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Reciprocal effects of phenothiazines and naphthalene sulfonamides on the external ATP-dependent permeability change in Chinese hamster ovary cells

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External ATP causes a great increase in passive permeability to phosphorylated metabolites in several transformed cells, but not in untransformed cells. We have previously demonstrated that the external ATP-dependent permeability change was induced in Chinese hamster ovary cells, CHO-K1, only in the presence of a mitochondrial inhibitor (rotenone, KCN) or a cytoskeleton-attacking agent, vinblastine (Kitagawa, T. and Akamatsu, Y. Biochim. Biophys. Acta 649, 76–82 (1981); 734, 25–32 (1983)). A similar ATP-dependent permeability change was also induced in CHO cells when the cells were treated with 10–30 μM trifluoperazine. This permeability change, like the previously mentioned ones, was found to be reversible and the treated cells remained viable. The permeability change induced by ATP and trifluoperazine was independent of changes in cellular ATP concentration and this property was the same as that of the permeability change with external ATP and vinblastine. Since trifluoperazine is known to interact with calmodulin and to inhibit calmodulin-dependent cellular functions, these results may indicate that calmodulin associated with the cytoskeleton plays an important role in control of the permeability change, although nonspecific perturbation by the drug of the membranes cannot be ruled out. Chlorpromazine and a naphthalene sulfonamide, W-7, also induced an ATP-dependent permeability change. However, these drugs, like mitochondrial inhibitors, reduced the cellular ATP concentration to induce the permeability change. Thus, a clear difference in the action of these drugs in intact cells was also shown in this study. Possible mechanisms for the ATP-dependent permeability change in mammalian cells are discussed.

Introduction

The selective permeability barrier of the plasma membrane is very important in maintaining cellular homeostasis in living cells. It was recently demonstrated that brief exposure of several transformed cells, such as 3T6, HeLa and B16 melanoma cells, to ATP caused a great increase in passive permeability, allowing passage through the plasma membrane of phosphorylated metabolites and ions [1–7]. Untransformed mouse fibroblasts, however, did not respond to ATP. In CHO-K1 cells, an external ATP-dependent permeability change was induced when the cells were treated with a mitochondrial inhibitor (rotenone, KCN) [8], or a cytoskeleton-attacking agent (vinblastine, cytochalasin B) [9]. The mitochondrial inhibitor reduced the cellular ATP concentration to achieve the effect of external ATP, whereas the cytoskeletal drugs induced the permeability change without a drastic change in the cellular ATP concentration. These results may indicate that reduc-

Abbreviations: W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide; W-5, *N*-(6-aminohexyl)-1-naphthalenesulfonamide.

tion of the cellular ATP level could cause some specific alterations in the cytoskeleton which are associated with the external ATP-dependent permeability change.

The structure and functions of the cytoskeleton were reported to be regulated by a ubiquitous Ca^{2+}-binding protein, calmodulin, which mediates many of the Ca^{2+}-dependent functions in eukaryotic cells [10–13]. In addition, vinca alkaloids which affect microtubules could inhibit calmodulin-dependent enzyme activities such as those of Ca^{2+}-ATPase [14] and phosphodiesterase [15]. These facts prompted us to determine whether calmodulin is involved in control of the ATP-dependent permeability change. In the present study, the effects of several phenothiazines and naphthalene sulfonamides, such as trifluoperazine and W-7, which were reported to inhibit various calmodulin-dependent processes [16–24], on passive permeability of CHO cells are examined. We report here that these drugs induce an external ATP-dependent passive permeability change in CHO cells. The possible effects of these drugs on the calmodulin-cytoskeleton complex and the membranes are discussed.

Materials and Methods

Chemicals. 2-Deoxy[1-^{3}H]glucose (15 Ci/mmol), 2-deoxy[1-^{14}C]glucose (59 mCi/mmol), 2-deoxy[1-^{14}C]glucose 6-phosphate (59 mCi/mmol) and [*N-methyl*-^{3}H]trifluoperazine (72 Ci/mmol) were obtained from Amersham International, U.K. Vinblastine sulfate, trifluoperazine-maleic acid and verapamil were kindly supplied by Shionogi Co., Ltd. (Tokyo), Yoshitomi Pharm. Industries (Osaka) and Eisai Co., Ltd. (Tokyo), respectively. W-7 and W-5 were purchased from Riken Co., Ltd., Tokyo. Chlorpromazine, chloroquine and all nucleotides used were obtained from Sigma, St. Louis, MO, U.S.A.

Cell culture. Chinese hamster ovary cells, clone K1 (CHO-K1), which were originally obtained from Flow Laboratories (Rockville, MD, U.S.A.), were cultured as described [8,9] in Ham's F12 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells, inoculated into 35-mm culture dishes at densities of $(1.5–2.5) \cdot 10^5$ cells per dish, were cultured at 37°C for 2–3 days and then used for the present experiments.

Measurement of passive permeability change. The passive permeability change was measured as described previously [7–9] by monitoring either efflux of acid-soluble radioactive materials from the monolayer cells or 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 buffer A containing the indicated additions. Buffer A comprised 0.1 M Tris-HCl (adjusted to 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, the 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 measure the *p*-nitrophenol formed at 410 nm.

Separation and characterization of radioactive sugars and sugar phosphates. To determine the proportions of radioactive sugars and sugar phosphates within the cells and in the medium for permeabilization, CHO cells were labeled with deoxy[^{3}H]glucose as above or deoxy[^{14}C]glucose (1 μCi/ml, 8.5 μM) for 3 h at 37°C in glucose-free medium, and aliquots extracted from the cells with hot water or the medium surrounding the ATP-treated cells were then applied to columns of Dowex-1 (formate form: Muromachi Kagaku Kogyo, Tokyo). Most of the free sugars containing deoxyglucose were eluted with 5 ml distilled water and the sugar phosphates were eluted further with 5 ml of a solution of 0.5 M ammonium formate/0.2 M formic acid as described [25]. Recovery of the applied radioactivity from each column was more than 90%.

Thin-layer chromatography was used to characterize further the radioactive materials using deoxy[^{14}C]glucose-labeled cells. Aliquots of the whole extracts, eluates with water and those with ammonium formate/formic acid as well as the ^{14}C-labeled standards were applied on cellulose glass

plates (E. Merck, Darmstadt, F.R.G.) and chromatograms were obtained with a solvent system of *n*-butanol/acetic acid/water (2:1:1, v/v; Ref. 25). After drying the plates, autoradiograms were made with Fuji RX film (Fuji Photo Film Co., Japan) for 5–7 days and the radioactive spots were measured.

Uptake of [^{3}H]trifluoperazine by CHO-K1 cells. CHO cells were washed twice with 0.15 M NaCl and then incubated at 37°C for the indicated periods with various concentrations of labeled trifluoperazine (0.075 μCi/ml, 10 μM) in buffer A at either pH 8.2 or 7.2. The cells were then washed three times with cold 0.15 M NaCl and lysed in 1 ml 0.5% SDS. The radioactivity of the lysates was measured with a liquid scintillation counter.

Measurement of intracellular ATP. Intracellular ATP was extracted from the 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 [26]. protein was determined by the method of Lowry et al. [27] with crystalline bovine serum albumin as a standard.

Results

Induction of a passive permeability change by external ATP and trifluoperazine

When CHO-K1 cells labeled with deoxy-[^{3}H]glucose were treated with 0.5 mM ATP and 20 μM trifluoperazine in buffer A (pH 8.2), a great increase in efflux of radioactive materials was observed (Fig. 1). This permeability change was rapid and completed within 15 min incubation. ATP or the drug alone did not induce a permeability change. More than 80% of the radioactivity within the labeled cells and of that released from the permeabilized cells was retained by Dowex-1 columns (formate form), and 0.2 M formic acid/0.5 M ammonium formate was required for elution, indicating that these radioactive materials are mainly composed of charged molecules (data not shown). Further characterization by cellulose thin-layer chromatography showed that the main radioactive materials were deoxy[^{3}H]glucose 6-phosphate (data not shown), as described previously [3].

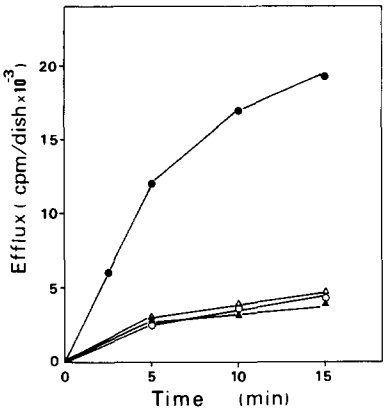

Fig. 1. Induction of a permeability change in CHO-K1 cells by treatment with ATP and trifluoperazine. CHO-K1 cells labeled with deoxy[^{3}H]glucose were incubated at 37°C in 1 ml buffer A (pH 8.2) containing the following additions: none, ○; 0.5 mM ATP, △; 20 μM trifluoperazine, ▲; 0.5 mM ATP + 20 μM trifluoperazine, ●. The indicated additions were made at the start of the incubation. After the indicated incubation period, radioactivity released into the medium was counted. The total radioactivity within the cells which could be extracted with 5% cold trichloroacetic acid was 22000 cpm/dish.

In the presence of ATP, 10–30 μM trifluoperazine was effective for induction of the permeability change (Fig. 2). These concentrations were in the range for the reported effects of trifluoperazine as a calmodulin antagonist [16–19]. However, higher

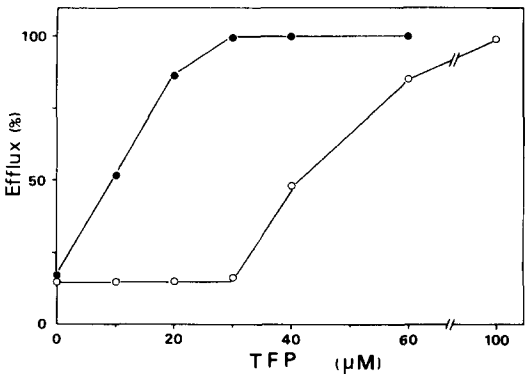

Fig. 2. Effect of trifluoperazine (TFP) concentration on the permeability change. CHO cells labeled with deoxy[^{3}H]glucose were incubated at 37°C for 10 min in buffer A (pH 8.2) with various concentrations of trifluoperazine in the presence (●) or absence (○) of 0.5 mM ATP. Efflux (%) of the radioactivity was calculated on the basis of the total radioactivity within the cells extracted with 5% cold trichloroacetic acid (26300 cpm/dish).

TABLE I

INCREASE IN HYDROLYSIS OF *p*-NITROPHENYL PHOSPHATE BY CHO CELLS TREATED WITH ATP AND TRIFLUOPERAZINE

Monolayer cultures of cells were incubated in 1 ml buffer A (pH 8.2) containing the indicated additions at 37°C for 10 min. Then, *p*-nitrophenyl phosphate (pNPP) was added at 5 mM to the medium directly and incubation was continued at 37°C for another 10 min. The supernatant was then removed for measurement of *p*-nitrophenol as described in Materials and Methods. TFP, trifluoperazine.

Treatment	Hydrolysis of pNPP (nmol/mg protein)
Buffer A (pH 8.2)	18.9
+0.5 mM ATP	22.5
+0.5 mM ATP+20 μM TFP	81.8
+20 μM TFP	18.5
+60 μM TFP	86.9

concentrations of trifluoperazine also produced an increase in the efflux. Similar results were observed when these permeability changes induced by trifluoperazine with or without external ATP were determined by the method of hydrolysis of *p*-nitrophenyl phosphate (Table I). These results suggest that ATP and trifluoperazine act synergistically to cause a massive increase in membrane permeability of CHO cells to phosphorylated metabolites.

Trifluoperazine and vinca alkaloids are amines and may alter the lysosomal pH. However, lysozomotropic agents, chloroquine (100 μM) and ammonium chloride (10 mM), did not affect the passive permeability in the presence or absence of external ATP (results not shown).

Dependency of the permeability change on the nucleotide and pH

The permeability change with trifluoperazine was preferentially modulated by external ATP. An ATP analogue, Ado*PP*[NH]*P*, and ADP were also shown to have moderate effects, but AMP and other nucleotide triphosphates were ineffective (Table II). More than 0.05 mM ATP was required for induction of the permeability change but the permeability change with Ado*PP*[NH]*P* or ADP became observable at 0.2–0.3 mM in the presence of trifluoperazine (data not shown). The ATP-dependent permeability changes were also dependent on pH, and were observed at pH 8.2 but not at pH 7.2 (Table II). These characteristics were fundamentally similar to those for CHO cells previously found with mitochondrial inhibitors or cytoskeleton-attacking agents [8,9]. In addition, the permea-

TABLE II

NUCLEOTIDE AND pH DEPENDENCY OF THE PERMEABILITY CHANGE

Efflux of deoxy[^{3}H]glucose-labeled materials from CHO cells was determined as described in Fig. 1 after incubation of the cells at 37°C for 10 min with the indicated treatment. Total radioactivity within the cells was 27100 cpm/dish. TFP, trifluoperazine.

Treatment	Efflux (%)
Buffer A (pH 8.2)	16.0
+20 μM TFP	13.6
+20 μM TFP+0.5 mM ATP	80.2
+20 μM TFP+0.5 mM Ado*PP*[NH]*P*	45.2
+20 μM TFP+0.5 mM ADP	44.5
+20 μM TFP+0.5 mM AMP	16.5
+20 μM TFP+0.5 mM GTP	17.0
+20 μM TFP+0.5 mM UTP	18.3
+100 μM TFP	100
Buffer A (pH 7.2)	15.0
+20 μM TFP	16.8
+20 μM TFP+0.5 mM ATP	16.5
+100 μM TFP	15.5

TABLE III

REVERSIBILITY OF THE PERMEABILITY CHANGE IN CHO CELLS TREATED WITH ATP AND TRIFLUOPERAZINE

CHO-K1 cells were treated as indicated at 37°C for 8 min to induce the permeability change. The cells were then incubated in F12 medium with 10% serum at 37°C for 2 h and the medium was replenished again. The cultures were further incubated at 37°C for 20 h and the numbers of viable cells in each dish were determined.

Treatment	Cell number (cells per dish) ($\times 10^{-6}$)
Buffer A (pH 8.2)	1.93
+0.5 mM ATP	1.79
+0.5 mM ATP+1 mM KCN	2.00
+0.5 mM ATP+20 μM TFP	1.81
+20 μM TFP	1.82
+100 μM TFP	0.12
Untreated	2.10

bility change induced by trifluoperazine alone showed a similar dependency on pH. However, this permeability change induced by trifluoperazine led to cell lysis, since cell growth of the trifluoperazine-treated cells in F12 medium containing 10% calf serum was significantly reduced (Table III). In contrast, the ATP-dependent permeability change with a low concentration of TFP as well as a mitochondrial inhibitor, KCN, was reversible and the permeabilized cells were viable in the medium even after the treatment.

Effect of trifluoperazine on intracellular ATP concentration

Since a permeability change with similar characteristics to those observed in the present study was induced in CHO cells treated with ATP and a mitochondrial inhibitor which lowered the intracellular ATP concentration [8], we examined the effect of trifluoperazine on the cellular ATP concentration under the conditions for the permeability change. As shown in Fig. 3, these drugs rapidly decreased the cellular ATP level during incubation at pH 8.2 for 10 min. This inhibitory effect on cellular ATP was also much less at pH 7.2, consistent with the effect on the permeability change, as shown in Table II.

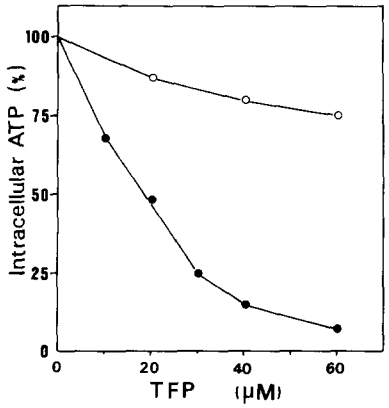

Fig. 3. Effect of trifluoperazine (TFP) on cellular ATP concentration in CHO cells. CHO cells were incubated with various concentrations of trifluoperazine at 37°C for 10 min in buffer A either at pH 8.2 (●) or pH 7.2 (○) and the cellular ATP concentrations within the cells were determined. The 100% values of the cells which were incubated in buffer A alone at pH 8.2 or pH 7.2 were 3.2 and 3.5 nmol/10^6 cells, respectively. The ATP concentration in untreated cells was 3.9 nmol/10^6 cells.

In order to determine whether the pH dependency of the effects of trifluoperazine on the permeability change and cellular ATP concentration were simply due to drug permeability, we measured the uptake of [^{3}H]trifluoperazine into cells at different pH values. As shown in Fig. 4, the incorporation of [^{3}H]trifluoperazine at pH 8.2 was about 2-fold greater than that at pH 7.2 in (a) a time- and (b) a concentration-dependent manner. Therefore, the pH-dependent permeability changes and depletion of cellular ATP induced by trifluoperazine could be partly ascribed to dependency of the drug uptake on the pH of the medium.

We next determined whether these changes with trifluoperazine in cellular ATP concentration were directly involved in the ATP-dependent and ATP-independent permeability changes. As reported previously, addition of excess glucose to the medium containing rotenone partly restored the reduced cellular ATP concentration, resulting in complete suppression of the external ATP-requiring permeability change (Table IV). However, in trifluoperazine-treated cultures, both permeability changes induced by trifluoperazine alone (60 μM) and trifluoperazine (30 μM) with external ATP were not affected by excess glucose, although the cellular ATP concentration was also restored with the glucose. These results clearly indicated that trifluoperazine-induced permeability changes were independent of the cellular ATP concentration.

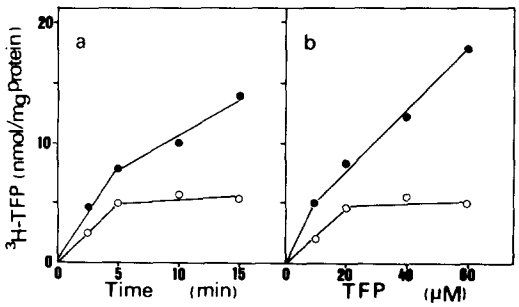

Fig. 4. Uptake of [^{3}H]trifluoperazine by CHO-K1 cells. The cells were incubated with 20 μM [^{3}H]trifluoperazine (0.15 μCi/ml) at 37°C in buffer A either at pH 8.2 (●) or at pH 7.2 (○) for the indicated periods (a). In experiment (b), similar incubations with various concentrations of trifluoperazine (TFP) were carried out for 10 min. After the incubation, the amounts of trifluoperazine taken up by the cells were determined as described in Materials and Methods.

TABLE IV

CHANGES IN CELLULAR ATP CONCENTRATION AND THE PERMEABILITY CHANGE IN CHO CELLS TREATED WITH ROTENONE OR TRIFLUOPERAZINE

CHO cells were treated as indicated in buffer A at pH 8.2 for 10 min at 37°C and the cellular ATP was extracted and determined. Another set of cells was labeled with deoxy[^{3}H]glucose and efflux of the labeled materials was determined after the cells were treated at 37°C for 10 min as indicated in the absence or presence of 0.5 mM ATP. Total radioactivity within the cells was 24100 cpm/dish. TFP, trifluoperazine.

Treatment	Cellular ATP (%)	Efflux (cpm per dish) ($\times 10^{-3}$)	
		−ATP	+ATP
Buffer A (pH 8.2)	100	5.44	6.75
+3 μM rotenone	6	6.24	24.0
+3 μM rotenone +10 mM glucose	54	–	7.57
+30 μM TFP	28	6.52	23.7
+30 μM TFP +10 mM glucose	64	–	21.2
+60 μM TFP	10	24.0	–
+60 μM TFP +10 mM glucose	66	20.9	–

Effects of chlorpromazine and naphthalene sulfonamides on passive permeability and cellular ATP concentration

In order to examine further the possibility that the external ATP-dependent permeability change involves a calmodulin-mediated process, effects of other calmodulin-interacting drugs on passive permeability of CHO cells were determined. Chlorpromazine [16–18] or a chlorinated methylsulfonamide (W-7) [23,24] at 30 μM also produced an external ATP-dependent permeability change in CHO cells at pH 8.2 (Table V). These drugs also significantly reduced the cellular ATP concentration at the alkaline pH. A chlorine-deficient analog of W-7, W-5, which was reported to have 100-fold less affinity for calmodulin [23], could induce neither the permeability change nor reduction of the cellular ATP concentration. However, a significant difference in the action of these drugs from that of trifluoperazine was evident, since restoration of the cellular ATP level on addition of glucose to the culture of chlorpromazine or W-7-treated cells resulted in suppression of the ATP-dependent permeability changes. These results indicate that chlorpromazine and W-7, like mitochondrial inhibitors, induced the permeability change by lowering the cellular ATP concentration, suggesting that these effects are not directly dependent on calmodulin.

TABLE V

EFFECTS OF CHLORPROMAZINE, W-7 AND W-5 ON THE PASSIVE PERMEABILITY CHANGE AND CELLULAR ATP CONCENTRATION

Efflux from labeled CHO cells treated as indicated with or without 0.5 mM ATP and changes in cellular ATP concentration with the indicated treatments were determined as described in Table IV. CPZ, chlorpromazine.

Treatment	Efflux (%)		Cellular ATP (%)
	−ATP	+ATP	
Buffer A (pH 8.2)	18.1	23.0	100
+30 μM CPZ	19.6	98.5	36
+30 μM CPZ +10 mM glucose	–	33.4	83
+30 μM W-7	18.5	96.1	11
+30 μM W-7 +10 mM glucose	–	23.5	82
+30 μM W-5	14.5	24.2	74

Discussion

The present results demonstrate that a passive permeability change is induced in CHO-K1 cells when the cells are treated with external ATP in the presence of 10–30 μM trifluoperazine. This permeability change requires more than 0.05 mM ATP and is observed at pH 8.2 but not at pH 7.2. Furthermore, this permeability change was shown to be reversible. These characteristics of the permeability change are similar to those previously found with mitochondrial inhibitors [8] and cytoskeleton-attacking agents [9]. As described, the cytoskeletal drug-induced permeability changes, unlike those with mitochondrial inhibitors, were independent of changes in the cellular ATP concentration [9]. The present permeability change induced by trifluoperazine was also independent of the cellular ATP concentration, suggesting that these permeability changes are controlled by a common mechanism.

Trifluoperazine is known to have a high binding affinity to calmodulin compared to many other

drugs [16] and therefore it has been used as a good tool to demonstrate the role of calmodulin in various cellular processes [16–22]. In addition, trifluoperazine-resistant mutants were recently isolated from a murine macrophage-like cell line and the mutants were altered as to the properties of calmodulin-binding proteins [28]. These facts suggest that selective binding of trifluoperazine to calmodulin occurs in intact cells, although the binding may be not specific under all circumstances. It has also been reported that calmodulin plays important roles in the assembly and functions of the cytoskeleton [11–13], and the subcellular localization of calmodulin is very close to that of microtubules [29]. In addition, vinca alkaloids could affect calmodulin-dependent enzyme activities [14,15]. Based on these lines of evidence, it is suggested that calmodulin associated with the cytoskeleton is involved in control of the ATP-dependent permeability change in CHO cells.

Modulation by calmodulin of a wide variety of functions is dependent on the cytoplasmic Ca^{2+} concentration [10,11]. The calmodulin antagonists used in the present studies selectively bind to calmodulin in a Ca^{2+}-dependent fashion [30]. In connection with this, it is of interest to note that addition of excess Ca^{2+} (2 mM) to the reaction mixture inhibited the ATP-dependent permeability change with vinblastine [9] or trifluoperazine (data not shown). This inhibitory effect of exogenous Ca^{2+} cannot be explained simply by chelation of the added ATP, since the permeability change induced by ATP and rotenone was not affected by the Ca^{2+}. These facts suggest that the exogenous Ca^{2+} acts on the cellular calmodulin-cytoskeleton complex in the permeability change.

However, it remains possible that the permeability change with trifluoperazine is mediated by a nonspecific perturbation of the plasma membrane due to the hydrophobic properties of the drug [31,32], although such calmodulin-independent effects of trifluoperazine on membranes require relatively higher concentrations of the drug [18,19]. In the present study, higher concentrations of trifluoperazine alone induced a permeability change possibly due to the membrane perturbation by the drug, since the permeability change was shown to be irreversible (Table II). But the drug may have more subtle effects on the membranes even at the lower concentrations. Therefore, further biochemical and molecular studies are required to obtain direct evidence of the involvement of calmodulin in the ATP-dependent permeability change.

The present studies also showed that other well-known calmodulin-interacting drugs such as chlorpromazine and W-7 induced a permeability change in the presence of ATP (Table V). W-5, a structural analog of W-7, could not induce such permeability changes. These results supported the idea that calmodulin is involved in this reaction. However, chlorpromazine and W-7, like mitochondrial inhibitors, were unexpectedly found to deplete cellular ATP when inducing the external ATP-dependent permeability change, since it was suppressed by addition of glucose that restored the cellular ATP concentration. These results clearly indicated that the actions of chlorpromazine and W-7 in intact cells are different from that of trifluoperazine under the present experimental conditions. It was reported that chlorpromazine inhibits various mitochondrial enzymes including F1-ATPase, which do not involve calmodulin [33,34], and reduces the cellular ATP level [35]. Therefore, the inhibitory effect of chlorpromazine as well as that of W-7 on mitochondrial ATP synthesis may be due to nonspecific binding to mitochondrial membranes. Trifluoperazine also reduced the cellular ATP concentration. The effects of calmodulin-interacting agents on cellular ATP described in this study were evident at alkaline pH and this was partly ascribed to the pH-dependent drug permeability (Fig. 4). During preparation of this paper, De and Weisman [36] also reported that trifluoperazine, chlorpromazine and W-7 could enhance the effect of external ATP on the passive permeability in transformed mouse fibroblasts without altering intracellular ATP levels.

Although the present and previous studies suggested involvement of the calmodulin-cytoskeleton complex in the external ATP-dependent permeability change, the mechanism of the permeability change in mammalian cells remains largely unknown. Earlier reports suggested involvement of a specific protein phosphorylation by external ATP in the plasma membrane [2–4,8,37], but results of recent studies were rather inconsistent with this model [38]. Recently, Weisman et al. [39] have shown that external ATP increases the membrane

permeability to Na^+ and K^+, leading to an increase in nucleotide permeability in transformed mouse fibroblasts. To obtain further insight into the biochemistry and regulation of the ATP-dependent permeability change, we developed a genetic approach and isolated CHO variants defective in the response to the external ATP (unpublished data). Characterization of these variants may throw light upon the mechanism by which passive permeability in mammalian cells is controlled.

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