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ACTIVATION OF THE ENDOPLASMIC RETICULUM Ca^{2+} PUMP OF PANCREATIC ACINI BY Ca^{2+} MOBILIZING HORMONES

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Stimulation of the pancreatic acinar cells with Ca^{2+} mobilizing hormones increased the ATP-dependent Ca^{2+} uptake into the ER of permeabilized cells. Activation of the ER Ca^{2+} pump resulted in increased apparent affinity for Ca^{2+} from 0.26 to 0.09uM and Vmax from 2.68 to 5.74 nmoles/mg prot./min. The apparent affinity of the pump for $VO_4^=$ was dependent on $[Ca^{2+}]$. Activation of the pump also decreased apparent affinity for $VO_4^=$ from 12 to 32uM at $[Ca^{2+}]$ of 0.138uM. These findings suggest that pump activation is due to acceleration of the rate of the conformational transition between the $VO_4^=(E_2)$ and $Ca^{2+}(E_1)$ sensitive forms of the pump.

The mechanism of Ca^{2+} transport by the endoplasmic reticulum (ER) received much attention in recent years when it was discovered that Ca^{2+} mobilizing hormones release Ca^{2+} from this organelle (1,2). There is now good evidence in many cells that Ca^{2+} release from the ER is mediated by inositol 1,4,5 trisphosphate (3-7) which appears to activate a Ca^{2+} channel in the ER membrane (8,9). Ca^{2+} uptake into the ER is mediated by an ATP and Mg^{2+} dependent, $VO_4^=$ sensitive Ca^{2+} pump (10,11), which resembles the better studied sarcoplasmic reticulum (SR) Ca^{2+} pumps of the skeletal and cardiac muscles (12,13). The details of Ca^{2+} uptake by the ER Ca^{2+} pump and pump behavior during and at the termination of cell stimulation are poorly understood. Our studies with gastric glands (14) and pancreatic acini (15) suggests that the ER Ca^{2+} pump might be activated during cell stimulation. Using permeabilized pancreatic acini we now provide direct evidence that the ER Ca^{2+} pump is activated by Ca^{2+} mobilizing hormones. Activation of the ER Ca^{2+} pump results in an increase in the apparent affinity for Ca^{2+}, a decrease in the apparent affinity for $VO_4^=$

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and an increase in Vmax. These findings are most compatible with a change in the rate of comformational transition between the $VO_4^=$ (E_2) and Ca^{2+} (E_1) sensitive forms of the pump.

METHODS

Pancreatic acini from 75-150g rats were prepared as previously described (31). The rate of cell permeabilization was estimated from measurements of Ethidium Bromide (Et.Br) fluorescence at 37°C, under continuous stirring and with excitation and emmission wavelengths of 365 and 580 nm and band widths of 3 and 12 respectively. For Et.Br fluorescence measurements, about 40 mg acini were washed once and resuspended in 2 ml of a solution containing (mM): KCl 120, $MgCl_2$ 3, Hepes-Na 10 (pH 7.1), Et.Br 0.01 DTPA (Diethylenetriamininepenta Acetic acid) 0.01, $CaCl_2$ 0.01 (uptake medium). To adjust the free Ca^{2+} concentration in all solutions, 0.2uM of the tetra K^+ form of Fura 2 was added to a sample of medium. Then increasing concentrations of EGTA were added and free Ca^{2+} was calculated from F,Fmin. and Fmax as before (32). Addition of 17uM EGTA to the uptake medium resulted in a free Ca^{2+} concentration to 0.22 uM. The rate and extent of permeabilization was reproducible as tested in 18 preparations.

Measurement of ATP dependent Ca^{2+} uptake: Acini (40mg/ml) were suspended in a solution containing (mM): NaCl 140, KCl 5, $MgCl_2$ 1, Hepes-Tris 10 (pH 7.4), glucose 10, pyruvate 10 and bovine serum albumin 1 mg/ml. The acini were incubated for 10 min. at 37°C with or without the hormones. They were then washed twice and resuspended in uptake medium with or without the hormone to which the following mitochondrial uptake blockers 10uM ruthenium red, 5ug/ml oligomicin and 1uM of the protonophor tetrachlorosalicylanilide (TCS) were added. After 1 minute incubation at 37°C 0.005% saponin were added and 3 minutes later aliquotes of 1 ml were transferred to 2 ml uptake medium containing mitochondrial blockers, ^{45}Ca and with or without 1.5 mM ATP. At the indicated times 0.5 ml of cell suspension were transferred to 10 ml of cold uptake medium containing 1 mM $LaCl_3$. The acini were washed three times with this medium and dissolved in 1 ml of 1M NaOH to measure ^{45}Ca radioactivity.

RESULTS AND DISCUSSION

For ER Ca^{2+} pump measurements, acini were permeabilized with the detergent saponin. Since it was desired to measure pump activity immediately after permeabilization, the rate and extent of permeabilization was measured by following ethidium bromide fluoresence (16). Maximal pump stimulation was achieved when permeabilzation was completed within 2-3 minutes (figure 1) and followed by a more than 1:1 dilution of saponin. Stimulation of the cells with 10^{-8}M cholecystokinin-octapeptide (CCK-OP) for 10 minutes increased the rate of ATP

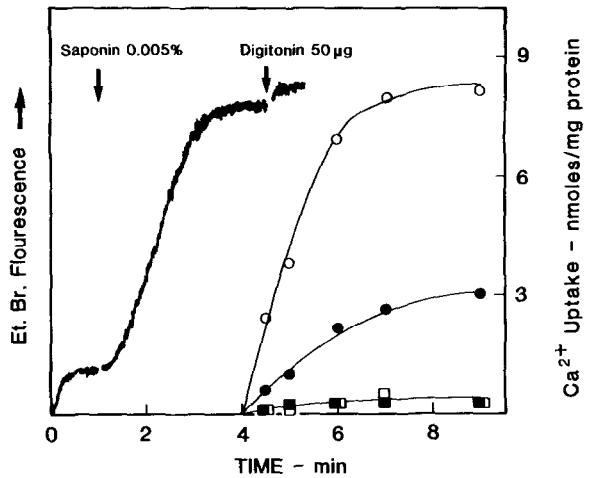

Figure 1. ATP dependent Ca^{2+} uptake into the ER of permeabilized resting and stimulated cells. Left panel-Pancreatic acini were added to 2 ml of uptake medium containing 10 uM Et.Br. When indicated, saponin and then digitonin were added to the medium. 0.005% saponin almost completely permeabilized the cells within 2.5 min. Right panel-Acini were incubated with (open symbols-stimulated) or without (close symbols-control) 10^{-8}M CCK-OP. The cells were then permeabilized and uptake in the presence (circles) or absence (squares) of 1.5 mM ATP was measured as described under methods.

dependent Ca^{2+} uptake into the ER. Similar results were obtained when the acini were stimulated with 100uM carbachol. Pump activation was observed in 34 out of 38 experiments similar to that in figure 1 and at various free Ca^{2+} concentrations. Variations in the degree of pump activation between experiments were noted. At about 0.22uM free Ca^{2+} (see below) pump rate was increased between 1.75-3.8 fold. A 3.8 fold increase is probably an underestimation of the true maximal value. Variation was likely due to the necessity to permeabilize the cells.

Figure 2 shows the rate of ATP dependent Ca^{2+} uptake as a function of the free Ca^{2+} concentration in permeabilized resting and stimulated acini. The Ca^{2+} dependency display simple saturation kinetic under both conditions. The apparent affinity for Ca^{2+} ($K_{0.5}$) of the ER Ca^{2+} pump of resting cells was 0.26 $\pm$ 0.04 uM with an overall maximal rate (Vmax) of about 2.68 $\pm$ 0.47 nmoles mg protein^{-1}.min^{-1}. The respective values for the ER Ca^{2+} pump of stimulated cells were $K_{0.5Ca}$ = 0.09 $\pm$ 0.03 uM and Vmax of about 5.74 $\pm$ 0.72 nmoles. mg protein $^{-1}$.min^{-1}.

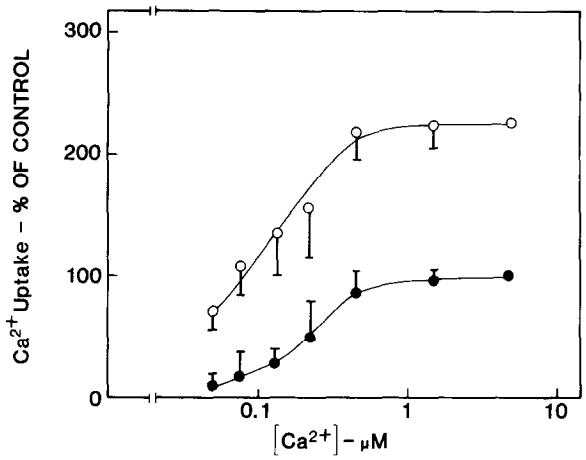

Figure 2. Ca^{2+} dependence of the ER Ca^{2+} pump of permeabilized resting and stimulated cells. ATP dependent Ca^{2+} uptake was measured in control (close symbols) and CCK-OP prestimulated (open symbols) acini as described in methods. During permeabilization all the acini were suspended in uptake medium containing 42 nM free Ca^{2+}. The permeabilized acini were transferred to uptake medium containing increasing concentrations of $CaCl_2$ to yield the indicated free Ca^{2+} concentrations. For measurement of Ca^{2+} uptake the acini were incubated at 37°C for: 0-5 minutes at free Ca^{2+} of 0.042-0.22 uM and for 0-2 minutes at free Ca^{2+} of 0.46-4.85 uM. Ca^{2+} uptake in the absence of ATP was measured at each free Ca^{2+} concentration and substracted from the uptake in the presence of ATP. The figure shows the mean ± SD of four separate experiments. The calculated kinetic parameters are given in the text.

The results in figure 2 indicate that both the apparent affinity for Ca^{2+} and the Vmax of the ER Ca^{2+} pump were altered by cell stimulation. These effects could result from modification of the true affinity of the pump for Ca^{2+} (KmCa)and the rates of all partial reactions in the pump

turnover cycle, similar to the effect of calmodulin on the plasma membrane Ca^{2+} pump (18), or a change of a rate limiting step in the turnover cycle which would affect both $K_{0.5Ca}$ and Vmax. As with other ATP dependent ion pumps (17, 19), including the SR Ca^{2+} pump (12, 20), it is likely that the conformational transition between the low (E_2) and the high (E_1) Ca^{2+} affinity forms of the pump is the rate limiting step.

To distinguish between these possibilities it was necessary to estimate the steady state distribution between the E_2 and E_1 forms of control and stimulated pump. $VO_4^=$ ions inhibit the pumps by binding to E_2 (21, 23) and in the case of the SR Ca^{2+}

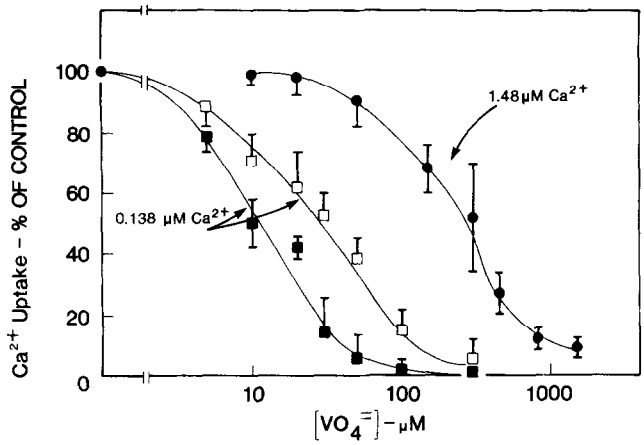

Figure 3. Inhibition of the ER Ca^{2+} pump by $VO_4^{=}$-effect of Ca^{2+} and cell stimulation. Acini were permeabilized in uptake medium containing the mitochondrial blockers and 0.042 uM Ca^{2+} and then diluted 1:2 into similar medium containing the indicated concentrations of $VO_4^{=}$ and sufficient $CaCl_2$ to give free Ca^{2+} of 0.138 or 1.48uM . The uptake medium was supplemented with, $MgCl_2$ to the same concentration as $VO_4^{=}$ in order to maintain the same free Mg^{2+} concentrations during Ca^{2+} uptake. This was particularly important at $VO_4^{=}$ concentrations above 0.3 mM. The figure shows the effect of $VO_4^{=}$ on Ca^{2+} uptake into the ER of resting (close symbols) and stimulated (open squares) cells at the indicated free Ca^{2+} concentrations. The effect of cell stimulation on $K_{0.5v}$ was measured within the same preparation while the effect of Ca^{2+} was measured in separate preparations. The figure shows the mean $\pm$ SD of 4-6 separate experiments. Stimulation of the cells increased the rate of Ca^{2+} uptake into the ER by about 3.5 fold.

pump, Ca^{2+} ions can relieve the inhibition by $VO_4^{=}$. Figure 3 shows that at a Ca^{2+} concentration of 0.138 uM, 50% inhibition of Ca^{2+} uptake ($K_{0.5v}$) into the ER of resting cells was achieved with 12 $\pm$ 3uM $VO_4^{=}$. Increasing Ca^{2+} concentrations to 1.48 uM increased the $K_{0.5v}$ to 230 $\pm$ 57 uM. In separate experiments we found that when cells preincubated with 63 nM Ca^{2+} and 100uM $VO_4^{=}$ were transferred to medium containing 4.85 uM Ca^{2+}, after a lag period, $VO_4^{=}$ inhibition was completely relieved (not shown). These observations indicate that the details of $VO_4^{=}$ inhibition of the ER and SR Ca^{2+} pumps are similar. In addition to the mode of $VO_4^{=}$ interaction there are several other similarities in the turnover cycle of the ER and the SR Ca^{2+} pumps (24-26). As with the SR Ca pump (23) $VO_4^{=}$ inhibition of the ER Ca^{2+} pump can therefore be used to determine the distribution of the pump between the E_2 and E_1 conformation during Ca^{2+} uptake. Figure 3 shows that in stimulated cells, the $K_{0.5v}$ for inhibition of Ca^{2+} uptake into the ER at 0.138 uM Ca^{2+} was increased to 32 $\pm$ 7 uM.

A change in the Vmax and the apparent affinities for both Ca^{2+} and $VO_4^{=}$ suggests that pump activation can be achieved by an increase in the rate of the conformational transition E_2 to E_1 without a change in K_{Ca} or K_V. The interaction of Ca^{2+} and $VO_4^{=}$ with the pump can then be described schematically as follows:

$$E_2 \overset{K_c}{\rightleftharpoons} E_1 \overset{K_{Ca}}{\rightleftharpoons} E_1Ca \quad \text{and} \quad E_2V \overset{K_v}{\rightleftharpoons} E_2 \overset{K_c}{\rightleftharpoons} E_1$$

where K_{Ca} and Kv are the true Ca^{2+} and $VO_4^{=}$ dissociation constants and Kc (= E_2/E_1) is the conformational equilibrium constant. Accordingly, the apparent Ca^{2+} and $VO_4^{=}$ dissociation constants can be described by the following equations respectively:

$$K_{0.5Ca}=K_{Ca}\ (1+K_C) \quad \text{and} \quad K_{0.5V}=K_V\ (1+1/K_C)$$

From the measured $K_{0.5Ca}$ and $K_{0.5V}$ of the ER pump in resting and stimulated cells it is possible to calculate a K_C of about 3.5 for unactivated pumps. A K_C of about 3 was found for the SR Ca^{2+} pump under similar conditions (23). K_C is reduced to 0.45 by cell stimulation, which indicates that upon activation the rate of the conformational change E_2-->E_1 is accelerated. Such an effect can most simply explaine the changes in the kinetic properties of the ER Ca^{2+} pump induced by cell stimulation.

The kinetic properties of the activated ER Ca^{2+} pump can explain the observation of partial reloading of the ER during the stimulation period (15), and in particular, the finding that Ca^{2+} reloading of the ER upon termination of cell stimulation occurs at or below resting levels of free cytosolic Ca^{2+}.

The cellular mechanism by which pump activation is achieved is not clear yet. The cardiac SR (27) and platelets ER (28) Ca^{2+} pumps could be activated by cAMP dependent protein kinase mediated phosphorylation. The former was shown to be activated also by Ca^{2+} and calmodulion dependent protein kinase (29) and protein kinase C (30). CCK-OP and carbachol increase free

cytosolic Ca^{2+} and activate protein kinase C in the pancreatic acinar cells (15, 33). Whether the hormonal induced activation of the ER Ca^{2+} pump is mediated by the protein kinases remains to be determined.

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