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Control of membrane permeability by external ATP in mammalian cells: isolation of an ATP-resistant variant from Chinese hamster ovary cells

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External ATP causes a great increase in the passive permeability of the plasma membrane for phosphorylated metabolites and other small molecules in cultured mammalian cells. We previously demonstrated that in CHO-K1 cells an ATP-dependent permeability change was induced in the presence of a mitochondrial inhibitor (KCN or rotenone), a cytoskeleton-attacking agent (vinblastine) and a calmodulin antagonist (trifluoperazine). These permeability changes were reversible but long exposure, for 30–60 min, to ATP together with a mitochondrial inhibitor significantly reduced the cell viability of the treated cells. Since this cell lysis was shown to be due to the ATP-dependent permeability change, we could isolate several clones resistant to the action of the external ATP from CHO-K1 cells after repeated treatment with ATP and rotenone. In 9.1 cells, one of the isolated clones, little or no ATP-dependent permeability change was observed in the presence of either a mitochondrial inhibitor, vinblastine or trifluoperazine. This CHO variant could be specifically resistant as to the change in membrane permeability induced by external ATP, since the permeabilities for the 2-deoxyglucose and drugs used in the present studies were similar to those in the case of the parent cells. These results suggest that a specific defect or alteration in the plasma membrane is involved in the ATP-dependent permeability change. It is also reported that Mg^{2+}-dependent ATPase activity was found on the cell surface of both CHO-K1 and 9.1 cells, and this activity was shown to be not involved in the permeability change controlled by external ATP.

Introduction

The selective permeability barrier of the plasma membrane is very important in maintaining the cellular homeostasis of living cells. It was recently demonstrated that the addition of ATP to several mammalian cells in culture, such as 3T6, B16 melanoma and HeLa cells, caused a great increase in passive permeability, allowing passage through the plasma membrane of phosphorylated metabolites and ions [1–7]. In CHO-K1 cells, an external ATP-dependent permeability change was induced when the cells were treated with a mitochondrial inhibitor (KCN or rotenone) [8], a cytoskeleton-attacking agent (vinblastine) [9], and a calmodulin antagonist (trifluoperazine) [10]. The mitochondrial inhibitor reduced the cellular ATP concentration to achieve the effect of external ATP, whereas vinblastine or trifluoperazine induced a permeability change without a drastic change in the cellular ATP concentration. Although the results of these studies suggested the important roles of the cellular ATP, cytoskeleton and calmodulin in the control of the permeability change, the mechanism by which external ATP regulates membrane permeability is largely unknown. To obtain further insight into the biochemistry, regulation and physiological role of the ATP-depen-

dent permeability change, studies on animal cell mutants defective in the ATP response would be very useful. We report here the initial isolation of an ATP-resistant variant from CHO-K1 cells and partial characterization of the variant cells.

Materials and Methods

Chemicals

2-Deoxy[1-^{3}H]glucose (16.2 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol) were obtained from Amersham International, U.K. Vinblastine sulfate was purchased from Shionogi Co. Ltd., Tokyo, Japan, and trifluoperazine was kindly supplied by Yoshitomi Pharm. Industries, Osaka, Japan. Nucleotides and other chemicals were obtained from Sigma, St. Louis, MO, U.S.A.

Cell culture

Chinese hamster ovary cells, clone K1 (CHO-K1), were obtained from Flow Laboratories (Rockville, MD, U.S.A.), and cultured as described previously [8–10] in Ham's F12 medium containing 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were seeded into 35-mm plastic dishes (Corning) at densities of $(1–3) \cdot 10^5$ cells/dish, cultured at 37°C for 2–3 days and then used for the present experiments.

Measurement of the passive permeability change

The passive permeability change was measured as described previously [7–10] by monitoring either the efflux of acid-soluble radioactive materials from monolayer cells or the hydrolysis of *p*-nitrophenyl phosphate. CHO cells were labeled for 3 h at 37°C with deoxy[^{3}H]glucose (0.25 μCi/ml, 1 μM) in glucose-free F12 medium containing 10% dialyzed calf serum. The labeled cells were washed twice with 0.15 M NaCl and then incubated at 37°C for 10–15 min with 1 ml of buffer A containing the indicated additions. Buffer A comprised 0.1 M Tris-HCl (pH 8.2 or 7.2)/0.05 M NaCl/0.05 mM $CaCl_2$. After the incubation, the radioactivity released into the medium was measured with a liquid scintillation counter. For studying the hydrolysis of *p*-nitrophenyl phosphate, cells treated in buffer A (pH 8.2) as indicated were further incubated at 37°C for 10 min with the same medium in the presence of 5 mM *p*-nitrophenyl phosphate. The supernatant was then removed and mixed with 0.1 ml of 1 M NaOH to determine the *p*-nitrophenol formed at 410 nm.

Regrowth of ATP-treated cells and isolation of ATP-resistant clones

$(1–1.5) \cdot 10^6$ CHO-K1 cells in 35-mm dishes were incubated at 37°C in buffer A (pH 8.2) containing 0.1–0.5 mM ATP and a mitochondrial inhibitor (KCN or rotenone) for the indicated period. After the incubation, the medium was removed carefully and replaced with 2 ml of F12 medium containing 10% serum. The cultures were incubated at 37°C for 2 h in a CO_2-atmosphere and then the medium was changed again for F12 medium containing serum to remove residual drugs in the cultures. The cells were further cultured at 37°C for 24 h and then the number of viable cells in each dish was determined after trypsinization and trypan blue-staining of the cells. For isolation of ATP-resistant cells, the few viable cells that remained on a dish after treatment with 0.5 mM ATP and 3 μM rotenone in buffer A (pH 8.2) for 60 min as described above were further cultured to a confluent state, and then this treatment was repeated. Then the viable cells were replated on 100 mm dishes at the density of 300 cells/dish and cultured for 1 week to obtain colonies. Relatively large colonies were randomly isolated and the ATP-dependent permeability change in each colony was determined. In about 70% of the isolated clones the ATP-sensitivity was decreased, the others showing normal sensitivity to ATP. One of the clones showing a reduced response to ATP (No. 9) was recloned further and an ATP-resistant clone, 9.1, was isolated.

Measurement of cellular ATP

Intracellular ATP was extracted from monolayer cells with ice-cold 0.4 M perchloric acid after the indicated treatment of the cells, and the ATP concentration was determined enzymatically with luciferin-luciferase, using a Packard Tri-Carb liquid scintillation spectrometer [11].

Growth inhibition by vinblastine and trifluoperazine

CHO-K1 or 9.1 cells were plated at the density

of $1.5 \cdot 10^5$ cells/dish and then incubated for 24 h. Various concentrations of the indicated drugs were then added to duplicate cultures and the cultures were further incubated for 2 days. The cells were then trypsinized, and the viable cells were counted after staining with trypan blue.

Measurement of 2-deoxy[^{3}H]glucose uptake

Cells cultured in 35-mm dishes were washed with phosphate-buffered saline and then incubated with 2-deoxy[^{3}H]glucose (0.25 μCi/ml, 10 μM) in 1 ml of either glucose-free F12 medium or buffer A (pH 8.2) at 37°C for 15 min. The uptake was linear for up to 30 min. After the incubation, the cells were washed twice with ice-cold phosphate-buffered saline and then the incorporated radioactivity was extracted with 1 ml of 5% cold trichloroacetic acid and counted as described [8]. Proteins were determined by the method of Lowry et al. [12] with bovine serum albumin as a standard.

Ecto-ATPase activity

Ecto-ATPase activity was determined as described [13] with a minor modification; intact cells were incubated in monolayer cultures with 0.5–1.0 mM [γ-^{32}P]ATP (2.5 μCi/ml) in buffer A (pH 8.2 or 7.2) for 5–15 min at 37°C, followed by determination of liberated $^{32}P_i$ after extraction with a two-phase separation system, isobutanol/benzene (1 : 1, v/v), with 1.25% ammonium molybdate and 0.15 M H_2SO_4. Radioactivity in the upper layer was counted with ACS II (Amersham). A control experiment with a dish without cells was always run in pallalel for correction for the nonenzymatic breakdown of added [γ-^{32}P]ATP.

Results

Isolation of external ATP-resistant cells

It was previously reported that the ATP-induced permeability change in CHO-K1 cells is reversible and that the permeabilized cells can grow when the membranes are sealed through incubation with F12 medium containing serum [9,10]. This reversibility of the permeabilization in CHO-K1 cells was further investigated as to whether or not longer incubation of the cells under the conditions for the permeability change affects the viability of the cells. CHO-K1 cells

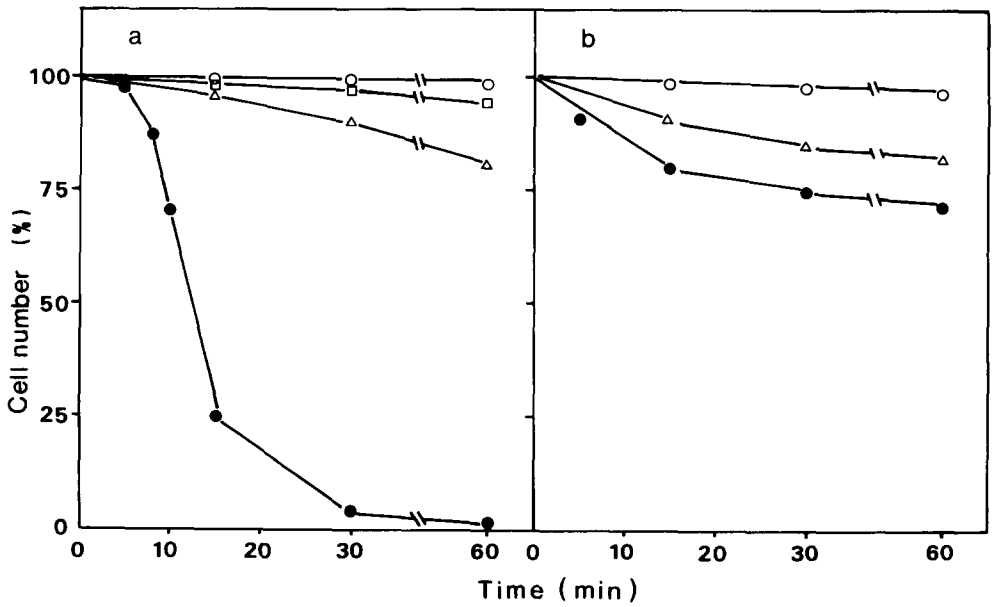

Fig. 1. Effect of exogenous ATP on the viability of cells. (a) Monolayer cultures of CHO cells were treated as indicated in buffer A (pH 8.2) at 37°C for various incubation times. Then the medium was replaced with fresh F12 medium containing 10% serum and the cells were incubated at 37°C for 2 h. After the incubation, the medium was replaced again and the cultures were further incubated at 37°C for 24 h. Then, the number of viable cells in each dish was determined. (b) Similar experiments were performed with an isolated ATP-resistant clone, 9.1. Buffer A alone (○); 0.5 mM ATP (△); 0.5 mM ATP+1 mM KCN (●); 1 mM KCN (□).

TABLE I

GROWTH INHIBITION AND PASSIVE PERMEABILITY CHANGE INDUCED BY EXOGENOUS ATP IN CHO-K1 CELLS

CHO-K1 cells were treated as indicated at 37°C for 30 min. The cells were then incubated with F12 medium containing 10% serum at 37°C for 2 h, and then the medium was replaced by fresh medium. The cultures were further incubated at 37°C for 24 h and then the number of viable cells in each dish was determined. The cell number (%) was calculated on the basis of the number of untreated cells ($1.75 \cdot 10^6$) per dish. In a separate experiment, the passive permeability change in CHO cells which had been labeled with deoxy[^{3}H]glucose was determined after incubation of the cells as indicated at 37°C for 10 min. The total radioactivity within the deoxy[^{3}H]glucose-labeled cells was 28500 cpm/dish.

Treatment	Cell number (%)	Efflux (%)
Buffer A (pH 8.2)	90.3	15.3
+0.5 mM ATP	88.0	18.8
+3 μM rotenone	2.2	87.5
+1 mM KCN	3.6	93.5
+20 mM glucose	89.1	22.8
+2 mM Mg^{2+}	78.3	16.8
Buffer A (pH 7.2)	98.8	18.3
+0.5 mM ATP+1 mM KCN	88.0	32.1

were treated with ATP and KCN in buffer A (pH 8.2) at 37°C for the indicated period and then the number of viable cells was determined after the treated cells had been further cultured with F12 medium containing 10% serum for 24 h (Fig. 1). As reported, the viability of the cells treated with ATP and KCN for up to 10 min, at which time the permeability change for phosphorylated metabolites is complete [8,9], was largely unchanged. However, longer exposure to the agents greatly reduced the number of viable cells, the number being less than 5% of that in the case of untreated cells when the treatment was continued for more than 30 min (Fig. 1a). This decrease in cell viability on ATP-treatment was shown to be mainly due to a permeability change caused by ATP, since both phenomena were well correlated with each other (Fig. 1a and Table I): firstly, these changes only occurred in the presence of ATP and KCN, and ATP or the drug alone did not induce them; secondly, similar results were obtained with another mitochondrial inhibitor, rotenone, instead of KCN; thirdly, addition of excess glucose or 2 mM Mg^{2+} to the reaction mixture, which inhibit the permeability change, also decreased the ATP-induced cell toxicity; and lastly, these reactions were pH-dependent, and ATP-induced cell toxicity and a permeability change were not observed at pH 7.2.

Since it was noticed during the course of these experiments that a few cells remained viable after the treatment with ATP and a mitochondrial inhibitor, these viable cells were further cultured and the same treatment was repeated, as described under Materials and Methods. Then several clones were randomly isolated from the viable cells. Most of the cloned cells were found to show decreased sensitivity to the ATP-induced cell toxicity, and the results for one of the isolated clones, 9.1, are shown in Fig. 1b.

Passive permeability change by external ATP in CHO-K1 and 9.1 cells

The external ATP-dependent passive permeability change was determined in both CHO-K1 and 9.1 cells. A great increase in the efflux of radioactive materials from CHO-K1 cells labeled with deoxy[^{3}H]glucose was observed when the cells were incubated with ATP in the presence of either KCN or vinblastine (Fig. 2a), as reported previously [8,9]. In 9.1 cells, however, the ATP-depen-

TABLE II

HYDROLYSIS OF *p*-NITROPHENYL PHOSPHATE BY CHO-K1 AND 9.1 CELLS TREATED WITH ATP

Monolayer cultures of CHO cells or 9.1 cells were incubated in 1 ml of buffer A (pH 8.2) containing the indicated additions at 37°C for 8 min. Then, 5 mM *p*-nitrophenyl phosphate was added to the medium directly and the incubation was continued at 37°C for another 10 min. The supernatant was removed for measurement of the *p*-nitrophenol produced.

Treatment	Hydrolysis of *p*-nitrophenyl phosphate (nmol/mg protein)	
	CHO-K1	9.1
Buffer A (pH 8.2)	17.6	15.2
+0.5 mM ATP	15.2	18.1
+1 mM KCN	116.3	27.2
+0.2 mM vinblastine	113.6	24.3
+1 mM KCN	15.1	15.2
+0.2 mM vinblastine	12.2	12.6
+0.1% Tween 80	101.1	109.1

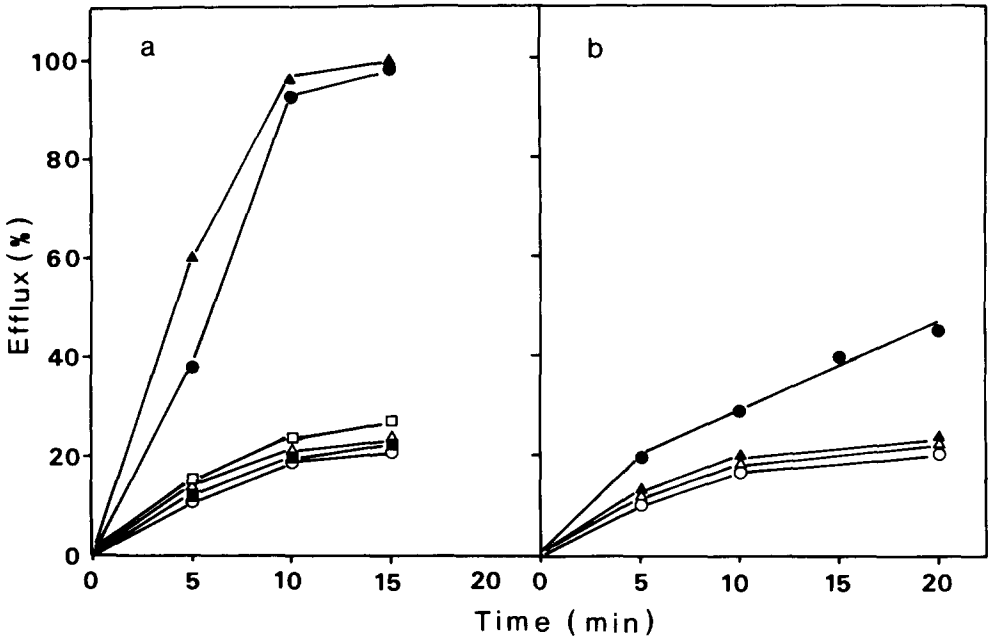

Fig. 2. Efflux of deoxy[^{3}H]glucose-labeled materials from CHO-K1 cells and 9.1 cells treated with exogenous ATP. CHO-K1 cells (a) or 9.1 cells (b) which had been labeled with deoxy[^{3}H]glucose (0.25 μCi/ml, 1 μM) were incubated at 37°C in buffer A (pH 8.2) containing the following additions: none (○); 0.5 mM ATP (△); 0.5 mM ATP+1 mM KCN (●); 1 mM KCN (□); 0.5 mM ATP+0.2 mM vinblastine (▲); 0.2 mM vinblastine (■). These additions were made at the start of the incubation. After the indicated incubation time, the radioactivity released into the medium was determined. The total radioactivities within the CHO cells or 9.1 cells were 52,700 cpm/dish and 44,400 cpm/dish, respectively.

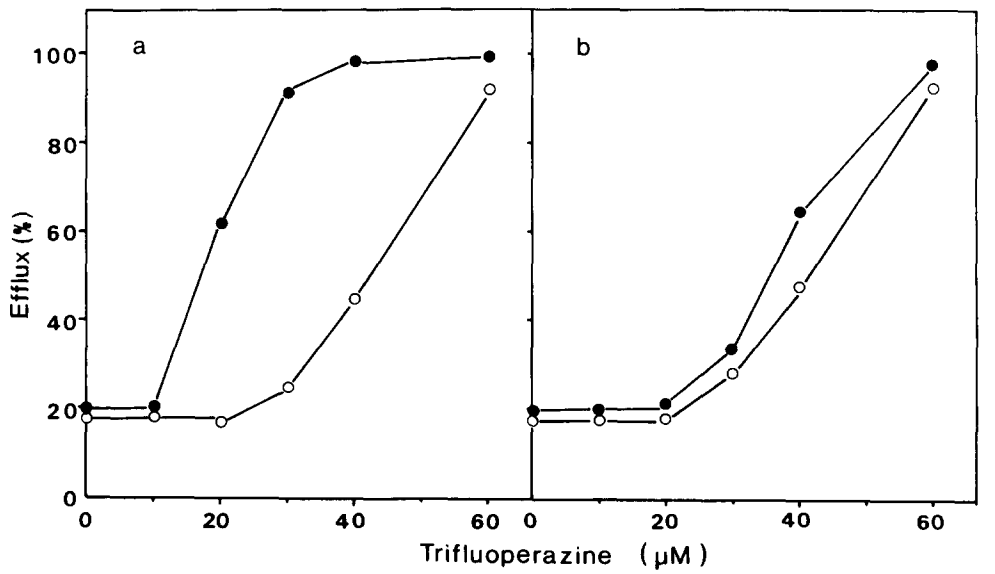

Fig. 3. Effect of trifluoperazine on the ATP-dependent permeability change. CHO cells (a) or 9.1 cells (b) were labeled with deoxy[^{3}H]glucose as described in Fig. 2 and then the cells were incubated in buffer A (pH 8.2) containing various concentrations of trifluoperazine (TFP) with (●) or without (○) 0.5 mM ATP. After incubation at 37°C for 10 min, the radioactivity released into the medium was measured and efflux (%) was calculated on the basis of the total radioactivity within the cells (CHO, 51900 cpm/dish; 9.1, 48700 cpm/dish).

TABLE III

EFFECTS OF MITOCHONDRIAL INHIBITORS ON THE CELLULAR ATP CONCENTRATION

CHO-K1 cells or 9.1 cells were treated as indicated at 37°C for 10 min, and the cellular ATP was extracted and determined.

Treatment	Cellular ATP (nmol/10^6 cells), (%)	
	CHO-K1	9.1
Untreated	4.00	4.30
Buffer A (pH 8.2)	3.20 (100)	3.60 (100)
+1 mM KCN	0.14 (5)	0.31 (8)
+3 μM rotenone	0.21 (7)	0.29 (8)
+10 mM glucose	1.72 (54)	1.60 (44)

dent permeability change was suppressed and little change in the efflux was observed with either KCN or vinblastine (Fig. 2b). This decrease in the sensitivity to external ATP of 9.1 cells was further confirmed when the permeability change with KCN or vinblastine was determined as to the entry and hydrolysis of added *p*-nitrophenyl phosphate (Table II). Indeed, when both types of cells were permeabilized with a non-ionic detergent, Tween 80, in a control experiment, no difference in the hydrolysis of *p*-nitrophenyl phosphate by these cells was found.

More recently, we demonstrated that ATP and a calmodulin antagonist, trifluoperazine, caused a synergistic increase in the membrane permeability of CHO-K1 cells, and a higher concentration of trifluoperazine alone caused an increase in the efflux of radioactive cellular metabolites [10]. These results were reproducible, as shown in Fig. 3a. In an ATP-resistant clone, 9.1, the synergistic effect of ATP and trifluoperazine on the induction of a permeability change was not seen but a permeability change due to a higher concentration of the drug alone was observed (Fig. 3b). These results indicate that the isolated 9.1 cells are defective in the response to external ATP.

Sensitivity of CHO-K1 and 9.1 cells to mitochondrial inhibitors, vinblastine and trifluoperazine

To determine whether 9.1 cells are specifically defective in the response to external ATP, the sensitivities to mitochondrial inhibitors, vinblastine and trifluoperazine, whose presence is required for an ATP response in CHO-K1 cells [8–10], of CHO-K1 and 9.1 cells were compared. Mitochondrial inhibitors, KCN and rotenone, similarly reduced the cellular ATP concentrations of these cells, and the reduced ATP concentrations were restored by 10 mM glucose, which indicated the similar sensitivities to these inhibitors of these cells (Table III). The sensitivities to vinblastine and trifluoperazine of these cells were also shown to be similar, as judged from the effects of these drugs on cell growth (Table IV). It was also noted that the permeabilities of 9.1 cells for these drugs seemed to be unchanged, since drug-resistance in animal cells is often due to reduced permeability for the drugs [15].

Uptake of 2-deoxyglucose by CHO-K1 and 9.1 cells

Since the isolated CHO clone, 9.1, was shown to be defective in the ATP response, it was of interest to determine if this variant is altered as to the membrane permeability for natural nutrients needed for cell growth. In CHO-K1 cells, the rates of the uptake of 2-deoxyglucose as an analogue of glucose in glucose-free F12 medium and in buffer A (pH 8.2) were $5.4 \cdot 10^{-11}$ and $4.3 \cdot 10^{-11}$ mol/mg protein per min, respectively. Similar values for the uptake were observed in 9.1 cells, $5.5 \cdot 10^{-11}$ and $4.8 \cdot 10^{-11}$ mol/mg protein per min in glucose-free F12 medium and in buffer A (pH 8.2),

TABLE IV

EFFECTS OF VINBLASTINE AND TRIFLUOPERAZINE (TFP) ON GROWTH OF CHO-K1 AND 9.1 CELLS

CHO-K1 and 9.1 cells were plated into 35 mm dishes at $1.5 \cdot 10^5$ cells per dish in F12 medium with 10% serum. After 24 h incubation, the drugs at the indicated concentrations were added to the cultures and the incubation was continued for a further 2 days. The number of viable cells in each dish was determined and the results are expressed as cell number (%) on the basis of that in the case of untreated cultures.

Drugs		Cell number (%)	
		CHO-K1	9.1
None		100	100
Vinblastine	0.01 μM	86.0	90.0
	0.05 μM	13.9	21.1
	0.1 μM	2.5	3.0
TFP	10 μM	56.2	74.5
	25 μM	4.4	2.8
	50 μM	1.0	1.5

respectively. These results taken together suggest that the membrane permeabilities in 9.1 cells for nutrients and several drugs are not altered.

Ecto-ATPase activity of CHO-K1 and 9.1 cells

The results presented here strongly suggest that the ATP-resistant property of 9.1 cells is a specific defect or alteration at the cell surface which interacts with external ATP. Possible target molecules are ATP-requiring ectoenzymes such as ATPases or protein kinases, whose existence at the cell surface has been reported in various types of mammalian cells [13,16–20]. However, the results of recent studies on ATP-induced permeabilization are against the involvement of an ectoprotein kinase-dependent membrane phosphorylation [10,21]. Therefore, we investigated as to whether or not ecto-ATPase activity participates in the permeability change in CHO-K1 cells and also whether or not change in the ATPase activity acquires the ATP-resistant phenotype. When intact CHO-K1 cells were incubated with [γ-^{32}P]ATP in buffer A (pH 8.2), ATP-hydrolyzing activity was observed only in the presence of Mg^{2+} (Table V). This activity was insensitive to ouabine, and Mg^{2+} could not be replaced by Ca^{2+}. These results indicate the presence of Mg^{2+}-dependent ATPase activity on the cell surface of the CHO-K1 cells. However, this ATPase activity cannot be involved in the control of the ATP-dependent permeability change, since it was observed without Mg^{2+} and was suppressed by more than 1 mM Mg^{2+} (Refs. 9, 22 and Table I). An ecto-ATPase activity showing similar characteristics was also found in 9.1 cells, and the specific activity of the ATPase was found to remain unchanged as that in the case of CHO-K1 cells was. These results together suggest that the ATPase activity found on the cell surface of CHO-K1 cells is not directly involved in the permeability change induced by external ATP, nor in an ATP-resistant phenotype of 9.1 cells.

TABLE V

ECTO-ATPase ACTIVITY OF CHO-K1 AND 9.1 CELLS

Monolayer cultures of cells were incubated with [^{32}P]ATP (2.5 μCi/ml, 1 mM) and the indicated additions in 1 ml of buffer A (pH 8.2 or 7.2) at 37°C for 15 min. After incubation, the liberated $^{32}P_i$ was extracted and measured as described under Materials and Methods.

Additions	Hydrolysis of ATP (nmol/10^6 cells)	
	CHO-K1	9.1
Buffer A (pH 8.2)	0.12	0.10
+5 mM $MgCl_2$	17.3	18.9
+5 mM $CaCl_2$	0.10	0.10
+5 mM $MgCl_2$ +1 mM ouabain	14.7	16.6
Buffer A (pH 7.2)	0.10	0.10
+5 mM $MgCl_2$	16.5	15.0

Discussion

In the present study, we observed that the permeabilization of CHO-K1 cells on treatment with external ATP and a mitochondrial inhibitor was reversible, as reported previously [10], but long exposure to these agents reduced the reversibility, leading to a remarkable decrease in the number of viable cells. This ATP-induced cell toxicity was shown to be due to the permeability change induced by external ATP. Then, by repeating this procedure, an ATP-resistant variant, 9.1, as to cell toxicity was spontaneously isolated from CHO-K1 cells. In this variant, no or little permeability change by external ATP was observed in the presence of either a mitochondrial inhibitor or vinblastine, whose modes of action on cellular function are different [9]. Furthermore, the ATP-dependent permeability change was also not seen in 9.1 cells when trifluoperazine was used as another drug for inducing the permeability change [10], although a permeability change with trifluoperazine alone at a higher concentration was observed similarly in both parent and the variant cells. In addition, membrane permeabilities for 2-deoxyglucose and the drugs used for the permeability changes in 9.1 cells were not altered at all. These results strongly suggest that the isolated clone, 9.1, is a specific membrane variant defective in a membrane permeability change induced by external ATP. Although genetic analysis of this cell has not been undertaken yet, the phenotype was very stable during serial passages for more than 2 months. Furthermore, subclones of 9.1 cells were to all be defective in the response to external ATP. These facts indicate the stable phenotype of this new membrane variant and exclude other

possibilities such as desensitization by the ATP-treatment.

External ATP-dependent permeability changes observed in several transformed cells including CHO-K1 cells have similar characteristics: temperature and pH dependencies, high specificity for added ATP, reversibility and no requirement for divalent ions like Ca^{2+} or Mg^{2+}. From this evidence we and other authors have assumed the involvement of an ATP-requiring enzymatic reaction on the cell surface [2,4,9,22]. However, the results of the present and previous studies on the ATP effect have shown that at least two kinds of well characterized enzyme activities on the cell surface, those of Mg^{2+}-ATPase and protein kinase [13,16–20], are not involved in the ATP-controlled membrane permeability, although the participation of other ectoenzymes remains to be investigated. Weisman et al. [23] recently raised the possibility that external ATP activates channel formation which allows the free movement of Na^+ and K^+, followed by an increase in the permeability for nucleotides which pass through the same or different channels. The reversibility of the ATP-induced permeabilization determined in the present study may depend on the nature of the created channels. Although we know little about the ATP-activated aqueous channels in mammalian cells, this ATP-resistant variant, 9.1, will be very useful for studying the nature and function of such ATP-interacting components in the plasma membrane associated with the permeability change. This as well as biochemical characterization of 9.1 warrant further experimental work in our laboratory.

External ATP was also reported to modulate various cell surface-dependent properties such as cell volume [24], ionic fluxes [25–27], morphology [28,29], and histamine release [29,30], as well as cell aggregation [31] and virus infection [32] in various types of cells. In spite of all these studies concerning the influence of external ATP on the cell surface of mammalian cells, the mechanisms and the physiological roles of the ATP are largely unknown. In the present study, several membrane-associated biological properties, such as cell growth, morphology, adhesiveness and the response to cyclic AMP of 9.1 cells were determined (data not shown). However, none of these biological properties were altered, suggesting that these properties are independent of the ATP-induced membrane permeability change.

It is also of interest to determine whether the ATP-dependent permeability change is critical with respect to malignant transformation, since this permeability change has been found in several transformed cells in culture [1–8]. By means of the ATP treatment, some drugs could be selectively incorporated into the transformed cells [6]. We are also in the process of isolating other mutants defective in the ATP-response from ATP-sensitive transformed cells by a similar procedure to that described here.

Further biochemical and genetic studies on these mutants will provide useful information on the regulation of passive permeability in mammalian cells.

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