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Permeability Change in Transformed Mouse Fibroblasts Caused by Ionophores, and its Relationship to Membrane Permeabilization by Exogenous ATP

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Summary. Electrogenic ionophores have been found to induce membrane permeabilization in Swiss mouse 3T3 cells that had undergone spontaneous transformation (3T6 cells). Cells attached to plastic dishes were loaded with [^{3}H] uridine, and then the medium was replaced by buffered salt solution at pH 7.8. The enhancement of membrane permeability was assayed by following the efflux of uridine nucleotides, normally impermeant substances. Titration with electrogenic ionophores, such as carbonylcyanide *m*-chlorophenylhydrazone (CCCP), SF-6847 and gramicidin D, markedly increased the membrane permeability within a very narrow range of ionophore concentration. Non-electrogenic ionophores, such as monensin and nigericin, did not affect membrane permeability. Measurements of the distribution of the lipophilic cation tetraphenylphosphonium (TPP^+) between the cells and their environment implied that the remarkable increase in permeability took place within a narrow range of membrane potential ($\Delta\psi$). The data could be explained by a $\Delta\psi$ threshold value, under which aqueous channels are opened in the plasma membrane. The effects exerted by electrogenic ionophores on the plasma membrane were found to be similar to those induced by exogenous ATP. In both cases rapid efflux of K^+, influx of Na^+ and reduction of $\Delta\psi$ preceded membrane permeabilization to low molecular weight, charged molecules, such as nucleotides. It is suggested that dissipation of $\Delta\psi$ induces conformational alterations in membranal components, and/or topological changes, such as aggregation of protein molecules, to form membranal aqueous channels. Electrogenic ionophores permeabilize both normal (3T3) and transformed (3T6) mouse fibroblasts, whereas ATP effects are specific for transformed cells. Thus, it is postulated that ATP acts *via* specific sites on the surface of transformed cells.

Key Words membrane potential · permeability · 3T6 cells · external ATP · ionophores · tetraphenylphosphonium

Introduction

The mechanism of permeation of nucleotides, phosphate esters and similar low molecular weight charged molecules through the plasma membrane of animal cells is uncertain. Because of their low lipid solubility the diffusion of these molecules through the lipid phase of the membrane is probably negligible (Plagemann & Wohlhueter, 1980). Cells can be rendered artificially permeable by various methods (Heppel & Makan, 1977) such as treatment with toluene (Moses & Richardson, 1970; Hilderman, Goldblatt & Deutscher, 1975), nonionic detergents (Kay, 1965; Malenkov et al., 1967; Carlson, Till & Ling, 1976), ionic detergents (Hodes, Palmer & Warren, 1960), osmotic shock (Kaltenbach, 1966; Castellot, Miller & Pardee, 1978), dextran sulfate (Kasahara, 1977) and exogenous ATP (Rozengurt & Heppel, 1975*a*). The latter is of special interest, since the increase in the passive permeability of the plasma membrane by exogenous ATP was found to be rapid, reversible, specific for ATP and selective for transformed cells (Rozengurt & Heppel, 1975*a*; Rozengurt, Heppel & Friedberg, 1977). The effect of exogenous ATP is more pronounced in the presence of respiration inhibitors, energy transfer inhibitors and uncouplers of oxidative phosphorylation (Rozengurt & Heppel, 1979).

Effects of exogenous ATP on membranal functions have been observed in various cell types. ATP-induced changes in cell volume (Hempling, Stewart & Gasic, 1969; Stewart, Gasic & Hempling, 1969; Rorive & Kleinzeller, 1972), and ion fluxes in Ehrlich ascites cells (Hempling et al., 1969; Landry & Lehninger, 1976), HeLa cells (Aiton & Lamb, 1975) and mast cells (Dahlquist, Diamant & Krüger, 1974; Krüger, Diamant & Dahlquist, 1974) have been reported. In mast cells, nucleotide permeability is also increased (Cockcroft & Gomperts, 1979*a*). Exogenous ATP affects aggregation and movement of fibroblasts (Jones, 1966; Knight, Jones & Jones, 1966), secretion of protons in frog gastric mucosa (Sanders et al., 1976) and insulin-stimulated glucose transport in adipocytes (Chang & Cuatrecasas, 1974). ATP has been postulated to be a neurotransmitter (Burnstock, 1974).

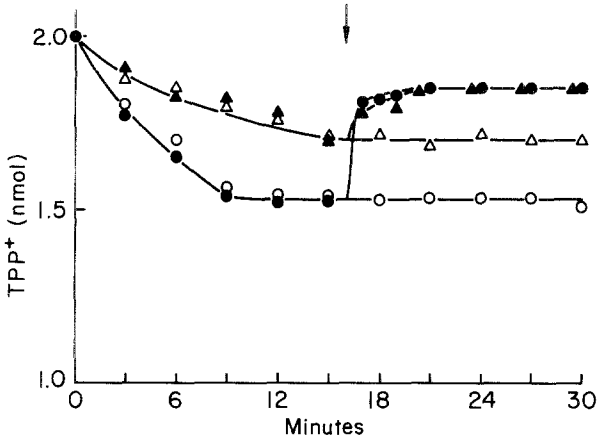

Fig. 1. TPP^+ uptake in 3T6 cells in the presence of either NaCl or KCl. Cell growth and TPP^+ uptake assays were performed as described in Materials and Methods. Incubation media contained 1 ml of 30 mM HEPES, pH 7.8 [the pH was adjusted with either NaOH (○,●) or KOH (△,▲)], 5 mg/ml dextran T-500 and either 125 mM NaCl (○,●) or 125 mM KCl (△,▲). The amount of TPP^+ in the extracellular medium at the indicated times is expressed in nmol. TPP^+ was added at zero time and after 16 min (arrow), the ionophore SF-6847 was added to a final concentration of 5 μM (●,▲) to dissipate the membrane potential. The membrane potential was determined to be −63 mV

Several possible mechanisms for membrane permeabilization were examined. The possibility that the effect of ATP is due to chelation of bivalent ions was ruled out, since other nucleotides and chelators do not permeabilize the membrane under the conditions used (Rozengurt et al., 1977). It is unlikely, as well, that hydrolysis products of ATP are the effective agents, since ADP, AMP, cyclic-AMP, pyrophosphate and phosphate do not affect membrane permeability (Rozengurt et al., 1977). The suggestion that phosphorylation and dephosphorylation of a 42,000 dalton membrane protein affect membrane permeability (Makan, 1981) has been ruled out, since protein phosphorylation appears to be unrelated to ATP-induced permeabilization (Weisman et al., 1984*a*).

Recently it was shown that addition of ATP induces ion fluxes and reduction of the electrical potential across the cell membrane ($\Delta\psi$) prior to the increase in nucleotide permeability (Weisman et al., 1984*b*). In view of this observation, a correlation between changes in $\Delta\psi$ and alterations in the passive membrane permeability induced by ionophores was examined. The mechanism underlying membrane permeabilization induced by ionophores and by exogenous ATP is discussed. A preliminary account of this work has been published (Friedberg et al., 1983).

Materials and Methods

Materials

[^{3}H]-uridine was obtained from New England Nuclear, Boston, Mass., and [^{3}H]-tetraphenylphosphonium bromide ([^{3}H]-TPP^+) from the Israel Atomic Energy Commission, Negev, Israel. SF-6847 was a gift from Y. Nishizawa, Sumitomo Chemical Co., Ltd., Osaka, Japan. Most other chemicals were obtained from Sigma, St. Louis, Mo., and were of the highest purity available.

Cell Cultures

Swiss mouse 3T3 and 3T6 cells (Todaro & Green, 1963) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS) as described (Rozengurt et al., 1977). Cells were used after reaching confluency.

Measurements of Efflux of Acid-Soluble Pools

The acid-soluble pool of 3T6 or 3T3 cells was labeled with [^{3}H]-uridine (1 μM, 0.5 μCi/ml) for 2 or 4 hr, respectively, in DMEM containing 5% FCS, at 37°C, in a humidified atmosphere containing 5% CO_2. After labeling, the medium was aspirated and the cells were washed three times with 0.15 M NaCl, containing 50 μM $CaCl_2$, and once with medium A, composed of 100 mM Tris HCl (pH 7.8 at 37°C), 50 mM NaCl, 50 μM $CaCl_2$ and 5 mg/ml dextran T-500, and finally incubated with 1 ml of medium A containing the indicated effectors. At the indicated time intervals, samples of 100 μl were withdrawn from the supernatant, for radioactivity measurements. Then the rest of the supernatant was aspirated, 1 ml of cold 5% trichloroacetic acid (TCA) was added to the dish, and after a 15- to 30-min incubation at 4°C the radioactivity in the TCA extract was measured. Radioactivity measurements were performed in a Beckman LS 7000 liquid scintillation counter, and the percent of the soluble pool released at each time point was calculated.

Measurement of TPP^+ Levels

Cells were prepared for TPP^+ uptake and efflux measurements the same way as for measurements of the acid-soluble pool except that radioactive uridine was not added. The cells were preincubated with 1.0 ml of medium A for 5 min at 37°C and then [^{3}H] TPP^+, 100 Ci/mol, was added to a final concentration of 2 μM. Samples of 50 μl were withdrawn from the supernatant at indicated time intervals (usually for 3 min) for radioactivity estimation to monitor the amount of TPP^+ taken up by, or released from the cells. The advantage of this method is that the measurements are performed without manipulating the cells, the same way as in flow dialysis assays (Ramos, Schuldiner & Kaback, 1976). The total amount of TPP^+ added to each dish was measured again at the end of the assay, by summation of the extracellular and intracellular TPP^+. The latter was extracted with 5% cold TCA for 20 min.

The electrical potential across the cell membrane ($\Delta\psi$) was calculated according to the Nernst equation:

$$\Delta\psi = \frac{RT}{F} \ln \frac{[TPP^+]_{IN}^{CORRECTED}}{[TPP^+]_{OUT}}. \quad (1)$$

R is the gas constant; T is the temperature in °K; F is the Faraday constant. $[TPP^+]_{IN}$ and $[TPP^+]_{OUT}$ are the concentrations of TPP^+ within the cells and in the surrounding medium, respectively. Correction for the $[TPP^+]_{IN}$ was made according to Lichtshtein et al. (Lichtshtein, Kaback & Blume, 1979*a*; Lichtshtein, Dunlop, Kaback & Blume, 1976*b*) and the $[TPP^+]_{IN}^{CORRECTED}$ is equal to the $[TPP^+]_{IN}$ in the presence of 125 mM NaCl minus $[TPP^+]_{IN}$ in the presence of 125 mM KCl. Figure 1 shows the time course of [^{3}H] TPP^+ accumulation in the presence of either NaCl or KCl. A steady-state level of TPP^+ accumulation was achieved in 10 to 15 min. Addition of the potent ionophore SF-6847 (5 μM), resulted in rapid efflux of TPP^+ revealing the potential-sensitive and insensitive portions of the [^{3}H]TPP^+ that were removed from the extracellular medium. The same pattern was obtained with other ionophores, such as CCCP (20 μM), gramicidin D (20 μM) or a combination of both.

As was previously emphasized, the correction factor for $[TPP^+]_{IN}$ is an approximation that may underestimate the contribution of intracellular organelles, such as mitochondria, that have an internally negative $\Delta\psi$ (Lichtshtein et al., 1979*a*). Therefore, the $\Delta\psi$ value determined by this method cannot be taken as an absolute qualitative measurement of the true $\Delta\psi$ value. However, the data obtained represent a valid qualitative determination of relative $\Delta\psi$ values (Tan & Tashjian, 1984). In addition, values obtained for $\Delta\psi$ by the TPP^+ method were in good correlation with electrophysiological measurements (Lichtshtein et al., 1979*a*).

Variations in the $\Delta\psi$ estimations were minimized, according to the method of calculation suggested by Sonenberg and coworkers (Cheng et al., 1980), by using the following equation:

$$\delta\Delta\psi = \Delta\psi_{II} - \Delta\psi_{I} = \frac{RT}{F} \ln \frac{[TPP^+]_{IN,II}/[TPP^+]_{OUT,II}}{[TPP^+]_{IN,I}/[TPP^+]_{OUT,I}}. \quad (2)$$

$\Delta\psi_I$ and $\Delta\psi_{II}$ are the electrical potentials across the membrane before and after addition of an effector, respectively. The other terms are as in Eq. (1).

Determination of Intracellular Water Volume

The internal water volume was estimated by using [^{3}H]-water (50 nCi/ml) and [^{14}C]-sorbitol (10 nCi/ml), as described (Rottenberg, 1979). The value obtained was 13 ± 0.6 μl water per mg of cell protein for 3T6 cells.

Assay of Sodium and Potassium Ions

Intracellular TCA pools, obtained after washing the cells six times with 0.1 M $MgCl_2$ (Smith & Rozengurt, 1978), were diluted in 1% LiCl (wt/vol) to less than 2 μg Na^+/ml or 1 μg K^+/ml. Standard curves were also constructed in 1% LiCl (wt/vol). Na^+ and K^+ in each sample was measured by flame emission spectrophotometry on a Varian AA-575 atomic absorption spectrophotometer.

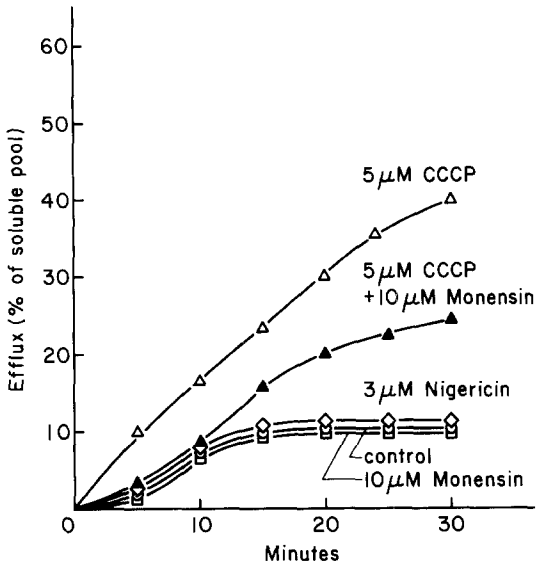

Fig. 2. Effect of ionophores on membrane permeability in 3T6 cells. Cell growth and permeability assays were performed as described in Materials and Methods. Additions: (○) None. (△) 5 μM CCCP. (□) 10 μM monensin. (◇) 3 μM nigericin. (▲) 5 μM CCCP and 10 μM monensin

Protein Determination

One ml of 1 M NaOH was added to each dish at the end of the experiment. After 30-min incubation at 37°C, samples were withdrawn for protein estimation according to Lowry et al. (1951).

Results

Electrogenic Ionophores Induce Cell Membrane Permeabilization

Addition of 5 μM carbonylcyanide *m*-chlorophenylhydrazone (CCCP), a protonophore which enables the electrogenic transfer of H^+ across the membrane, to 3T6 cells in medium A, resulted in a pronounced increase in the efflux of the [^{3}H]-uridine-labeled intracellular soluble pool (Fig. 2). Similar results were obtained with the protonophore SF-6847 (*data not shown*). The nonelectrogenic ionophores, nigericin (3 μM) and monensin (10 μM) which mediate the exchange of H^+ for K^+, and H^+ for Na^+, respectively, had no effect on the rate of the efflux. Furthermore, when monensin was added together with CCCP, the rate of efflux was reduced, as compared to CCCP by itself (Fig. 2). As was previously shown (Rozengurt & Heppel, 1979) the combination of CCCP and ATP induces a high rate of efflux (Fig. 3). This effect of CCCP and ATP can

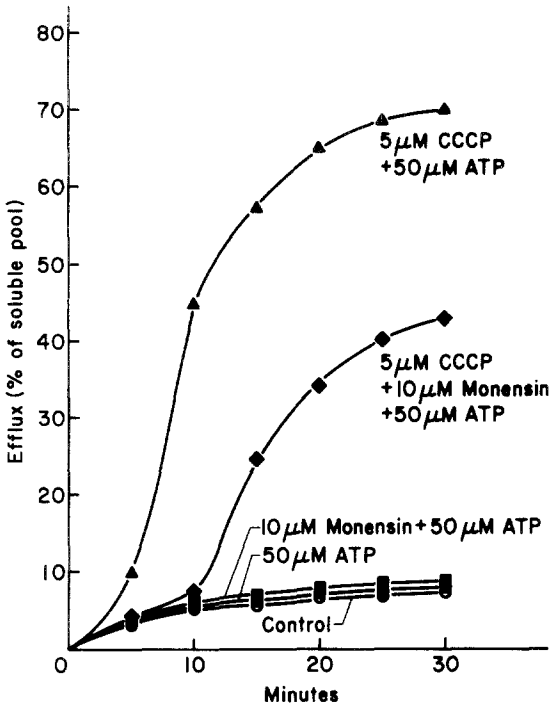

Fig. 3. Effect of ATP and ionophores on membrane permeability in 3T6 cells. Cell growth and permeability assays were performed as described in Materials and Methods. Additions: (○) None. (●) 50 μM ATP. (■) 10 μM monensin and 50 μM ATP. (▲) 5 μM CCCP and 50 μM ATP. (◆) 5 μM CCCP, 10 μM monensin and 50 μM ATP

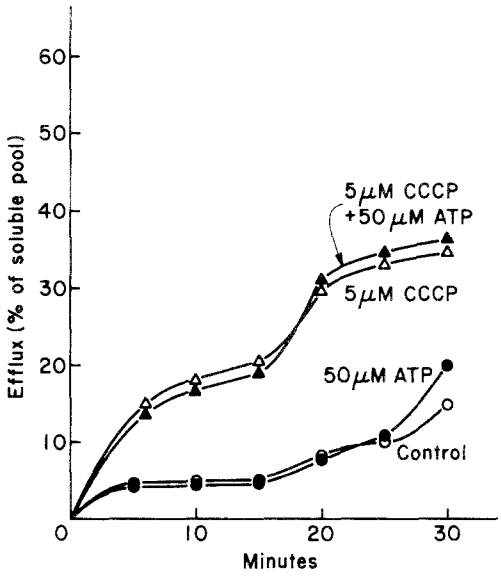

Fig. 4. Effect of CCCP and ATP on membrane permeability in 3T3 cells. Cell growth and permeability assays were performed as described in Materials and Methods. Additions: (○) None. (●) 50 μM ATP. (△) 5 μM CCCP. (▲) 5 μM CCCP and 50 μM ATP

be titrated down to levels of CCCP that do not increase efflux alone (*see below*). The efflux induced by CCCP and ATP was considerably diminished upon addition of monensin (Fig. 3). The effect of monensin could be explained by the nonelectrogenic influx of Na^+, which activates the plasma membrane (Na^+,K^+)-ATPase (Rozengurt & Heppel, 1975*b*; Smith & Rozengurt, 1978; Lichtshtein et al., 1979*a*,*b*). The activation of this enzyme, which reacts as an electrogenic ion pump, results in the increase of membrane potential, concomitant with reduction of the efflux.

The electrogenic ionophore CCCP induced cell membrane permeabilization in 3T3 cells, although to a somewhat lesser extent than 3T6 cells (Fig. 4). However, in contrast to 3T6 cells, there was no additional permeabilization of 3T3 cells upon addition of ATP. These data are in accord with the insensitivity to ATP that was observed in these cells (Rozengurt et al., 1977).

An Ionophore Concentration-Dependent Threshold for Cell Membrane Permeabilization

Titration studies with CCCP have shown a sharp increase in the efflux of the [^{3}H]-uridine-labeled soluble pool, between 2 and 3 μM CCCP (Fig. 5). A similar pattern of efflux *vs.* ionophore concentration was obtained with gramicidin D, an electrogenic, channel-forming ionophore (Fig. 6). Again, a sharp increase in efflux was observed with an increase of ionophore concentration from 4 to 5 μM, whereas below or above these concentrations the variations in the efflux were small.

Changes in $\Delta\psi$ were estimated from the distribution of the lipophilic, radioactive cation [^{3}H]-TPP^+ between the cells and their environment [Eq. (2)]. As shown in Figs. 5 and 6, there is no sharp change in the steady-state levels of TPP^+ within the cells as a function of ionophore concentration. Figure 6 shows that the sharp increase in membrane permeability took place in parallel to a relatively small and gradual decrease in $\Delta\psi$, indicating a $\Delta\psi$ threshold for membrane permeabilization by electrogenic ionophores. A reduction of $\Delta\psi$ in the range of 25 to 35 mV induced by gramicidin D, was followed by the marked increase in the plasma membrane permeability.

Replacement of NaCl by KCl in the Medium Induces Membrane Permeabilization

Replacement of NaCl by KCl in medium A resulted in an increase in the membrane permeability (Fig. 7). This is an additional correlation between the reduction of $\Delta\psi$ and an increase in membrane permeability, since external K^+ dissipates $\Delta\psi$ (Fig. 1). Furthermore, the cells became susceptible to low levels of ATP in the presence of a high concentra-

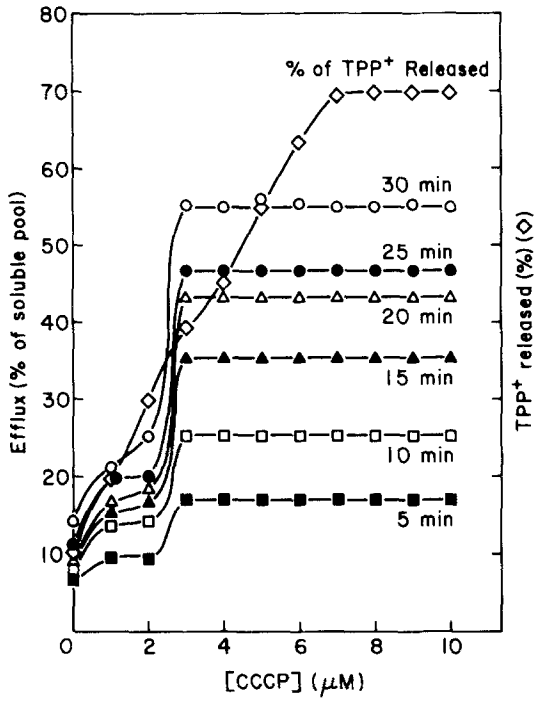

Fig. 5. Effect of CCCP concentration on the permeability and the TPP^+ level in 3T6 cells. Cell growth, permeability assays and TPP^+ uptake assays were performed as described in Materials and Methods. The percent of the acid-soluble pool that was released upon addition of various concentrations of CCCP was determined at the indicated time intervals: (■) 5, (□) 10, (▲) 15, (△) 20, (●) 25 and (○) 30 min after addition of CCCP. The percent of TPP^+ released (◇) was calculated from the difference in the steady-state levels of TPP^+ before and after CCCP addition (not corrected for the potential-insensitive portion of TPP^+)

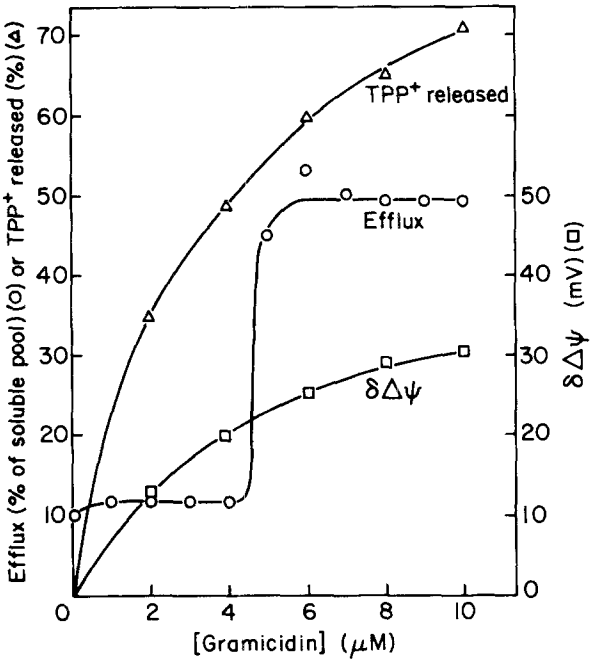

Fig. 6. Effect of gramicidin D concentration on the permeability and TPP^+ level in 3T6 cells. Cell growth, permeability assays and TPP^+ uptake assays were performed as described in Materials and Methods. (○) Percent of the acid-soluble pool that was released after 30-min incubation. (△) Percent of TPP^+ released, calculated from the difference in the steady-state levels of TPP^+ before and after addition of gramicidin D (not corrected for the potential-insensitive portion of TPP^+). (□) The reduction in the membrane potential ($\delta\Delta\psi$) calculated according to Eq. (2), using corrected values of TPP^+ levels

tion of K^+ (Fig. 7), as was the case with electrogenic ionophores (Fig. 3).

Exogenous ATP and Electrogenic Ionophores have Similar Effects on Membrane Depolarization, Ion Fluxes and Membrane Permeability

Addition of ATP to cells that had accumulated TPP^+ caused loss of the lipophilic cation (Fig. 8). The TPP^+ loss during the first 3 min, before nucleotide efflux took place, suggests that membrane depolarization preceded membrane permeabilization. This conclusion is supported by the effect of gramicidin D, an ionophore for monovalent cations, which induced membrane depolarization similar to that obtained with ATP (Fig. 8), as well as efflux of the intracellular soluble pool (Fig. 6). Additional similarity between exogenous ATP and gramicidin was observed when their effects on ion fluxes were examined. Figure 9 shows that exogenous ATP caused immediate and rapid K^+ efflux and Na^+ influx in 3T6 cells. This is probably the reason for the rapid membrane depolarization. The reduction of the cellular level of K^+ and the increase in the cellular content of Na^+ (Fig. 9), preceded the efflux of the [^{3}H]-uridine-labeled soluble pool (Weisman et al., 1984*b*). The ion fluxes induced by the ionophore gramicidin D were similar to those obtained with ATP (Fig. 9).

Titration studies with ATP have shown an increase in the efflux of the [^{3}H]-uridine-labeled pool between 50 to 200 μM ATP, whereas the changes in the efflux above or below these concentrations were negligible (Fig. 10). There is a similarity between the concentration dependance of the efflux in the case of ATP (Fig. 10) and the effects of ionophores (Figs. 5 and 6), although the effect of ATP is less pronounced.

Discussion

The data presented in this study suggest that $\Delta\psi$ has a major role in the regulation of the passive permeability of the plasma membrane in cultured 3T6 cells.

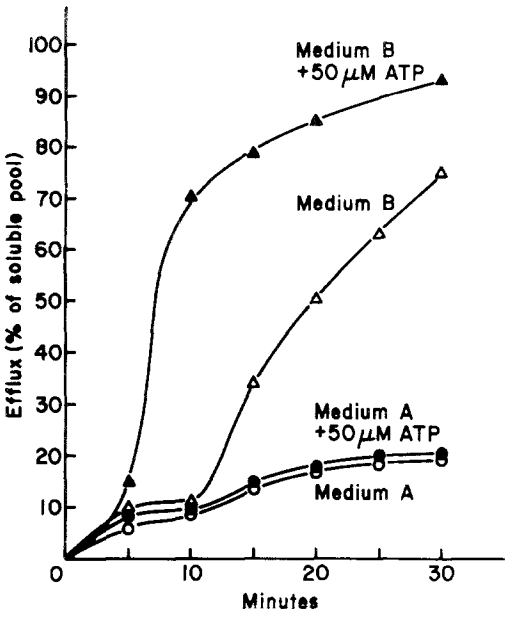

Fig. 7. Effect of potassium ions on membrane permeability in 3T6 cells. Cell growth and permeability assays were performed as described in Materials and Methods. (○) Medium A which contains 50 mM NaCl. (△) Medium B which is similar to Medium A except the NaCl was replaced by KCl. (●) Medium A and 50 μM ATP. (▲) Medium B and 50 μM ATP

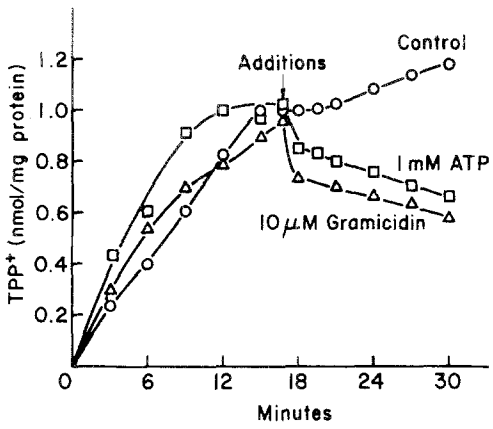

Fig. 8. Effect of ATP and gramicidin D on TPP^+ levels in 3T6 cells. Cell growth and TPP^+ uptake assays were performed as described in Materials and Methods. Additions were made at 16 min (arrow). (○) None. (△) 10 μM gramicidin D. (□) 1.0 mM ATP

This conclusion is supported by the following observations. Dissipation of $\Delta\psi$ by several electrogenic ionophores was followed by efflux of the intracellular soluble pools of nucleotides, phosphate esters and certain other normally impermeant compounds. The membrane permeabilization was obtained with either carrier-type ionophores, such as CCCP, or with channel-forming ionophores, such as gramicidin D. The effect of CCCP (Fig. 5) was found to be more pronounced than that of gramicidin D (Fig. 6). This difference may be due to reported side effects of CCCP such as its effect on sulfhydryl groups (Kaback et al., 1974). Nigericin and monensin, which mediate nonelectrogenic exchange of H^+ for K^+ or Na^+, respectively, did not affect membrane permeability. However, in HeLa cells, prolonged incubation with nigericin has been reported to increase membrane permeability to ions and macromolecules (Alonso & Carrasco, 1982). In the present study monensin abrogates the CCCP-induced permeabilization (Figs. 2, 3). This can be attributed to the ability of monensin to increase the $\Delta\psi$ (Lichtshtein et al., 1979*b*) through activation of the plasma membrane enzyme (Na^+,K^+)-ATPase. This enzyme is an electrogenic ion pump, regulated by the intracellular level of Na^+, and thus could be activated by the monensin-mediated Na^+ influx (Rozengurt & Heppel, 1975*b*; Smith & Rozengurt, 1978; Lichtshtein et al., 1979*a,b*). Other studies showed that dissipation of $\Delta\psi$ by high concentrations of external K^+ was followed by efflux of the intracellular soluble pool. A high K^+ concentration causes depolarization of the plasma membrane, but its effect on the $\Delta\psi$ of intracellular membranes is negligible (Lichtshtein et al., 1979*a,b*). The similarity between the effects of K^+ and ionophores suggest that the apparent effects of the latter are mainly on the plasma membrane.

A gradual increase in the ionophore concentration resulted in dissipation of $\Delta\psi$ and increase in membrane permeability. However, the patterns of the two processes are markedly different. Whereas the reduction of $\Delta\psi$ with increase in the ionophore concentration is gradual, the change in the membrane permeability is sudden and sharp. Thus, in a certain range of $\Delta\psi$, a relatively small reduction in the membrane potential causes a considerable increase in membrane permeability. Apparently, a $\Delta\psi$ threshold is a part of the mechanism underlying plasma membrane permeabilization.

There are similarities between the effects of exogenous ATP and the effects of electrogenic ionophores, as expressed in the induction of ion fluxes, reduction of $\Delta\psi$ and increase in passive plasma membrane permeability. These similarities suggest that some steps in the mechanism by which exogenous ATP permeabilizes the plasma membrane are similar, or even the same, as in the mechanism of permeabilization by ionophores. This possibility has been further studied (Weisman et al., 1984*b*), and there are some apparent differences between the two effectors. Permeabilization by ATP is more pronounced than that induced by ionophores.

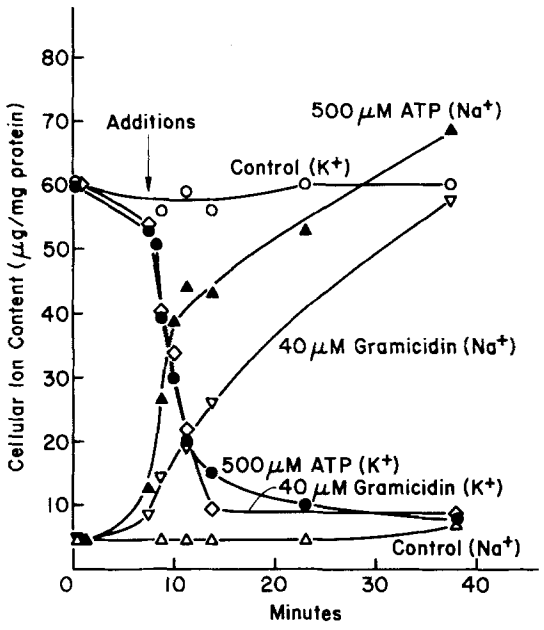

Fig. 9. Effect of ATP and gramicidin D on cellular sodium and potassium ion content. Cell growth and Na^+ and K^+ measurements were performed as described in Materials and Methods. The experiments were performed in medium I, composed of 30 mM HEPES, pH 7.8, 120 mM NaCl, 5 mM KCl and 5 mg/ml dextran T-500. Additions were made at 8 min (arrow). (○,●,◇) K^+ content expressed as μg K^+ per mg protein. Additions: (○) None. (●) 500 μM ATP. (◇) 40 μM gramicidin D. (△,▲,▽) Na^+ content expressed as μg Na^+ per mg protein. Additions: (△) None. (▲) 500 μM ATP. (▽) 40 μM gramicidin D

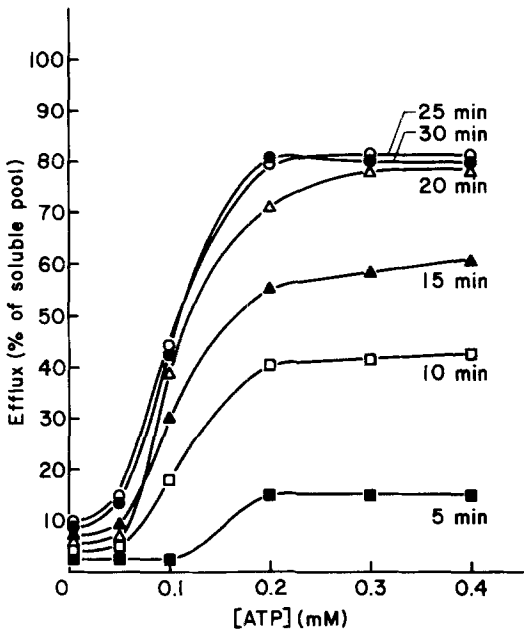

Fig. 10. Effect of ATP concentration on the permeability of 3T6 cells. Cell growth and permeability assays were performed as described in Materials and Methods. The percent of the acid-soluble pool released upon addition of various concentrations of ATP was determined at the indicated time intervals: (■) 5, (□) 10, (▲) 15, (△) 20, (○) 25 and (●) 30 min after addition of ATP

Since ATP is not an ionophore, the induction of ion fluxes and membrane depolarization by this nucleotide should be mediated by membranal components. As the effect of ATP is specific, and much more pronounced in 3T6 than in 3T3 cells, it is postulated that ATP acts *via* specific sites on the cell surface, which are more abundant in transformed cells.

Previous studies have suggested that the exogenous ATP-dependent increase in the plasma membrane permeability to nucleotides is regulated by the level of intracellular ATP (Rozengurt & Heppel, 1979). The data in this and other studies (Weisman et al., 1984*b*) suggest that alterations in $\Delta\psi$ are affecting membrane permeability. Since low concentrations of ionophores reduce both $\Delta\psi$ (Figs. 5, 6) and the level of intracellular ATP (Rozengurt & Heppel, 1979), it is difficult to determine whether the former or the latter effect induces cell membrane permeabilization to nucleotides. However, recent experiments with ouabain (Weisman et al., 1984*b*) and Ca^{2+}-calmodulin antagonists (De & Weisman, 1984) indicate that nucleotide permeability can be induced without affecting intracellular ATP levels. These compounds cause influx of Na^+ and efflux of K^+ suggesting that they act through dissipation of $\Delta\psi$.

There are several cell types for which exogenous ATP has been found to affect movement of ions across the cell membrane. ATP-induced ion fluxes were observed in HeLa (Aiton & Lamb, 1975), mast (Dahlquist et al., 1974; Krüger et al., 1974) and Ehrlich ascites cells (Hempling et al., 1969; Landry & Lehninger, 1977). ATP-induced changes in cell volume were reported for HeLa (Stewart et al., 1969; Hempling et al., 1969; Rorive & Kleinzeller, 1972) and mast cells (Dahlquist et al., 1974; Krüger et al., 1974). In mast cells, a low ATP concentration stimulates histamine secretion, whereas higher concentrations induce membrane permeabilization (Cockcroft & Gomperts, 1979*a*,*b*; Bennett, Cockcroft & Gomperts, 1981). It has been suggested that these phenomena are mediated by ATP receptors (Cockcroft & Gomperts, 1980; Bennett et al., 1981).

The data presented in this study suggest that the sequence of events upon addition of ATP to susceptible cells is initiated by interaction of ATP with specific sites on the cell surface. The nature of these sites is not known, but their interaction with ATP induced ion fluxes across the plasma membrane and

reduction of $\Delta\psi$. It is suggested that the dissipation of $\Delta\psi$ induces conformational changes of membranal components, leading to generation of aqueous channels. Another possibility is that dissipation of $\Delta\psi$ affects the topology of the membrane, and induces aggregation of membranal proteins to form aqueous channels, which enable the transfer of normally impermeant solutes through the cell membrane.

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