J. Membrane Biol. 86, 189–196 (1985)

The Journal of
Membrane Biology
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Topical Review

Permeabilization of Transformed Cells in Culture by External ATP

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Introduction

This review is primarily concerned with the effect of external ATP on passive permeability of transformed cells in culture. Adenosine triphosphate causes the formation of abnormal channels for aqueous solutes that ordinarily do not cross the plasma membrane, including such compounds as nucleoside mono-, di- and triphosphates, phosphate esters and coenzymes. The effect has been seen in many transformed cells and has been intensively studied in transformed mouse fibroblasts, but it was not observed in a variety of untransformed cells. Untransformed cells which have a secretory function represent a major exception to this statement, and for this reason the effect of external ATP on mast cells will be briefly discussed. Many studies have been concerned with various effects of exogenous ATP on cultured cells, cell suspensions freshly isolated from tissues and other preparations commonly used in physiology and pharmacology. A number of these investigations have been concerned with ion fluxes induced by ATP. The literature is extensive and cannot be covered in this short review. However, a limited discussion of these investigations is included because some of the findings are directly relevant to permeabilization of transformed cells by external ATP.

Experiments designed to gain some insight into the mechanism of ATP-dependent permeabilization will be reviewed. Finally, methods for permeabilizing mammalian cells with greatly reduced concentrations of external ATP will be discussed. These procedures include the use of ionophores, manipulation of osmotic conditions, treatment with ouabain or with agents that reduce the level of intracellular ATP, and also the use of inhibitors of Ca^{++}-calmodulin systems.

Key Words passive permeability · external ATP · transformed cells

Effect of External ATP on the Permeability of Transformed Mammalian Cells: Description of the Phenomenon

The effect of external ATP on plasma membrane permeability in 3T6 cells, a spontaneously transformed mouse fibroblast line, has been studied over a period of years in several laboratories. Work on this project was begun in the laboratory of Dr. E. Rozengurt [73]. It was found that cell cultures, incubated in an ATP-containing alkaline medium low in divalent cations, became freely permeable to nucleotides and phosphate esters [28, 73, 75]. The permeability change was detected by following the entry of *p*-nitrophenyl phosphate or by measuring the efflux of nucleotides that were labeled because the cells had previously been incubated with [^{3}H] adenosine or [^{3}H] uridine (Fig. 1). Other transformed cell lines were also found to be sensitive to external ATP, including SV3T3, 3T12, PY3T3, transformed rat kidney cells, mouse melanoma lines, Ehrlich ascites cells, HeLa cells, L929 cells and transformed Chinese hamster ovary cells ([47, 48, 57, 58]; *unpublished results*). Most of the transformed cells respond to 100–250 μM ATP and to much lower concentrations under certain conditions, which will be described in this review. Sensitivity to external ATP is probably not a general property of transformed cells. As an example, 3T3 cells transformed by murine sarcoma virus do not respond [48]. Vari-

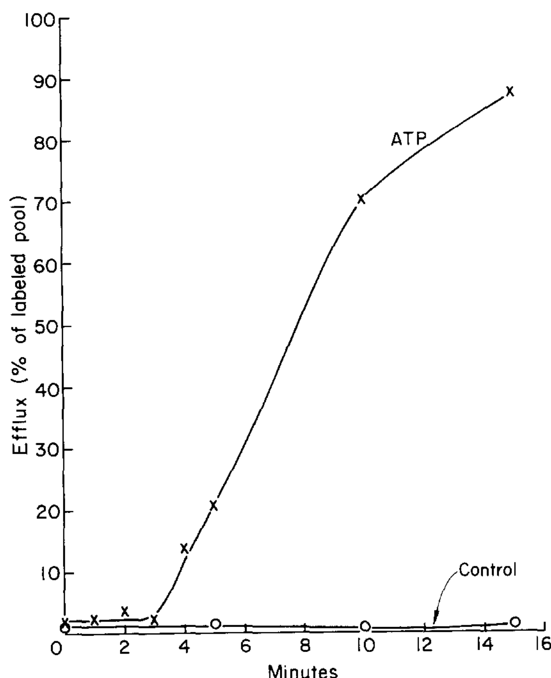

Fig. 1. 3T6 cells were grown to confluency in 35 mm plastic dishes and labeled with [^{3}H]adenosine as described [92]. They were washed with saline and incubated at 37°C with 1 ml of medium I [30 mM HEPES, pH 7.8, 120 mM NaCl, 5 mM KCl and 5 mg/ml Dextran T-500 (Pharmacia)]. There was an initial burst of efflux of labeled adenosine from the intracellular pool, which ended within 7 min. At this point (0 min on the graph), ATP was added to some of the dishes, to a concentration of 150 μM. Individual dishes were sampled at intervals and the efflux of radioactive nucleotides was measured. The extent of subsequent efflux is expressed as a percent of the initial labeled pool

ous untransformed cells are resistant to ATP-dependent permeabilization, including 3T3 mouse fibroblasts, NRK cells, secondary cultures of mouse embryo fibroblasts and human lung fibroblasts [28, 57, 73]. Permeabilization by external ATP has been demonstrated in transformed cells grown in monolayer or suspension cultures [57]. The process does not depend on cell density as it will occur in sparse or overgrown cultures (*unpublished results*). The reaction is highly specific for ATP. Compounds found to be inactive include ADP, AMP, GTP, CTP, UTP, inorganic phosphate, inorganic pyrophosphate and triphosphate, EGTA, EDTA and vanadate [28, 75; *unpublished observations*]. Only γ-thio ATP is also effective, although it acts more slowly and at higher concentrations than required for ATP [57].

ATP-dependent permeabilization of transformed cells is assayed in various isotonic buffers, including Tris-HCl or HEPES. The optimum pH for the process is 7.6 to 7.8, although a slow rate of leakage of nucleotides is observed at a physiological pH of 7.4. It is critical to the permeabilization process that the concentration of Ca^{2+} and Mg^{2+} be reduced below that of ATP. It is possible to demonstrate permeabilization when ATP and divalent cations are present at equivalent concentrations, but the rate of efflux of nucleotides decreases sharply at ratios of Mg^{2+} + Ca^{2+}/ATP greater than 1. It seems probable that the active agent is ATP^{4-} as was observed for permeabilization of mast cells by Cockcroft and Gomperts [19, 20]. Permeabilization of transformed mouse fibroblasts is routinely assayed in the presence of 50 μM $CaCl_2$, which helps the cells adhere to the culture dish.

There is a lag period after addition of external ATP to transformed cells before efflux of a prelabeled intracellular pool of nucleotides is detected. This lag phase lasts for 3–5 min at 37°C and can be prolonged to 10 min at 25°C [75]. The lag period has facilitated the investigation of cellular changes that occur before the cells become permeable to nucleotides (*see below*). Nucleotide efflux, once detected, continues for about 20 min until the intracellular pool of labeled nucleotides is equilibrated with the extracellular fluid. The cells remain impermeable for macromolecules during this time, but subsequently leakage of RNA and proteins, including lactate dehydrogenase, is observed [92].

The effect of external ATP on transformed cells is reversible at least for up to 25 min. Permeability for nucleotides is reversed by shifting to a neutral buffer containing physiological concentrations of divalent cations [75] or by addition of substrates which cause generation of intracellular ATP [58]. The latter was accomplished by adding a suitable concentration of ADP, NAD and glucose 6-phosphate to the medium. These compounds enter permeabilized cells and generate ATP by glycolysis, thereby sealing the cells. Sealing also occurs upon the addition of pyruvate, ADP and inorganic phosphate, which generate intracellular ATP by oxidative phosphorylation. These and other findings (*see below*) lead to the conclusion that ATP-dependent permeabilization is under reciprocal control of intra- and extracellular ATP concentrations. Several cycles of permeabilization and sealing of the cells are possible without affecting subsequent appearance or rate of growth [75]. Accordingly, the permeabilization procedure could be used to introduce normally impermeant compounds into transformed cells. As an example, 1-β-D-arabinofuranosyl cytidine 5′-triphosphate was sealed into a culture of 3T6 cells, resulting in the inhibition of DNA synthesis [45]. Similarly, the protein synthesis inhibitors GDPCP and sparsomycin were sealed inside of 3T6 cells [45]. In fact, the intracellular concentration of

many metabolites, coenzymes, etc., can be manipulated by addition of compounds to the medium surrounding permeabilized cells. Thus, it was possible to study control of glycolysis and the pentose phosphate cycle [60] and protein synthesis [46] in 3T6 cells made permeable with external ATP.

Studies Concerned with the Mechanism of ATP-Dependent Permeabilization of Transformed Cells

In trying to elucidate the mechanism of ATP-dependent permeabilization it was important at the outset to determine if factors present in fetal calf serum played a significant role. Accordingly, cultures of 3T6 cells were plated and grown in serum-free medium supplemented with insulin and epidermal growth factor. On being transferred to permeabilization buffer, they showed the same response to external ATP as did cells grown in the presence of serum, and sealing occurred under the same conditions described above [28]. These results clearly demonstrate that ATP interacts with an effector system derived from the cells, and serum factors are not involved.

An attractive hypothesis was the notion that phosphorylation of a membrane-associated protein by external ATP resulted in the formation of aqueous channels. Several studies yielded data consistent with this idea [28, 59]. In a later investigation the phosphorylation of certain proteins by exogenous ATP was confirmed, but it was established that this was unrelated to the permeabilization process [93]. In spite of considerable effort, we have been unable to detect any protein whose phosphorylation could be correlated with the formation of aqueous channels in the plasma membrane.

An early finding regarding the mechanism of permeabilization was that the concentration of intracellular ATP can modulate the formation of channels dependent on external ATP. The concentration of external ATP required to permeabilize transformed cells can be reduced to 25–50 μM when the levels of intracellular ATP are lowered by starvation or by treatment with agents that inhibit oxidative phosphorylation or respiration [72, 74]. These include cyanide, dinitrophenol, CCCP, antimycin, rotenone, rutamycin, oligomycin and diamide. For example, as shown in Fig. 2, permeabilization does not occur with rotenone or a low concentration of ATP alone, but these compounds together act synergistically to induce nucleotide efflux. Furthermore, ATP-dependent permeabilization does not occur in the presence of glucose (*unpublished observations*) and, as mentioned above,

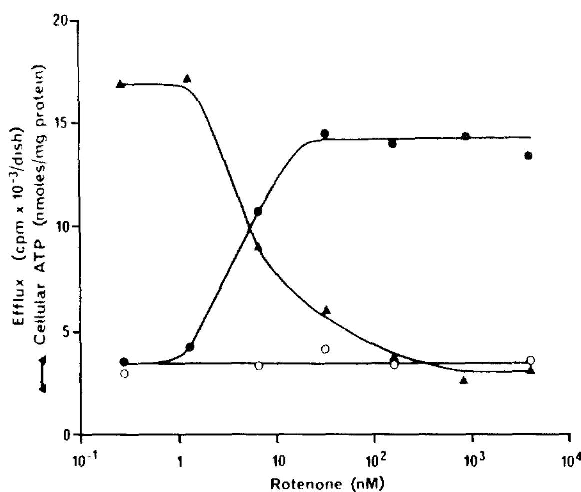

Fig. 2. Effect of different concentrations of rotenone on level of intracellular ATP and in enhancing the effect of external ATP. Cultures of 3T6 cells were labeled with [^{3}H]2-deoxyglucose as described in reference [74]. The cultures were washed and incubated in an alkaline buffered medium in the presence of different concentrations of rotenone for 20 min. Some of the cultures received 50 μM ATP 5 min after being exposed to rotenone. The "basal" efflux in the presence of rotenone (○), or in the presence of 50 μM ATP (not shown, but identical), consisted mostly of [^{3}H]2-deoxyglucose itself. The stimulated efflux in the presence of rotenone + 50 μM ATP (●), was shown to be [^{3}H]2-deoxyglucose 6-phosphate. Cultures exposed to rotenone were extracted with 0.4 N $HClO_4$ and the ATP content (▲) was determined. Note that efflux of the phosphate ester was increasingly stimulated as the level of intracellular ATP fell. Figure reproduced with permission of *J. Biol. Chem.*

sealing of permeabilized cells is facilitated by addition of glycolytic factors which serve to increase intracellular ATP levels [58]. These results indicate the importance of both intracellular and extracellular ATP concentrations in the regulation of a normal plasma membrane barrier to charged molecules.

We then decided to investigate the early events that occur during the lag period following the administration of external ATP, since early cellular responses might yield further insight into the mechanism of permeabilization. It was found that exogenous ATP rapidly induces Na^+ influx and K^+ efflux in transformed cells. These changes reach a steady state prior to the increase in passive membrane permeability for normally impermeant molecules [92]. In addition, a 35% reduction in the concentration of intracellular ATP is detected, associated with an increase in the levels of ADP and AMP [92]. This response also occurs prior to detection of aqueous channel formation. The massive hydrolysis of intracellular ATP is not entirely due to activation of the (Na^+,K^+)ATPase in response to the influx of Na^+ since ouabain, a specific inhibitor of this plasma

membrane enzyme, acts synergistically with ATP to induce permeabilization and the early effects associated with this process (*see below*).

Exogenous ATP also causes a significant reduction in plasma membrane potential, concomitant with activation of the Na^+/K^+ flux and reduction of intracellular ATP [31, 92]. Alterations in membrane potential were measured using the lipophilic cation, tetraphenylphosphonium (TPP^+) [54]. This compound distributes according to the total cellular potential so that one must be cautious in concluding that alterations in TPP^+ distribution represent a reduction of the plasma membrane component of the total potential. Fortunately, other evidence also indicates that a decrease in membrane potential is involved in permeabilization. Partial replacement of medium Na^+ with K^+, which specifically lowers plasma membrane potential [54], acts synergistically with exogenous ATP to induce aqueous channel formation in transformed cells [31].

Other evidence supports the role of monovalent cation fluxes in the mechanism of ATP-dependent permeabilization. For example, ouabain serves to enhance the effect of exogenous ATP. Concentrations of ouabain and ATP, which by themselves are ineffective, induce permeabilization when added simultaneously [92]. This differs from the synergism observed with inhibitors of respiration and oxidative phosphorylation because ouabain does not reduce the level of intracellular ATP in the concentrations used. Ouabain alone increases the level of intracellular Na^+ and causes a loss of K^+ from transformed, 3T6, cells as well as from untransformed, 3T3, cultures. However, 3T3 cells do not become permeabilized, even in the presence of ouabain [92].

Exogenous ATP causes a rapid increase in the volume of 3T6 cells (G. Weisman, *unpublished results*) as measured by changes in the distribution of [^3H] 3-0-methyl glucose. This swelling is presumably in response to the ATP-dependent ion fluxes just described. The swollen cells recover their original volume after 5 min due to induction of aqueous channels, which occurs with a 3–5 min lag. The ATP-dependent swelling has been seen in other cell lines (*see* last section) and swelling also occurs under conditions that increase sensitivity to exogenous ATP, including replacement of extracellular Na^+ with K^+ and an increase in extracellular pH. We have found that swelling is not essential for the process of ATP-dependent permeabilization. Cells swollen by incubation in a medium of low ionic and osmotic strength show greatly increased sensitivity to exogenous ATP. Hypotonic swelling can be prevented by addition of *either* NaCl or sucrose to maintain isotonic conditions. However, only NaCl reduces sensitivity to exogenous ATP; addition of sucrose has no effect. Furthermore, cell volume can be reduced by addition of either NaCl or sucrose to obtain hypertonic conditions. Permeabilization by 125 μM ATP was inhibited in the medium of high ionic strength but hyperosmotic conditions obtained with sucrose did not affect the process of permeabilization (*unpublished observations*). Thus, it appears that permeabilization is affected in direct relation to the ionic strength and not the osmolarity of the medium. Changes in ionic strength are known to modify the electrostatic surface potential of membranes [5, 63], which may relate to the effects of ionic strength on transformed cells.

The permeabilization process has been characterized further. The mechanism of ATP-dependent channel formation is anion-selective, preferring chloride ion. Nitrate is much less effective and replacement of chloride in the medium by thiocyanate prevents permeabilization by external ATP. These results suggest that activation of a bidirectional membrane channel transporting Na^-, K^+ and Cl^- is required to induce membrane permeability for normally impermeant compounds. Reports of a membrane cotransporter with similar specificities [7, 33, 35, 38, 87] led us to test diuretics such as furosemide and bumetanide, which are known to be inhibitors of this cotransporter. Both furosemide and bumetanide were found to inhibit permeabilization (G. Weisman, *unpublished observations*). However, $^{86}Rb^+$ uptake, taken as a measure of Na^+, K^+, Cl^- cotransporter activity, was sensitive to furosemide at 1/4 the concentration needed to inhibit ATP-dependent permeabilization. In addition, anion selectivity was somewhat more rigid in the studies on $^{86}Rb^+$ uptake. These results indicate that if the Na^+, K^+, Cl^- cotransporter is involved in permeabilization its properties have been significantly altered in the presence of exogenous ATP.

There is yet another aspect to the mechanism of ATP-dependent permeabilization of transformed cells. The divalent cation, Ca^{2+}, appears to have a specific inhibitory effect which is not shared by Mg^{2+}. First of all, Ca^{2+}, but not Mg^{2+} is able to seal permeabilized cells at an alkaline pH, although both cations function at a neutral pH [59]. Furthermore, it was found that La^{3+} and Tb^{3+} inhibit permeabilization when present at one-fifth the concentration of external ATP [26, 59]. These trivalent cations have been reported to replace Ca^{2+} in modulating many enzymatic reactions [32, 40, 79, 91]. They form a 1 : 1 complex with ATP over a wide range of ATP concentrations [85, 88] and on this basis their inhibitory effect cannot be explained simply as a result of reducing the concentration of free ATP, the active agent in permeabilization. Assignment of

a specific role for Ca^{2+} itself as an inhibitor of permeabilization is complicated by the fact that the concentration required approaches that needed to chelate most of the ATP.

Other evidence suggests a role for the Ca^{2+}-calmodulin complex in the regulation of permeabilization by external ATP. Antagonists of this complex, such as trifluoperazine, chlorpromazine and W-7 [41] were found to enhance the effect of external ATP in 3T6 cells [26]. This occurs without a reduction in the concentration of intracellular ATP, as is the case for synergism seen with energy inhibitors mentioned earlier in this review. It is known that some effects of these drugs may be due to nonspecific membrane interactions which are independent of Ca^{2+}-calmodulin antagonism. Evidence against this possibility in regard to permeabilization was obtained with W-5, a compound similar to W-7 in structure and hydrophobic properties. W-5 does not inhibit Ca^{2+}-calmodulin requiring reactions [41] and, in contrast to W-7, does not enhance the effect of external ATP [26].

The results detailed above suggest the following working hypothesis for the mechanism of ATP-dependent permeabilization in transformed cells: External ATP interacts with a plasma membrane receptor, which results in the activation of specific ion channels, leading to a massive loss of K^+ and entry of Na^+, and a decrease in the plasma membrane potential. These effects directly induce plasma membrane channels for nucleotides and other impermeant small molecules. Solid evidence for an extracellular ATP receptor involved in the permeabilization process has not yet been obtained. However, in preliminary experiments it was observed that when 3T6 cells were treated with ^{32}P-azido ATP and ultraviolet light under conditions similar to those used in permeabilization experiments, only a few membrane proteins were labeled. The role of these proteins in permeabilization is currently being investigated.

Another interesting development concerns the effect of dithiothreitol on ATP-dependent permeabilization. It was found that brief incubation of 3T6 cells in growth medium containing 1–3 mM dithiothreitol completely prevented subsequent ATP-dependent permeabilization. This effect of dithiothreitol was reversed in only 10 min by washing the monolayer of cells and then incubating them with oxidized glutathione. The working hypothesis was that a membrane protein involved in permeabilization contains a critical disulfide bond susceptible to reduction by dithiothreitol and reoxidation by oxidized glutathione. Strong support for this hypothesis resulted from experiments with glutathione maleimide, which has been shown to be an impermeant alkylating agent, unable to cross the plasma membrane [1, 8, 37]. This compound rendered the dithiothreitol-induced inhibition of permeabilization insensitive to reversal by oxidized glutathione (G. Weisman, *unpublished results*). Synthesis of [^{35}S]-labeled glutathione-maleimide was carried out in the hope of localizing the site of interaction, but material obtained so far was not of sufficiently high specific activity to give convincing results. It happens that the effect of dithiothreitol is inhibited in media of high ionic strength in exactly the same way as ATP-dependent permeabilization and the two phenomena have similar pH requirements. These results encourage us to consider that dithiothreitol and ATP may react with the same site on the plasma membrane, a site which might be identified by means of a radioactive label.

External ATP has a direct effect on the topology of the plasma membrane. Freeze fracture electron micrographs of plasma membranes from 3T6 cells treated for 3 min with ATP show markedly different characteristics when compared with untreated controls (Fig. 3). Thus, it may be that ATP interacts with an extracellular receptor to cause gross physical changes in the plasma membrane that induce the permeabilization process.

Effect of External ATP on Certain Untransformed, Secreting Cells

Certain untransformed cells with a secretory function respond to extracellular ATP. The most extensive investigations have been carried out with mast cells, which release histamine when incubated with ATP in the presence of Ca^{2+} [27]. It was shown that extracellular ATP induced an influx of radioactively labeled Na^+ in isolated rat mast cells [23] and caused the cells to swell [10, 84]. In later work [25], it was concluded that exogenous ATP increases the permeability of the mast cell membrane for Na^+ and K^+, and that these changes are necessary but not sufficient for histamine release.

A thorough analysis of the action of external ATP on mast cells was carried out by Cockcroft and Gomperts [19–21]. In agreement with earlier work [24], they found that the effective agonist form of ATP is ATP^{4-}. Thus, it would correspond to the very small concentration of ATP that is not complexed to divalent cations in most physiological media. The secretion of histamine is optimal at 2 μM ATP^{4-} and this concentration of ATP^{4-} also causes increased incorporation of [^{32}P]P_i into phosphatidylinositol. Higher concentrations (above 2.9 μM) of ATP^{4-} cause inhibition of secretion and phosphatidylinositol labeling and permit leakage of ^{32}P-labeled metabolites. These effects of ATP are spe-

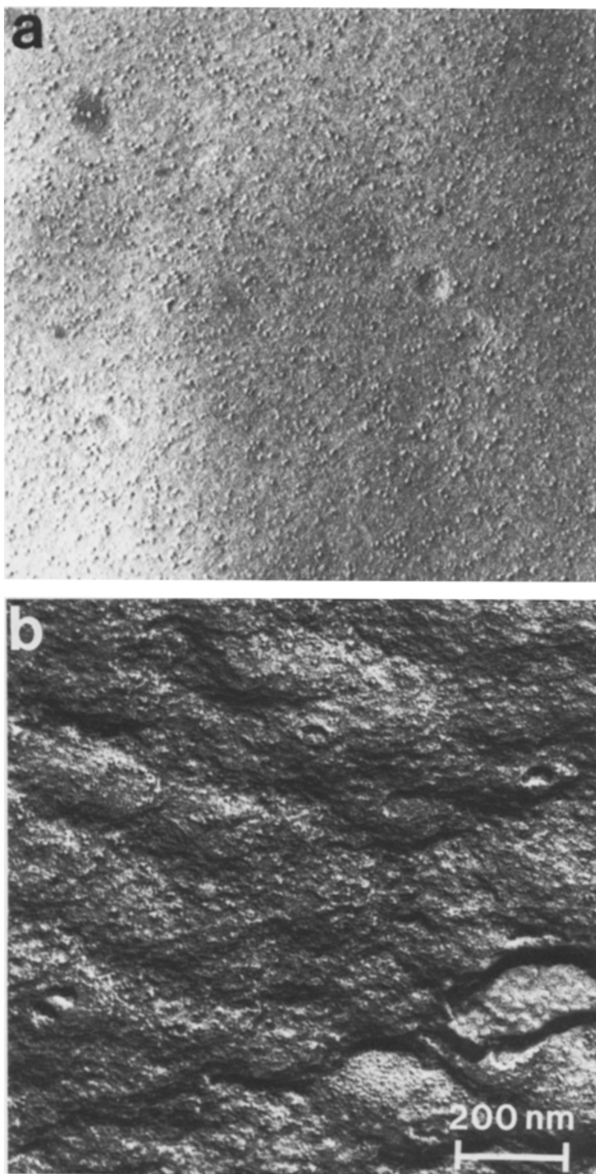

Fig. 3. 3T6 cells were washed and incubated in medium I for 7 min at 37°C as described in Fig. 1. Then, 150 μM ATP was added as indicated for 3 min at 37°C, and the cells were washed with medium I and chilled to 0°C. Freeze-fracture samples of the plasma membranes were prepared, and carbon-platinum replicas of the fractured membrane were examined by transmission electron microscopy. These preparations were made by Maria Toivio-Kinnucan, Auburn University. The freeze-fracture patterns show a normal plasma membrane morphology when prepared from 3T6 cells incubated in the absence of ATP. When cells were incubated in the presence of ATP, the freeze fracture patterns are dramatically altered, showing large 200 nm invaginations in the plane of the membrane. Such patterns are also seen in freeze-fracture patterns from membranes undergoing blebbing or when cells are treated with cytoskeletal disrupting agents. (*a*) Absence of ATP. (*b*) Presence of ATP

cific. The analogue adenylylimido diphosphate was without activity, as were CTP, GTP, ADP and adenosine [19]. In a later study [21] eighteen additional analogues of ATP were tested. Only 2′-deoxy ATP, 9-β-D-arabinofuranosyl ATP, [$\beta\gamma$-thio] ATP and ATP itself were found to stimulate histamine secretion and to permit leakage of ^{32}P-labeled metabolites. For three of the agonists that were examined in both systems, the concentrations required to cause metabolite leakage and to inhibit histamine secretion appear to coincide. Accordingly, it was suggested "that these are both manifestations of a common cellular phenomenon initiated by ligands binding to the ATP^{4-} receptor" [21]. Histamine release and the labeling of phosphatidylinositol are dependent on extracellular Ca^{2+} while metabolite release is not Ca^{2+} dependent. The permeabilization of transformed cells by exogenous ATP, discussed above, appears to be analogous to Ca^{2+}-independent release of intracellular metabolites by ATP^{4-} in mast cells.

The ATP-dependent leakage of metabolites in mast cells is rapidly reversed upon adding Mg^{2+} [36]. Fluorescence measurements using 3,3′-diethylthiadicarbocyanine iodide [90] indicate that plasma membrane potential, abolished by external ATP, recovers following sealing by Mg^{2+}. Using this procedure it was possible to seal in non-hydrolyzable GTP analogues and to demonstrate their role in secretion [36]. The nuclear stain, ethidium bromide, is able to permeate ATP-treated cells and can be used as a probe to detect membrane permeability lesions. The ATP-induced permeabilization of mast cells can also be used to allow entry of ^{45}Ca-EDTA [9]. Under these conditions secretion of histamine is controlled by manipulating Ca^{2+} between 1–20 μM.

Other Effects of External ATP

Studies on ATP-dependent permeabilization have thus far been limited to relatively few transformed cell lines. However, many investigations have been carried out concerned with a variety of other effects of exogenous ATP on transformed and untransformed cell cultures, freshly isolated mammalian cells and different kinds of tissue preparations. ATP and certain related compounds have been reported to be neurotransmitters and a substantial literature exists dealing with purinergic receptors on excitable membranes involved in physiological processes. This field has been adequately reviewed [14, 15] and

will not be covered here. Ecto-ATPases and ecto-protein kinases have also been described (*see*, for example, [16, 17, 44, 51, 52, 61, 70, 76]), suggesting a role for exogenous ATP. In this section we will be mainly concerned with effects of external ATP on ion fluxes across the plasma membrane.

Some years ago it was reported that external ATP caused swelling of TA3 ascites tumor cells [34, 83] and fibroblasts [43]. The ATP-induced volume changes are associated with increased ion permeability in TA3 cells [39], kidney tubules [71] and red blood cells [29, 65, 69, 77]. Similar effects on ion permeability were observed in HeLa cells [3], NN astrocytes, N-18 neuroblastoma, GL-26 glioblastoma and L-929 fibroblasts [86], myoblasts and myotubes [49], and hepatocytes [13]. Other studies indicate that 0.1 mM ATP causes a 20-fold stimulation in the rate of K^+/K^+ exchange in HeLa cells [4] and a stimulation of Cl^- secretion in MDCK cells [81]. External ATP (3.5 mM) also causes selective enhancement of plasma membrane permeability to Na^+ relative to K^+ in mouse neuroblastoma Neuro-2A cells, as measured by electrophysiological techniques [89]. ATP-regulated K^+ channels have been reported in cardiac muscle [62] and hepatocytes [13]. Quite recently it was observed that mouse fibroblastic L cells responded to exogenous ATP ($\geq$0.2 mM) with a transient hyperpolarization due to increased membrane permeability to K^+ [64]. However, intracellular injection of up to 3 mM ATP produced no detectable changes in the membrane potential. Extracellular ADP was also effective in these studies, suggesting a role for P2 purinergic receptors in this phenomenon. The authors speculate that ATP (or ADP), which are known to be released from aggregated platelets and injured cells, are regulatory mediators for activation of the mechanism of wound healing in fibroblasts. In fact, ATP is also released from hypoxic cells [18, 30], stimulated nerve cells [2, 42, 80], constricting muscle [11, 12] and electrically stimulated brain slices [56, 68].

External ATP has been reported to affect the Ca^{2+} permeability of the plasma membrane. Uptake of Ca^{2+} in the presence of Mg^{2+} and ATP has been observed in chick embryo fibroblasts [66]. Exogenous ATP also enhances Ca^{2+} uptake in intact thymocytes [55], kidney tubules [71], Ehrlich ascites tumor cells [53], and MDCK cells [82]. Incubation with ATP has been reported to increase the concentration of cAMP in intestinal epithelial cells [50], P388 mouse lymphoma cells [6], adipocytes [22], and fibroblasts [94]. External ATP can also induce shape changes in erythrocyte ghosts [78] and can immobilize amoebas [67]. These results taken together indicate that effects of exogenous ATP are widespread, although the physiological significance has not been established in every case.

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Received 15 April 1985