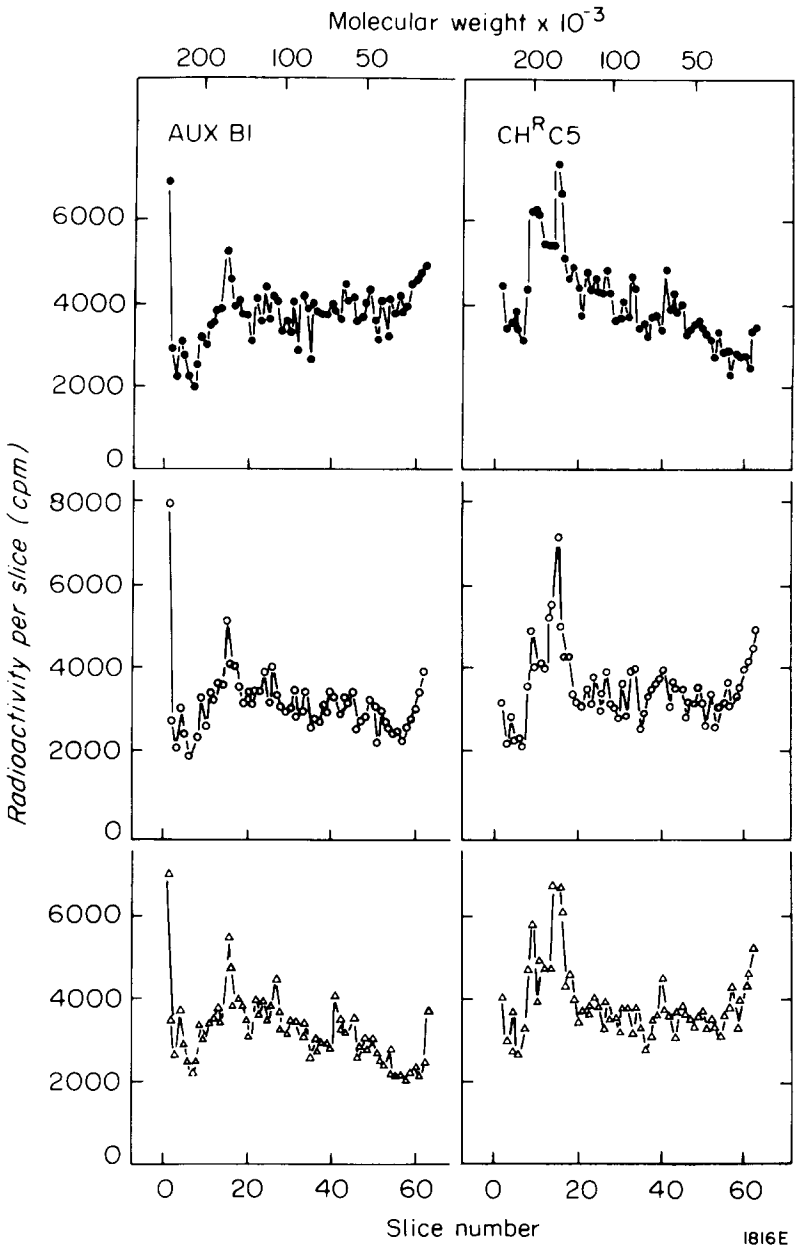

Fig. 5. Effect of cyclic AMP on membrane phosphorylation profile. Phosphorylation reactions were carried out in the absence (●———●) or presence of 10^{-7} M (○———○) or 10^{-5} M (△———△) cyclic AMP. Experimental conditions were as described in Materials and Methods.

appear to be cyclic AMP-dependent contrary to that reported for a number of other membrane-associated kinases [17,18].

Membrane-associated phosphoprotein phosphatase

For a membrane phosphorylation to be involved in the active permeability

TABLE II

EFFECT OF CYCLIC AMP ON MEMBRANE PHOSPHORYLATION

Phosphorylation of membranes was performed as in Table I. Control values were obtained from appropriate samples without added cyclic AMP. Values are the averages of 4 or 5 experiments ±S.D.

Cyclic AMP (M)	Phosphorylation of membrane proteins (percent of control)	
	AUXB1	CH^RC5
$1 \cdot 10^{-8}$	98 ± 12	97 ± 12
$1 \cdot 10^{-7}$	98 ± 19	100 ± 11
$1 \cdot 10^{-6}$	109 ± 20	117 ± 16
$1 \cdot 10^{-5}$	99 ± 15	94 ± 5
$1 \cdot 10^{-4}$	90 ± 13	92 ± 17

barrier, the membrane must be able to rapidly phosphorylate and dephosphorylate the membrane components involved in order to be able to explain the rapid changes in drug permeability seen upon addition of cyanide [4]. To determine if there was a phosphoprotein phosphatase activity also associated with these membranes, the ability of isolated membranes from mutant and parental cells to dephosphorylate phosphoprotamine was measured. As can be seen in Fig. 6, a membrane-associated phosphoprotein phosphatase was found to be associated with the membranes from both AUXB1 and CH^RC5. The activity of this phosphatase was found to be identical for the membranes from both cell lines (Fig. 6). This activity could also be completely inhibited by the addition of 10 mM $ZnCl_2$ (Fig. 6) which is consistent with the properties of other phosphoprotein phosphatase [13].

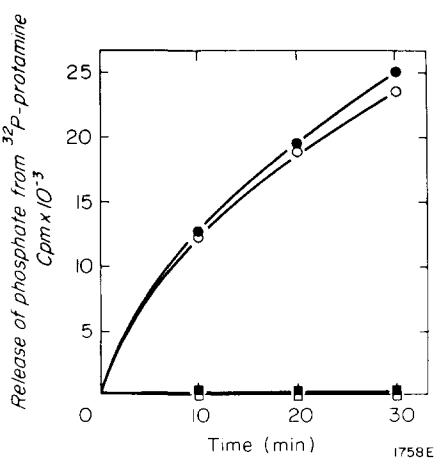

Fig. 6. Measurement of phosphoprotein phosphatase activity on isolated membranes. Membranes from AUXB1 (open symbols) and CH^RC5 (closed symbols) were assayed for phosphatase activity in the absence (circles) and presence (squares) of 10 mM $ZnCl_2$ by the method of Maeno and Greengard [13].

Discussion

In this paper we have presented evidence in support of the view that the potentiation of colchicine uptake in CHO cells by metabolic inhibitors [4] is mediated through their effect on cellular ATP levels. We have shown that the ATP level changes rapidly upon addition of cyanide and increases again after the addition of glucose (Fig. 1) with rates consistent with the kinetics of stimulation of colchicine uptake by cyanide and its reversal by glucose [4]. In addition, we have shown that the mutant cell line CH^RC4 maintains its drug permeability barrier at much lower ATP levels. First, lower levels of glucose are required to reverse the cyanide-induced potentiation of colchicine uptake in the mutant CH^RC4 [4] even though it metabolizes glucose to generate ATP at slower rates than the parental cell line (Figs. 1 and 2). Second, by generating cells with various ATP levels we have been able to demonstrate directly that the mutant can maintain its permeability barrier at much lower ATP levels that can the parental cell line (Fig. 3). These data show that the colchicine-resistant mutants have an alteration in this active drug permeability barrier. This more efficient permeability barrier could be the reason for the much reduced drug permeability of these mutants at normal ATP levels.

In this paper we have also presented evidence in vitro for the existence of a membrane-associated protein kinase-phosphoprotein phosphatase system which may be involved in the phosphorylation and dephosphorylation of various membrane proteins in vivo. One of the major differences in the protein phosphorylation pattern observed between the parental and mutant cell lines was the enhanced phosphorylation of two high molecular weight protein peaks (Fig. 4). The phosphoprotein phosphatase activity of these isolated membranes seems to be the same in both mutant and parental cell lines (Fig. 6). In preliminary experiments, evidence has been obtained that this phosphatase will also dephosphorylate membrane proteins but does not seem very specific for any one protein (unpublished data). It will need to be investigated further whether differences in phosphatase activity play a role in the delayed response to cyanide-induced stimulation seen in the mutant cell lines.

Since it is of importance to determine whether or not the pattern of phosphorylation observed in vitro reflects that in vivo, we have attempted to label these proteins in vivo by incorporating [^{32}P]orthophosphate into whole cells and analyzing the membranes of these cells by polyacrylamide gel electrophoresis. So far we have been unable to detect the phosphorylation of these proteins using this procedure. This could be due to a number of different reasons. For example, the label could be removed by the phosphatase during the membrane isolation or perhaps not enough label could be incorporated for the phosphorylation of these proteins to be detected.

Juliano et al. [15] have reported the presence of a surface glycoprotein on the mutant cell lines which is absent or in decreased amounts in the parental cell line. This glycoprotein (the "P" glycoprotein) also appears to be one of the major proteins phosphorylated in the mutant cell membranes (Fig. 4). Evidence for this stems from the fact that the "P" glycoprotein (labeled by [^{3}H]glucosamine) and the phosphorylated peak co-migrate on acrylamide gel electrophoresis (Fig. 4). Furthermore the correlation of the amount of the phos-

phorylated protein with the degree of resistance in the two independently selected mutants CH^RC4 and CH^RC5, and the lack of this phosphorylated protein in the CH^RC5 revertant I10-1 (Fig. 4), is similar to that reported for the "P" glycoprotein [16]. The strong correlation between the amount of this glycoprotein and the relative resistance of these cells to colchicine have led Juliano and Ling [16] to postulate the involvement of this protein in the resistant phenotype.

The evidence that this "P" glycoprotein was also phosphorylated by the membrane-associated kinase suggests a role for this glycoprotein in the maintenance of the active permeability barrier against colchicine. The mere presence of the "P" glycoprotein is not enough for maintenance of a decreased permeability since in the presence of cyanide the rate of colchicine uptake is stimulated to a point where the rate of drug uptake becomes the same for both the mutant and parental cell lines [4]. Thus we propose that this glycoprotein must be in a phosphorylated state in order to maintain a decreased drug permeability. The presence of larger amounts of the "P" glycoprotein in the mutant cells would then result in a much lower permeability to a variety of drugs. In addition, if the effect of metabolic inhibition is to block the phosphorylation of the "P" glycoprotein through a decrease in the ATP level, then the resulting decrease in phosphorylated "P" glycoprotein should yield a stimulation in drug uptake.

Numerous workers have found that metabolic depletion results in a variety of alterations in membrane structure. For example, in chicken erythrocytes metabolic depletion has been reported to result in an increase in the exposure of the membrane lipids toward phospholipase C [19,20], phospholipase A [21] and trinitrobenzene sulfonic acid [21]. Metabolic depletion has also been reported to have effects on lectin-induced agglutination in transformed cells [22] and human erythrocytes [23,24] as well as inducing shape changes in human erythrocytes [25–28]. In the CHO cell system, metabolic depletion results in marked changes in membrane permeability to a variety of drugs [4]. It seems possible that all these effects are mediated through a phosphorylation-dephosphorylation of membrane proteins.

The mechanism whereby the phosphorylated "P" glycoprotein might be able to maintain a decreased permeability for compounds such as colchicine, which enter these cells via an unmediated mechanism [3], is not known. However, these data are consistent with the model proposed by Ling [29] in which this glycoprotein is involved in the modulation of membrane lipid fluidity in a manner resulting in decreased drug permeability. It would be of considerable interest to determine the molecular mechanism whereby this glycoprotein could modulate membrane lipid fluidity.

Acknowledgements

This work was supported by grants from the Medical Research Council of Canada (MT-5708) and the National Cancer Institute of Canada and by a contract from the National Institutes of Health of the United States (1CP 43331). One of us (S.A.C.) was supported by a Medical Research Council of Canada Studentship.

References

1 Ling, V. and Thompson, L.H. (1974) J. Cell. Physiol. 83, 103—116
2 Bech-Hansen, N.T., Till, J.E. and Ling, V. (1976) J. Cell. Physiol. 88, 23—32
3 Carlsen, S.A., Till, J.E. and Ling, V. (1976) Biochim. Biophys. Acta 455, 900—912
4 See, Y.P., Carlsen, S.A., Till, J.E. and Ling, V. (1974) Biochim. Biophys. Acta 373, 242—252
5 McBurney, M.W. and Whitmore, G.F. (1974) Cell. 2, 173—182
6 Thompson, L.H. and Baker, R.M. (1974) In Methods in Cell Biology (Prescott, D.M., ed.). Vol. VI pp. 209—281, Academic Press, New York
7 Stanners, C.P., Elicieri, G. and Green, H. (1971) Nat. New. Biol. 230, 52—54
8 Stanley, P.E. and Williams, S.G. (1969) Anal. Biochem. 29, 381—392
9 Johnstone, R.M. (1974) Biochim. Biophys. Acta 356, 319—330
10 Brunette, D.M. and Till, J.E. (1971) J. Membrane Biol. 5, 215—224
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
12 Bray, G.A. (1960) Anal. Biochem. 1, 279—285
13 Maeno, H. and Greengard, P. (1972) J. Biol. Chem. 247, 3269—3277
14 Laemmli, U.K. (1970) Nature 227, 680—685
15 Juliano, R., Ling, V. and Graves, J. (1976) J. Supramol. Struct. 4, 521—526
16 Juliano, R.L. and Ling, V. (1976) Biochim. Biophys. Acta 455, 152—162
17 Azhar, S. and Menon, K.M.J. (1975) Biochem. J. 151, 23—36
18 Roses, A.D. and Appel, S.H. (1973) J. Biol. Chem. 248, 1408—1411
19 Frish, A., Gazitt, Y. and Loyter, A. (1973) Biochim. Biophys. Acta 291, 690—700
20 Gazitt, Y., Ohad, I. and Loyter, A. (1975) Biochim. Biophys. Acta 382, 65—72
21 Gazitt, T., Ohad, I. and Loyter, A. (1976) Biochim. Biophys. Acta 436, 1—14
22 Vlodavsky, I., Inbar, M. and Sachs, L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1780—1784
23 Singer, J.A. and Morrison, M. (1975) Biochim. Biophys. Acta 406, 553—563
24 Singer, J.A. and Morrison, M. (1976) Biochim. Biophys. Acta 426, 123—131
25 Weed, R.I., LaCelle, P.L. and Merrill, E.W. (1969) J. Clin. Invest. 48, 795—809
26 Shohet, S.B. and Haley, J.E. (1972) Nouv. Rev. Fr. D'Hémat. 12, 761—770
27 Lichtman, M.A. and Weed, R.I. (1972) Nouv. Rev. Fr. D'Hémat. 12, 799—814
28 Palek, J., Stewart, G. and Lionetti, F.J. (1974) Blood 44, 583—597
29 Ling, V. (1975) Can. J. Genet. Cytol. 17, 503—515
30 Hokin, L.E. Sastry, P.S., Galsworthy, P.R. and Yoda, A. (1965) Proc. Natl. Acad. Sci. U.S. 54, 177-184