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Inositol 1,4,5-Trisphosphate-induced Increase in Ca^{2+}-ATPase Activity in the Microsomes of Tracheal Epithelial Cells

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=ABSTRACT=

Membrane vesicles were prepared by differential centrifugation from epithelial cells of porcine trachea. Total activity of microsomal ATPases was measured spectrophotometrically by a coupled enzyme assay. The steady-state activity of the enzyme was 329±10 nmol/min·mg protein. Thapsigargin, a specific antagonist of intracellular Ca^{2+}-ATPase, inhibited about 50% of the activity, leaving 178±18 nmol/min·mg protein (n=6), indicating that the Ca^{2+}-ATPase is one of the major microsomal ATPases. The microsomes used in this study appeared to be tight-sealed vesicles since they showed saturation in $^{45}Ca^{2+}$ uptake experiments. Inositol 1,4,5-trisphosphate ($InsP_3$, 4 μM), an agonist of $InsP_3$-sensitive Ca^{2+} release channel ($InsP_3$ receptor), and Ca-ionophore A23187 (10 μM) induced $^{45}Ca^{2+}$ releases of 20% and 50% of stored $^{45}Ca^{2+}$, respectively. The addition of 10 μM $InsP_3$ also increased the microsomal ATPase activity from 282±8 nmol/min·mg protein to 334±21 nmol/min·mg protein in the intact vesicles. Similar increase in the activity was observed by making microsomes leaky (uncoupling) using the Ca-ionophore A23187. $InsP_3$-induced effects were blocked by either thapsigargin or heparin suggesting that: 1) the $InsP_3$-induced increase in ATPase activity is mediated by microsomal Ca^{2+}-ATPase, and 2) dissipation of Ca^{2+} gradient across the microsomal membrane is responsible for the $InsP_3$-induced effect. In order to test the dependence of the Ca^{2+}-ATPase activity on the activity of $InsP_3$ receptors, the activity of ATPases was monitored in various concentrations of free Ca^{2+} using EGTA-Ca^{2+} buffers. The Ca^{2+}-dependent biphasic change is the well-known character of $InsP_3$ receptor but not of microsomal Ca^{2+}-ATPase in non-excitable cells; however, the activity of microsomal ATPase appeared biphasic and a maximal activity of 397±36 nmol/min·mg protein was obtained in the solution containing 100 nM free Ca^{2+}. Below or above this concentration, the activity of ATPases was lower. These results strongly support a positive correlation of microsomal Ca^{2+}-ATPases to the $InsP_3$ receptors in epithelial microsomes.

Key Words: Porcine tracheal epithelium, Ca^{2+}-ATPase, $InsP_3$ receptor, Coupling, $^{45}Ca^{2+}$ uptake and release

INTRODUCTION

Epithelial cells in the airway play a major role in transepithelial secretion and mucociliary clearance which are necessary functions for a fundamental lung defence mechanism (Wanner, 1977). Coordination of the cellular activity of these multicellular system is required for the defence mechanism and is often mediated by intercellular communication

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through the gap junctional connections (Spray & Bennet, 1985). The signals that pass between cells remain poorly-defined although both Ca^{2+} and inositol 1,4,5-trisphosphate ($InsP_3$) are potential candidates (Saez et al, 1989). Both Ca^{2+} and $InsP_3$ also play a major role as intracellular messengers in these cells (Boitano et al, 1992; Lansley et al, 1992). In this respect, the airway epithelium can be used as an excellent model to study the intracellular communication as well as intercellular communication.

When a tracheal epithelium is stimulated, a wave of increase in intracellular Ca^{2+} spreads out across the stimulated cell in the form of an intracellular Ca^{2+} wave (Sanderson et al, 1990). The intracellular Ca^{2+} wave is initiated successfully in the Ca^{2+}-free medium suggesting that the Ca^{2+} is released from the intracellular Ca^{2+} stores. These cells indeed have intracellular Ca^{2+} stores which are activated by receptor-mediated activation of an $InsP_3$-second messenger system (Hansen et al, 1995). Ca^{2+} stores have both $InsP_3$ receptor channels which release the stored Ca^{2+} into cytoplasm and Ca^{2+}-ATPases (organellar Ca^{2+} pumps) which sequester the cytoplasmic Ca^{2+} into the lumen of Ca^{2+} stores by the expense of ATP. Boitano et al (1992) have successfully demonstrated that the intracellular Ca^{2+} wave is mediated by $InsP_3$-induced Ca^{2+} release from the $InsP_3$-sensitive Ca^{2+} stores. They were able to inhibit the intracellular Ca^{2+} wave: 1) by the injection of heparin, an antagonist of the $InsP_3$ receptor, and 2) by the depletion of $InsP_3$-sensitive Ca^{2+} stores with a specific Ca^{2+}-ATPase inhibitor, thapsigargin.

In order to characterize the intracellular Ca^{2+} stores, we have prepared microsomes from porcine airway epithelia and investigated ionic transport across the microsomal membrane. We have found a positive correlation in the activities of the Ca^{2+}-ATPase and the $InsP_3$ receptor. The activation of $InsP_3$ receptors increased the activity of Ca^{2+}-ATPases in intact microsomes only and this was not observed in leaky microsomes. These findings suggest that the pumping activity of the Ca^{2+}-ATPase is increased by the efflux of luminal Ca^{2+} through the $InsP_3$ receptors.

METHODS

Materials

$^{45}CaCl_2$ was purchased from Du Pont-NEN Research Products (Boston, MA, USA). Triton X-100 and $MgCl_2$ were supplied by Wako Pure Chemical LTD (Japan). All other chemicals, drugs, and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Porcine tracheas of freshly killed pigs were generously supplied by a local slaughterhouse in Cheongju, Chungbuk.

Preparation of microsomes from the epithelial cells of porcine trachea

The trachea was slit longitudinally and the mucosa was dissected from its cartilaginous backing. The mucosa was ground by a food processor in a solution containing; 130 mM NaCl, 5 mM KCl, 25 mM Hepes (pH 7.2), 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Glucose. The homogenate was filtered through three layers of cheesecloth and the filtrate was centrifuged at 3,000 rpm ($1,500\times g$). The supernatant was centrifuged again for 30 minutes at 10,000 rpm ($11,000\times g$) to remove the mitochondrial fractions and cell debris. The suspension of high speed centrifugation was centrifuged twice for 1 hour at 24,000 rpm in a type TFT70.38 rotor (Kontron Instruments, Milan, Italy). The pellet containing membrane vesicles was resuspended in 0.3 M sucrose, 100 mM KCl, 5 mM Na-Pipes, pH 6.8, frozen in liquid nitrogen, and stored at -80℃ before use. The concentration of protein was determined by the Lowry method (Lowry et al, 1951).

Measurement of ATPase activity

ATPase activity of microsomal vesicles from

porcine airway was measured by the method of Niggli et al (1979). Briefly, the activity was monitored in a solution containing 120 mM KCl, 30 mM Hepes, pH 7.4, 1 mM $MgCl_2$, 0.5 mM ATP, 50 μM $CaCl_2$, 0.4 mM NADH, 2 mM phosphoenolpyruvate, 1 IU/ml pyruvate kinase, and 1 IU/ml lactate dehydrogenase. The formation of ADP by Ca^{2+}-ATPase activity is quantitatively coupled to the oxidation of NADH, and the oxidation of NADH in the reaction solution decreases the absorbance at 340 nm. The absorbance at 340 nm was continuously monitored and the activity was calculated from the slope of the decrease in absorbance. Although mitochondrial contamination was minimized by differential centrifugation, ATPases of contaminated mitochondria were inhibited by potassium cyanide treatment during the analysis.

Ca^{2+}-dependence of Ca^{2+}-ATPase activity

The Ca^{2+}-dependence of microsomal ATPases was measured in solutions containing various concentrations of free Ca^{2+}. The concentration of free Ca^{2+} was determined by the addition of various amounts of EGTA and calculated by using a computer program that used the stability constants of Fabiato (1988). To determine the activity of ATPases in leaky microsomal preparations, either Triton X-100 (0.001%) or the Ca-ionophore A23187 (10 μM) was added to permeabilize the microsomal membrane, and the activity was measured in a similar way.

Measurement of microsomal $^{45}Ca^{2+}$ uptake

$^{45}Ca^{2+}$ uptake was performed in a solution containing 120 mM KCl, 30 mM Hepes, pH 7.4, 1 mM $MgCl_2$, 0.5 mM ATP, 1.83 μM $^{45}CaCl_2$ and 50 μM $CaCl_2$. The radioactivity of $^{45}Ca^{2+}$ uptaken by the microsomes was determined by the filtration method (Valdivia et al, 1992). Calcium uptake was initiated by the addition of 1 mM ATP. The microsomes were washed on a Whatman filter (GF/B). The washing solution contained 10 mM Ca^{2+} to block the release of Ca^{2+} through the $InsP_3$ receptors in the microsomal membrane.

RESULTS

Effect of thapsigargin on microsomal ATPases

The decrease in absorbance at 340 nm represents the steady-state activity of the microsomal ATPases shown in Fig. 1A. The absorbance in the control experiment decreased linearly for 10 minutes, until NADH in the reaction solution was depleted. The activity was calculated from the slope of the decrease. When 10 μM thapsigargin was added, the rate of decrease in the absorbance was reduced. The

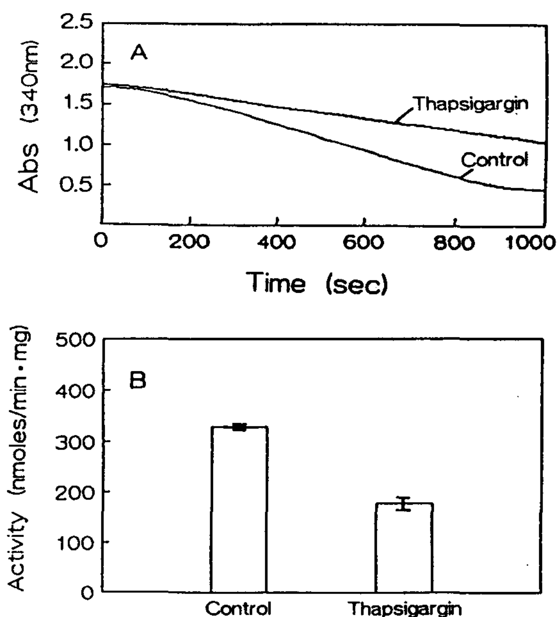

Fig. 1. Effects of thapsigargin on the microsomal ATPases. (A) The total ATPase activity was monitored by the decrease in absorbance at 340 nm in the control solution (see Methods). The effect of thapsigargin (10 μM) on ATPase activity was compared with that of the control experiment. (B) The average total ATPase activity with and without thapsigargin. The ATPase activity is expressed as the time-dependent decrease in the concentration of NADH per milligram of microsomal protein.

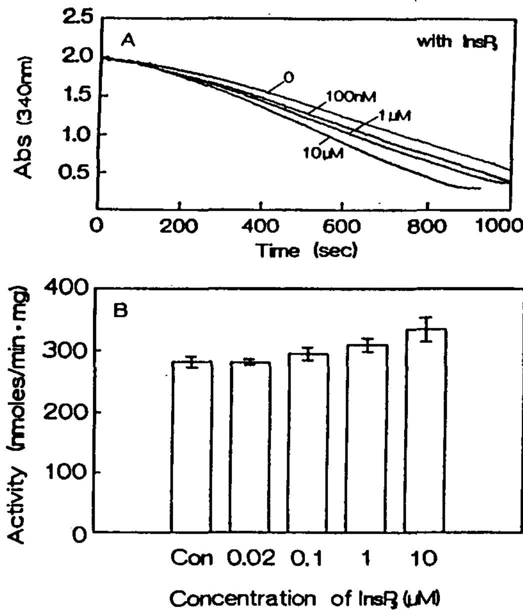

Fig. 2. $InsP_3$ increases the total activity of microsomal ATPases. (A) Effect of $InsP_3$ on the microsomal ATPase activity measured by absorbance changes at 340 nm. (B) The average activities with various concentrations of $InsP_3$ from 20 nM to 10 μM. The activities are expressed with standard deviations calculated from 3-5 experiments.

average activities in the control experiment and in the experiment with thapsigargin were 329±10 nmol/min.mg protein (n=10) and 178±18 nmol/min.mg protein (n=6), respectively (Fig. 1B).

$InsP_3$-dependent increase in microsomal Ca^{2+}-ATPase activity

In order to understand the functional relation between the Ca^{2+}-ATPase and the $InsP_3$ receptor on the microsomal vesicles, we measured the effect of $InsP_3$ on the activity of microsomal Ca^{2+}-ATPase using a preparation of intact vesicles. $InsP_3$ increased the activity of microsomal ATPases in Ca^{2+} concentrations above 100 nM (Fig. 2). The activities were 282±8, 281±7, 296±11, 310±12, and 334 ±20 nmol/min.mg protein in $InsP_3$ concentrations of 0 nM, 20 nM, 100 nM, 1 μM, and 10 μM,

Table 1. Activities of microsomal ATPases in various conditions

	Control	$InsP_3$[a]	Thapsigargin[b] +$InsP_3$[a]
Intact microsomes	329±20[c]	371±13	165±19
Leaky microsomes[d]	377±22	373±7	149±10

a: Inositol 1,4,5-trisphosphate, 4 μM, b: Thapsigargin, 10 μM, c: Unit of the activity; nmoles/min.mg protein, d: Microsomes treated with Ca-ionophore A23187 (10 μM), Data expressed with standard deviation (n=3~5).

respectively. The effect of $InsP_3$ was dose-dependent and 10 μM $InsP_3$ increased the activity about 20%.

To determine whether $InsP_3$-induced ATPase activity is due to the activation of the microsomal Ca^{2+}-ATPase, we investigated the effect of thapsigargin on the microsomal ATPases. $InsP_3$ (4 μM) increased ATPase activity by ~13% in intact microsomes (Table 1). However, thapsigargin decreased the activity to ~45%, as shown in Fig. 1, and the simultaneous addition of $InsP_3$ did not increase the enzyme activity at all. Similar results were observed in leaky microsomes. The activity of microsomal Ca^{2+}-ATPase was uncoupled by making microsomes leaky with 10 μM Ca-ionophore A23187. The increase in ATPase activity was ~15% in the Ca-ionophore-treated microsomes and $InsP_3$ did not further increase the activity. These results indicate that the $InsP_3$-induced increase in the ATPase activity is mediated by the microsomal Ca^{2+}-ATPase.

The idea of $InsP_3$-induced activation of the microsomal Ca^{2+}-ATPase is supported by the results, as shown in Fig. 3., which verify that the microsomes used in these experiments are tight-sealed vesicles. Efflux of luminal Ca^{2+} was measured by $^{45}Ca^{2+}$ uptake and release experiments. The time course of $^{45}Ca^{2+}$ uptake is shown in Fig. 3. The uptake was measured by a filtration method after incubations of 5, 15, 30, 60, 300, 600 seconds. Uptake of $^{45}Ca^{2+}$ appeared to be biphasic; a rapid

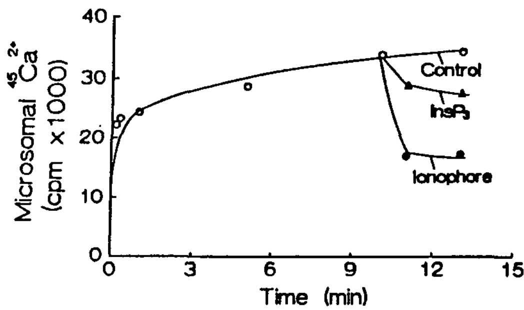

Fig. 3. Time course of $^{45}Ca^{2+}$ uptake and release. The uptake experiment was done in a control solution containing 50 μM Ca^{2+} and 1.83 μM $^{45}Ca^{2+}$. The microsomes were washed by filtering through a Whatman filter (GF/B). The washing solution contained 10 mM Ca^{2+} to block the $^{45}Ca^{2+}$ release during the washing procedure. Mitochondrial ATPases were inhibited by cyanide treatment. After 10 minutes of uptake either InsP$_3$ (4 μM) or Ca-ionophore A23187 (10 μM) was added. The data represent 5 different experiments.

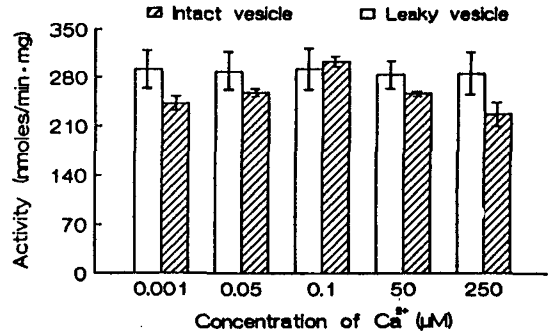

Fig. 4. The average activities of microsomal ATPases at various concentrations of Ca^{2+}. Each activity was measured in the presence of 4 μM InsP$_3$. Ca^{2+}-dependence of ATPase activity in the intact microsomes is shown slashed. Concentration of free Ca^{2+} was adjusted by the addition of EGTA and calculated by a computer program that used the stability constants of Fabiato (1988). The average activities of microsomal ATPase in the leaky microsomes are shown blank.

uptake followed by a slow uptake. The rapid uptake of 80% saturation was achieved within 1 minute and it took about 10 minutes for full saturation. Release of stored $^{45}Ca^{2+}$ was measured after 10 minutes of uptake in the presence of 4 μM InsP$_3$ or 10 μM Ca-ionophore A23187. The ionophore released ~50% of the stored $^{45}Ca^{2+}$ and 4 μM InsP$_3$ released ~20%. Since InsP$_3$ released the microsomal $^{45}Ca^{2+}$ by activating InsP$_3$-sensitive Ca^{2+} release channels, significant parts of microsomes must have originated from the intracellular Ca^{2+} stores which contain InsP$_3$ receptors as well as Ca^{2+}-ATPases.

Ca^{2+}-dependence of InsP$_3$ effect

The effect of InsP$_3$ on the microsomal Ca^{2+}-ATPase was measured in the solutions containing various concentrations of free Ca^{2+} shown in Fig. 4. If there are any functional connections between Ca^{2+}-ATPases and InsP$_3$ receptors, we might be able to see the Ca^{2+}-dependence of microsomal Ca^{2+}-ATPases in only intact microsomes because of the Ca^{2+}-dependence of InsP$_3$ receptor. When the concentration of free Ca^{2+} in the reaction solution was changed from 1 nM to 250 μM, the activity of Ca^{2+}-ATPase in the intact microsomes appeared biphasic as expected. The activities were 242±11, 257±5, 303±8, 257±4, and 226±17 nmol/min·mg protein in free Ca^{2+} concentrations of 1 nM, 50 nM, 100 nM, 50 μM and 250 μM, respectively. The maximal activity of 303±8 nmol/min·mg protein was obtained in the medium containing 100 nM free Ca^{2+}. Below or above this concentration the activity was decreased and it was decreased to ~70% of the maximum at 250 μM Ca^{2+}. To make sure that the Ca^{2+}-dependence of the microsomal Ca^{2+}-ATPase is not an endogenous character of the Ca^{2+}-ATPase itself, the microsomes were treated with the Ca-ionophore A23187 (10 μM), and the microsomal membrane was permeabilized to Ca^{2+}. The activities in the leaky microsomes were 291±31, 288±28, 292±34, 284±21, and 286±35 nmol/min·mg protein in free Ca^{2+} concentrations of 1 nM, 50 nM, 100 nM, 50 μM, and 250 μM, respectively. The Ca-ionophore completely removed the Ca^{2+}-depend-

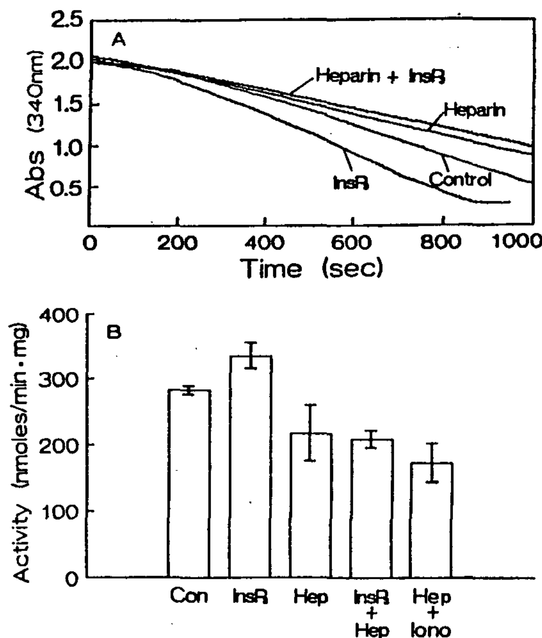

Fig. 5. Effect of heparin on the $InsP_3$-induced increase in the Ca^{2+}-ATPase activity. (A) The time courses of absorbance change in different conditions. The concentrations of $InsP_3$ and heparin were 4 μM and 100 μg/ml, respectively. (B) The average activities measured in various conditions. The concentrations of $InsP_3$, heparin, and Ca-ionophore were 4 μM, 100 μg/ml, 10 μM, respectively. Standard deviations were calculated from 3-5 experiments.

ence of Ca^{2+}-ATPase activity and the specific activity was independent of extravesicular Ca^{2+} concentration.

Heparin effects on the microsomal Ca^{2+}-ATPase

If the effect of $InsP_3$ on the microsomal Ca^{2+}-ATPases is due to the activation of the $InsP_3$ receptors in the intact microsomes, the $InsP_3$ effect will be blocked by heparin. In Fig. 5, $InsP_3$ increased the ATPase activity from 282 ± 8 nmol/min·mg protein to 334 ± 20 nmol/min·mg protein; however, heparin (100 μg/ml) decreased the activity to 217 ± 46 nmol/min·mg protein (Fig. 5B, Hep). The stimulatory effect of $InsP_3$ was not observed in the presence of heparin, and the activity in this condition was 209 ± 15 nmol/min·mg protein, very similar to the value obtained with only heparin (Fig. 5B, Hep+$InsP_3$). Heparin further decreased the activity in the Ca-ionophore-treated leaky vesicles to 174 ± 30 nmol/min·mg protein (Fig. 5B, Hep+Iono).

DISCUSSION

In this study we have shown that extravesicular $InsP_3$ increases the activity of microsomal Ca^{2+}-ATPase in intact microsomes. Since $InsP_3$ binds to the $InsP_3$ receptor and there is no evidence that $InsP_3$ directly activates the microsomal Ca^{2+}-ATPase, the simplest model to explain the $InsP_3$-induced effect is the efflux of luminal Ca^{2+} through the Ca^{2+} release channel, dissipation of Ca^{2+} gradient, and increase in the Ca^{2+} pump activity. This model was supported by the results obtained with thapsigargin and heparin. Thapsigargin and heparin blocked the $InsP_3$-induced activation of Ca^{2+}-ATPase in the intact vesicles, as shown in Table 1 and Fig. 5 respectively.

Thapsigargin blocked the total ATPase activity more than 50%, suggesting that more than 50% of total ATPase activity was mediated by microsomal Ca^{2+}-ATPases (Fig. 1 and Table 1). The major part of total activity, thapsigargin-insensitive activity, still existed (~40% after the inhibition). This residual activity may be explained by following: 1) the microsomes used in this experiment may contain other types of ATPases, and 2) thspsigargin may not completely block the activity of microsomal Ca^{2+}-ATPase in tracheal epithelial cells. Existence of other ATPases is possible because the activity of microsomal ATPase is still high even at 1 nM Ca^{2+}, as shown in Fig. 4A. The characteristics of epithelial Ca^{2+}-ATPases are poorly known in tracheal cells; however, purified intracellular Ca^{2+}-ATPase from muscle cells has almost no activity at this Ca^{2+} concentration (Sasaki et al, 1992; Hughes et al,

1994). Although thapsigargin completely blocks the activity of sarcoplasmic reticulum Ca^{2+}-ATPases in muscle cells (Lytton et al, 1991; Wictome et al, 1992), it may have less effect on the Ca^{2+}-ATPases from nonexcitable cells (Ozawa et al, 1995).

The results shown in Fig. 3 suggest that the microsomes in our preparation are tight-sealed vesicles. $InsP_3$ released ~20% of stored $^{45}Ca^{2+}$ and this would increase the pump activity. $InsP_3$-induced uncoupling will be maximal in the leaky microsomes and, if the model is adequate, the activity of ATPases will be also maximal in the leaky microsomes. Interestingly, $InsP_3$ increased the ATPase activity by as much as the activity shown in the leaky microsomes (Table 1). This seems to suggest that all the microsomes originated from the $InsP_3$-sensitive Ca^{2+} stores; however, we have found that tracheal epithelial cells have another type of major Ca^{2+} store, known as caffeine-sensitive Ca^{2+} stores (Kim et al, 1996). These two types of Ca^{2+} stores appeared to be physically independent because $^{45}Ca^{2+}$ release from these two stores was additive. This observation leaves us to answer the following questions: what part of total $^{45}Ca^{2+}$ release is mediated by the caffeine-sensitive Ca^{2+} stores in tracheal cells, and how this release contributes to the increase in the Ca^{2+}-ATPase activity.

We may be able to explain the contribution of microsomes from the caffeine-sensitive Ca^{2+} stores to the total activity of Ca^{2+}-ATPase in the following way: since the activity of the caffeine-sensitive Ca^{2+} release channel (ryanodine receptor) is dependent on the Ca^{2+} concentration and is maximal at 0.1 to 1 μM Ca^{2+}, the caffeine-sensitive channel would have some basal activity under our control conditions. This would release luminal Ca^{2+} quite enough for a microsome to be considered leaky. If the microsomes from caffeine-sensitve Ca^{2+} stores become leaky, there will be no contribution to the total pump activity because the Ca^{2+}-ATPase activity will be already maximal in these microsomal fractions. This argument is supported by there being no significant increase in the pump activity with application of caffeine to intact microsomes (unpublished observation).

Heparin decreased ATPase activity below the control level in the leaky microsomes and $InsP_3$ did not further increase the activity in the presence of heparin (Fig. 5). There are two possible explanations of the heparin effect. In the first place, the $InsP_3$ receptor may have endogenous activity and heparin may inhibit this activity; secondly, heparin may block the microsomal Ca^{2+}-ATPase as well as $InsP_3$ receptor. The first idea is less plausible because no endogenous activity of $InsP_3$ receptor has been reported without $InsP_3$ (Champeil et al, 1989; Finch et al, 1991; De Young and Keizer, 1992). The second case is more reasonable since heparin still inhibits ATPase activity in the leaky microsomes and this suggests a direct inhibition of ATPase by heparin. Nonspecific effects of heparin have been reported and this character of heparin limits its general usage *in vivo* (Miyazaki et al, 1993).

The biphasic effect of Ca^{2+} on the activity of the $InsP_3$ receptor has been called Ca^{2+}-dependent activation and inactivation of the $InsP_3$ receptor (Bezprozvanny et al, 1991; Marshall and Taylor, 1993; Bootman et al, 1995). Since no (or poor) Ca^{2+}-dependence of purified intracellular Ca^{2+}-ATP ase activity has been reported in nonexcitable cells (Ezaki et al, 1992), we hypothesize the Ca^{2+}-dependence of microsomal Ca^{2+}-ATPases in only intact microsomes if there are functional connections between Ca^{2+}-ATPases and $InsP_3$ receptors. We have tested this possibility by the following: 1) seeing whether $InsP_3$ directly increases the activity of microsomal Ca^{2+}-ATPases in the leaky microsomes and 2) seeing whether the activity of Ca^{2+}-ATPases depends on extravesicular Ca^{2+}. We have found negative results in both cases. $InsP_3$ did not increase the activity of Ca^{2+}-ATPases in the leaky microsomes, and the activity of Ca^{2+}-ATPases did not depend on extravesicular Ca^{2+} (Fig. 4). These results imply that the activation of $InsP_3$ receptors in the

microsomes increases the activity of the microsomal Ca^{2+}-ATPase in intact microsomes because of the dissipation of the Ca^{2+} gradient across the microsomal membrane, suggesting that there is indirect coupling between these two microsomal components in the tracheal epithelium.

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REFERENCES

Bezprozvanny I, Watras J & Erlich EB (1991) Bell-shaped calcium-responce curves of Ins(1,4,5)P_3- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351,** 751-754

Boitano S, Dirksen ER & Sanderson MJ (1992) Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science* **258,** 292-295

Bootman MD, Missiaen L, Parys JB, De Smedt H & Casteels R (1995) Control of inositol 1,4,5-trisphosphate-induced Ca^{2+} release by cytosolic Ca^{2+}. *Biochem J* **306,** 445-451

Champeil P, Combettes L, Berthon B, Douce E, Orlowski S & Claret M (1989) Fast kinetics of calcium release induced by myo-inositol trisphosphate in permeabilized rat hepatocytes. *J Biol Chem* **264,** 17665-17673

De Young GW & Keizer J (1992) A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillation in Ca^{2+} concentration. *Proc Natl Acad Sci USA* **89,** 9895-9899

Ezaki J, Himeno M & Kato K (1992) Purification and characterization of (Ca^{2+}-Mg^{2+})-ATPase in rat liver lysosomal membranes. *J Biochem* **112,** 33-39

Fabiato A (1988) Computer programs for calculating total from specified free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods in Enzymol* **175,** 378-471

Finch EA, Turner TJ & Goldin SM (1991) Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* **252,** 443-446

Hansen M, Boitano S, Dirksen ER & Sanderson MJ (1995) A role for phospholipase C activity but not ryanodine receptors in the initiation and propagation of intercellular calcium waves. *J Cell Sci* **108,** 2583-2590

Hughes G, Starling AP, East JM & Lee AG (1994) Mechanism of inhibition of the Ca^{2+}-ATPase by spermine and other polycationic compounds. *Biochem* **33,** 4745-4754

Kim YK & Cho HJ (1996) Two types of intracellular Ca^{2+} pools in the porcine tracheal epithelial cells. Biophys J 70, in press (Abstract)

Lansley AB, Sanderson MJ & Dirksen ER (1992) Control of the beat cycle of respiratory tract cilia by Ca^{2+} and cAMP. *Am J Physiol 263 (Lung Cell Mol Physiol)* **7,** L232-L242

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* **193,** 265-275

Lytton J, Westlin M & Hanley MH (1991) Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *J Biol Chem* **266,** 17067-17071

Marshall ICB & Taylor CW (1993) Regulation of inositol 1,4,5-trisphosphate receptors. *J Exp Biol* **184,** 161-182

Miyazaki S, Shirakawa H, Nakada K & Honda Y (1993) Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Develop Biol* **158,** 62-78

Niggli V, Penniston JT & Carafoli E (1979) Purification of the (Ca^{2+}-Mg^{2+})-ATPase from human erythrocyte membranes using a calmodulin affinity column. *J Biol Chem* **254,** 9955-9958

Ozawa T, Thevenod F & Schulz I (1995) Characterization of two different Ca^{2+} uptake and IP_3-sensitive Ca^{2+} release mechanisms in microsomal Ca^{2+} pools of rat pancreatic acinar cells. *J Membr Biol* **144,** 111-120

Saez JC, Conner JA, Spray DC & Bennett MVL (1989) Hepatocyte gap junctions are permeable to the second messengers inositol 1,4,5-trisphosphate and to calcium ions. *Proc Natl Acad Sci USA* **86,** 2708-2712

Sanderson MJ, Charles AC & Dirksen ER (1990) Mechanical stimulation and intercellular communication increases intracellular Ca^{2+} in epithelial cells. *Cell Regul* **1,** 585-596

Sasaki T, Inui M, Kimura Y, Kuzuya T & Tada M

(1992) Molecular mechanism of regulation of Ca^{2+} pump ATPase by phospholamban in cardiac sarcoplasmic reticulum. *J Biol Chem* **267,** 1674-1679

Spray DC & Bennet MVL (1985) Physiology and pharmacology of gap junctions. *Ann Rev Physiol* **47,** 281-303

Valdivia C, Vaughan D, Potter BVL & Coronado R (1992) Fast release of $^{45}Ca^{2+}$ induced by inositol 1,4,5-trisphosphate and Ca^{2+} in the sarcoplasmic reticulum of rabbit skeletal muscle: evidence for two types of Ca^{2+} release channels. *Biophys J* **61,** 1184-1193

Wanner A (1977) Clinical aspects of mucociliary transport. *Am Rev Resp Dis* **116,** 73-125

Wictome M, Henderson I, Lee AG & East JM (1992) Mechanism of inhibition of the calcium pump of sarcoplasmic reticulum by thapsigargin. *Biochem J* **283,** 525-529