# Regulation of Epithelial Calcium Channels by Protein Kinases

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Calcium is the most abundant cation in the body, mainly in the mineral phase of bones (99%). In addition, calcium serves as a universal intracellular messenger in the cell due to the fact that, unlike other cations such as potassium and sodium, extracellular free calcium concentration is approximately 10,000 times that of intracellular free calcium. Calcium enters the cell, and in turn, the body through ion channels which are tightly regulated.

Our project examined the functional regulation of the recently identified vitamin D responsive epithelial calcium channels, CaT1 and CaT2, which are gatekeepers for calcium absorption and reabsorption. Our hypothesis was that CaT1 and CaT2 are under regulation by phosphorylation mediated by protein kinases A and C, as there are consensus phosphorylation sites in the two proteins. To test the hypothesis, we expressed CaT1 and CaT2 in African clawed frog eggs and examined whether the functions of the channels are affected by activation of protein kinase A or C. We expected this project to provide some insight into how functions of these calcium channels are regulated. Our initial results show a decrease in activity in the calcium channels after it was exposed to a protein kinase C activator.

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## Introduction

Calcium is extremely important in the body as a major component of the bone and as an intracellular messenger. Too much or too little calcium can cause problems in the body. Calcium enters the body through tightly regulated ion channels. CaT1 and CaT2 are recently identified vitamin D regulated calcium selective channels, which are regarded as gatekeepers for calcium absorption and reabsorption. One way the body adjusts calcium homeostasis is to regulate the activity of the calcium channels. In this study, we tested whether the CaT1/CaT2-mediated calcium transport is regulated by protein kinases A and C. We found that CaT1 was upregulated by protein kinase C whereas CaT2 was not. Activation of protein kinase A decreased the activity of CaT2 but not CaT1.

## Methods

### *Xenopus laevis* Oocyte Preparation

Oocytes were surgically removed from anesthetized frogs under tricaine. They were separated and defoliated using collagenase A and were washed 6 times with Barth's solution. They were then incubated for 4 hours with pyruvic acid, penicillin, and gentamicin at 18°C. After injection, oocytes were incubated for 1 to 2 days. The medium was changed every day.

### mRNA Translation

CaT1 and CaT2 DNA were linearized by using Not I enzyme. mRNA was transcribed *in vitro,* using mMESSAGE mMACHINE kit (Ambion, Austin, TX). Transcription was confirmed by running an agarose gel, and concentration was determined by absorbance readings.

### Injection of Oocytes

The oocytes were injected with 50 nL of either water, CaT1, or CaT2 RNA (25ng/oocyte).

### Voltage Clamp

After the mRNA had been expressed in the oocytes, they were tested under voltage clamp to see if 1,2-Dioctanoyl-sn-glycerol (DoG), Sp-Adenosine 3′,5′-cyclic monophosphothioate (Sp-cAMP), or Phorbol 12-Myristate 13-Acetate (PMA) had any effect on the functions of the protein channels. Two solutions were prepared, in addition to the tested solutions. One was a choline solution (100mM choline, 2mM potassium, 1mM magnesium, and 10 mM HEPES, pH 7.5). The other was a sodium solution (sodium in the place of choline). The oocytes were first put into the choline solution. After the current readings stabilized, they were switched over to the sodium solution and the difference was noted. This was repeated 3 times. Next, the oocytes were placed into DMSO. They were incubated for 15 minutes with the voltage clamp turned off. They were put into the choline solution. After they stabilized, they were put into the sodium solution. This was repeated 3 times, and any changes were noted. This was repeated with DoG, cAMP, and PMA.

### $^{45}$Ca Uptake

Oocytes (7 to 9/group) were put into a bath of either DoG (1.0μM), Sp-cAMP (0.1mM), PMA (0.5mM), or DMSO (vehicle) (0.01%) for 30 minutes. They were then washed and put into an uptake medium (100mM Na, 2mM K, 1mM Mg, 10mM HEPES,

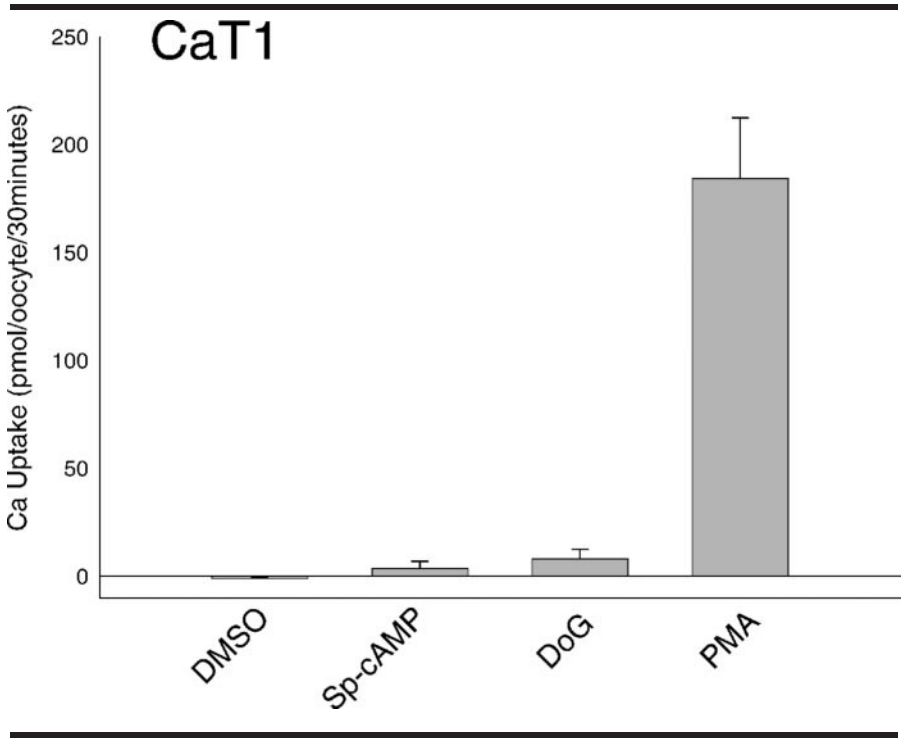


**Fig 1. Results for the oocytes expressing CaT1**

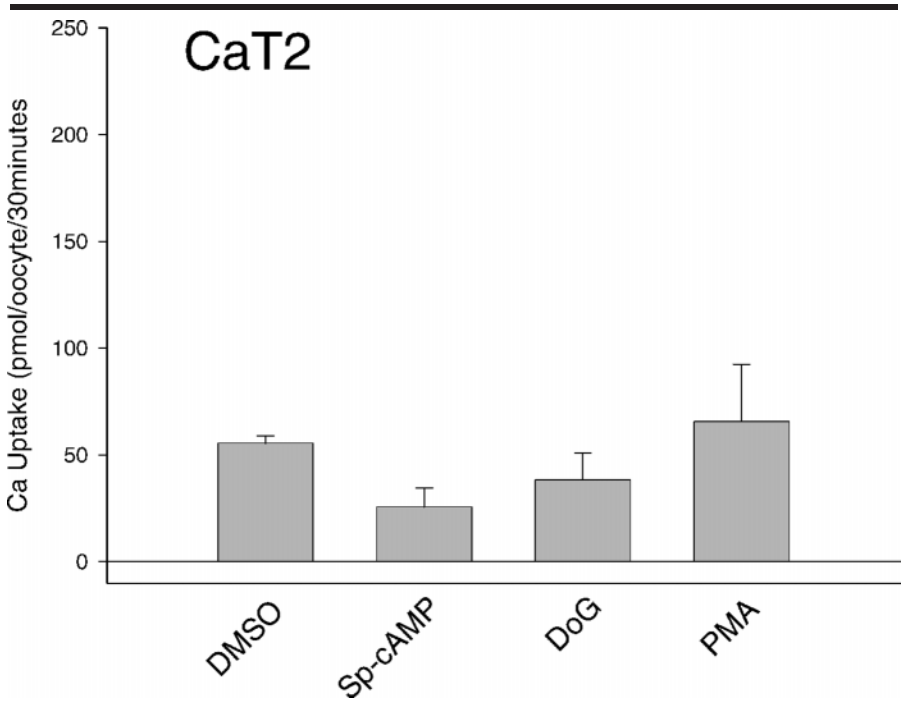


**Fig 2. Results for the oocytes expressing CaT2**

and 1 mM $Ca^{2+}$ with $^{45}Ca$ tracer) for 30 minutes. The uptake was stopped by washing with ice cold medium without $^{45}Ca$. The oocytes were then lysed with 10% SDS detergent and the $^{45}Ca$ content was measured with a scintillation counter.

## Data Analysis

$^{45}Ca$ uptake data were analyzed using SigmaPlot software (SPSS, Chicago, IL) and are presented as mean value ± standard error.

Figure 1 illustrates the results for the oocytes expressing CaT1 after taking into what effect the drugs had on the oocyte itself. These values were determined by taking the readings from the CaT1 oocytes and subtracting the readings from the water-injected control oocytes. The only significant change noted was with the oocytes exposed to PMA. There was a very large increase in calcium uptake.

The same maneuver was applied to CaT2-expressing oocytes (Figure 2). In contrast to CaT1, the CaT2-mediated calcium uptake was decreased significantly only in the oocytes exposed to Sp-cAMP. The changes in the calcium uptake values may have been a result of the phosphorylation of the channel proteins by the protein kinases. The phosphorylation of the channel proteins may in turn trigger the insertion of the channel proteins into the plasma membrane or trigger internalization of the channel proteins from the membrane. We concluded that the CaT1 activity is increased by activation of protein kinase C (by PMA) whereas CaT2 activity is decreased by activation of protein kinase A (by Sp-cAMP) in *Xenopus* oocytes.

## CONCLUSION

We hope that a better understanding of the regulation of calcium transport could be used to find cures to diseases such as osteoporosis and other calcium-related diseases.