THE ROLE OF INOSITOL 1,4,5-TRISPHOSPHATE
IN MOBILIZING CALCIUM FROM
INTRACELLULAR STORES IN
THE SALIVARY GLANDS OF
AMBLYOMMA AMERICANUM
(L.)

By
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Bachelor of Science
in Arts and Sciences
Oklahoma State University
Stillwater, Oklahoma
1987

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the degree of
MASTER OF SCIENCE
May, 1989
R. A. S. F. Cop. 2.
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(L.)

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ACKNOWLEDGEMENTS

The author would like to thank Dr. Richard Essenberg, Professor, Department of Biochemistry, and Dr. Jack Dillwith, Assistant Professor, Department of Entomology, for their understanding, guidance, and advice with this project. These individuals served on the author's graduate committee.

I would also like to thank Dr. John Sauer, Regents Professor, Department of Entomology, for his patience, encouragement, and guidance. You are a very dedicated and admirable person.

I want to express my appreciation to Dr. Katherine Kocan for her assistance with the electron microscopy and to Dr. Larry Claypool for his help with the statistics.

To Dr. Jan McSwain, Jim Tucker, Dr. Kent Shelby, Dr. Altaf Qureshi, Lorry Newsom, and Becky Mumma, I wish to express my overwhelming thanks. It has been an enjoyable experience getting to know and work with each of you.

I appreciate the continual support of my parents O.E. and Mary Jane, and my brother Ken.

I want to thank my friends Randy, Kenny and Cary for their encouragement and support.

This research was supported by NIH Grant No. AI-26158 from the National Institute of Health.
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INTRODUCTION

This thesis is a complete manuscript to be submitted for publication. This manuscript is being submitted to *Insect Biochemistry*. This thesis appears in the format of the journal in which it is being submitted.
THE ROLE OF INOSITOL 1,4,5-TRISPHOSPHATE
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ABSTRACT

Isolated tick salivary glands, permeabilized with digitonin in the presence of the mitochondrial and non-mitochondrial Ca\(^{2+}\) uptake inhibitors, sodium azide and vanadate, released Ca\(^{2+}\) in response to 20 µM inositol 1,4,5-trisphosphate (IP\(_3\)). Inositol 1-phosphate (IP\(_1\)) and inositol 1,4-bisphosphate (IP\(_2\)) appeared to stimulate an uptake of Ca\(^{2+}\) into whole glands. IP\(_3\) caused release of Ca\(^{2+}\) from a 100,000 g microsome enriched pellet; however, IP\(_1\) and IP\(_2\) were ineffective in stimulating an uptake or efflux of Ca\(^{2+}\). The combined 900 g and 11,500 g pellets showed no significant release of Ca\(^{2+}\) in response to addition of IP\(_3\). IP\(_3\) concentrations as low as 1 µM are capable of stimulating a significant release of Ca\(^{2+}\) from microsomes. Results suggest that intracellular Ca\(^{2+}\) is mobilized from microsomal intracellular stores in response to agonists which increase cytosolic IP\(_3\) in tick salivary glands. Results also suggest a possible role for IP\(_1\) or IP\(_2\) or both in stimulating an uptake of Ca\(^{2+}\) into vanadate and azide-insensitive intracellular pools.
INTRODUCTION

Michell (1975) noted that plasma membrane-associated phosphoinositide metabolism correlates with many physiological agonists that raise intracellular calcium. Subsequently, a variety of intermediate products of hormone stimulated inositol lipid metabolism were proposed as agonists for mobilizing cytoplasmic calcium (Thomas et al., 1984). Inositol 1,4,5-trisphosphate (IP$_3$), a product of agonist-induced phosphatidylinositol 4,5-bisphosphate metabolism, was shown to release calcium rapidly from the endoplasmic reticulum of permeabilized cells such as rat pancreas and hepatocytes (Berridge, 1986; Streb et al., 1984). Kinetic analysis has shown that [$^{32}$P] IP$_3$ binds rapidly to the microsomal membranes (Spat et al., 1986). Spat et al. (1986) further demonstrated a specific, high affinity binding site for IP$_3$ on the microsomal fraction of rat liver which corresponds with the ability of IP$_3$ to release Ca$^{2+}$. Inositol 1,4-bisphosphate (IP$_2$) and inositol 4,5-bisphosphate at 1 µM had no effect on IP$_3$ binding (Spat et al., 1986). Berridge (1986) demonstrated that the vicinal phosphates on the 4 and 5 positions are essential for releasing calcium from intracellular stores and the phosphate on the opposite side of the ring enhances the affinity of IP$_3$ for its putative receptor. Unlike inositol 1,4,5-trisphosphate, neither inositol 1,3,4-trisphosphate nor inositol 1,3,4,5-tetrakisphosphate are able to mobilize
intracellular calcium (Tilly et al., 1987).

Activation of an endoplasmic reticulum Ca^{2+}-ATPase pump ensures rapid reloading of the endoplasmic reticulum with Ca^{2+} even at low extracellular Ca^{2+}. ATP dependent Ca^{2+} uptake into the endoplasmic reticulum of permeabilized IP_3-prestimulated cells is higher than that into the endoplasmic reticulum of unstimulated cells (Muallen et al., 1988). Non-mitochondrial calcium uptake in rat pancreatic acinar cells can be completely inhibited by 2 mM vanadate, (Streb and Schultz, 1983), and mitochondrial calcium uptake can be inhibited by azide (Streb et al., 1984). Ten micromolar GTP enhances IP_3-induced calcium release from liver microsomes (Dawson, 1985; Ueda, 1986).

Calcium is important in tick salivary gland function (Sauer and Essenberg, 1984). Dopamine and cyclic AMP stimulated fluid secretion by isolated salivary glands is inhibited by low extracellular Ca^{2+} (Needham and Sauer, 1979). Cyclic AMP-dependent phosphodiesterase activity is activated and inhibited by submicromolar and micromolar Ca^{2+} respectively (McMullen et al., 1980), and protein phosphatase activity is inhibited by micromolar Ca^{2+} (Williams et al., 1988). More recently a factor present in the tick's brain was shown to increase the level of inositol trisphosphate demonstrating the existence of the phosphoinositide signal transduction pathway in tick salivary glands (McSwain et al., 1989). This study demonstrates that IP_3 mobilizes Ca^{2+} from intracellular
stores of permeabilized tick salivary glands and that the subcellular location is likely the microsomal fraction. Unexpectedly, it appears that the immediate products of IP$_3$ metabolism, IP$_1$ and IP$_2$ may stimulate an uptake of Ca$^{2+}$ into a non-mitochondrial component of the salivary glands.
MATERIALS AND METHODS

**Materials.** D-myo-inositol 1,4,5-trisphosphate (95% or greater purity) and D-myo-inositol 1-phosphate were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Indiana. D-myo-inositol 1,4-bisphosphate (98% pure), vanadium oxide, and digitonin were from Sigma Chemical Company, St. Louis, Missouri. TMB-8 [8-(diethylamino)-3,4,5-trimethoxybenzoate] was from Calbiochem Corporation, San Diego, California.

**Tissue Preparation.** Adult lone star ticks, *Amblyomma americanum* (L.), were reared following the methods of Patrick and Hair, (1975). Rapidly feeding female ticks (200-800 mg) were dissected at 4 °C in buffer containing 0.1 M MOPS and 20 mM EGTA at pH 6.8.

**Calcium determination in whole salivary glands and subcellular fractions.** The whole salivary glands or subcellular tissue fractions were placed in a 1 ml reaction medium containing 20 mM HEPES (pH 7.4), 100 mM KCl, 5 mM MgCl\(_2\), 5 mM ATP, 10 µM GTP, 0.1 mM digitonin, 2 mM vanadate, and 0.1 M sodium azide following a modified procedure for measuring Ca\(^{2+}\) release from microsomes (Enouf et. al., 1987). With subcellular tissue fractions, digitonin was deleted from the reaction buffer. A miniature calcium electrode (MI-600, Microelectrodes, Inc., Londonderry, New
Hampshire) was pre-calibrated at 1 µM Ca^{2+} using a Corning calcium standard, and then placed into the medium with a micro-reference electrode (MI-402, Microelectrodes, Inc., Londonderry, New Hampshire). The reaction medium was continuously stirred. The IP₁, IP₂, IP₃, and calcium-free H₂O controls were separately added to the reaction medium to determine tissue responses. Inositol phosphates and H₂O were added sequentially at 4 minute intervals. Except for dose response experiments, 20 µM IP₁, IP₂, and IP₃ was used in all experiments. The free medium level of Ca^{2+} was monitored continuously with the use of a signal amplifier (Johnson Research Foundation, Philadelphia, Pa.) and a Bell and Howell oscillographic recorder (Figure 1).

**Subcellular fractionation.** Fractions were prepared according to a modified procedure of McSwain et al., 1987. Sixty glands were used in each assay. The glands were homogenized in 1 ml of medium containing 0.25 M sucrose, 10 mM tricine buffer (pH 7.2), 10 mM MgCl₂, 0.05% p-amino-benzamidine, and 5% (w/v) polyethylene glycol. The crude homogenate was centrifuged at 900 g for 10 min and the pellet was washed twice. The 900 g supernatant was centrifuged at 11,500 g for 10 min and the pellet was washed twice. The 11,500 g supernatant was centrifuged at 100,000 g for 60 min to yield the 100,000 g pellet.

**Electron microscopy.** The 100,000 g pellet was collected
as described above and fixed according to the procedures of McSwain et al., 1987. Thin sections were obtained with a Sorvall MT-2 ultramicrotome and stained with methanolic uranyl acetate and lead citrate. Sections were examined and photographed with a JEOL cx 2 transmission electron microscope.
RESULTS

Determination of changes in free medium calcium in response to inositol phosphates added to permeabilized salivary glands and subcellular fractions

Changes in medium Ca\(^{2+}\) were continuously monitored for 4 minutes in the presence or absence of either single applications or sequential changes of IP\(_1\), IP\(_2\) and IP\(_3\) (Figure 1). Water controls were subtracted from the observed changes. Each experiment was performed in triplicate except Figure 4b represents four replications. An analysis of variance procedure (Steel and Torrie, 1980) was used to evaluate statistical significance of data. A typical chart recording of one experiment after sequential additions of IP\(_3\), H\(_2\)O, IP\(_2\), H\(_2\)O, IP\(_1\), and H\(_2\)O respectively at 4 minute intervals is indicated in Figure 2. Few oscillations in levels of medium Ca\(^{2+}\) (Berridge and Galione, 1986) were observed possibly because the Ca\(^{2+}\) uptake inhibitors vanadate and azide were present in the bathing medium.

Inositol phosphate effects on whole permeabilized salivary glands

IP\(_3\) stimulated a significant increase in bathing medium Ca\(^{2+}\) (p < 0.05) whereas IP\(_1\) and IP\(_2\) produced a significant decrease (p < 0.05) (Figure 3). Additions of IP\(_3\) followed by H\(_2\)O, IP\(_2\) and H\(_2\)O at 4 minute intervals stimulated a release of Ca\(^{2+}\) in response to IP\(_3\) (Figure 4a), but not IP\(_2\). TMB-8, a
calcium release inhibitor (Lydan and O'Day, 1988), completely inhibited the effect of IP$_3$ (Figure 4b). When IP$_2$ was added to whole permeabilized glands prior to adding IP$_3$ (Figure 5), a significant ($p < 0.05$) decrease in medium Ca$^{2+}$ (tissue uptake) was observed while subsequent addition of IP$_3$ stimulated a release of Ca$^{2+}$ ($p < 0.05$).

**Inositol phosphate effects on fractionated salivary gland tissue**

The 100,000 g pellet (Figure 6) contained a homogenous mixture of free or attached ribosomes and small membrane-bound vesicles (microsomes). These results are comparable to those observed by McSwain et al. (1987) in the microsomal fraction of tick salivary glands. The addition of IP$_3$ to the 100,000 g pellet (Figure 7) resulted in a significant release of Ca$^{2+}$. There were no significant changes in the bathing medium Ca$^{2+}$ after adding IP$_1$, IP$_2$ or IP$_3$ to combined non-microsomal fractions (900 g and 11,500 g pellets) (Figure 8). Sequential additions of increasing concentrations of IP$_3$ to microsomal tissue (Figure 9) indicated that 1 µM IP$_3$ stimulated a significant ($p < 0.05$) release of Ca$^{2+}$ from microsomes although 20 µM concentrations were more effective. IP$_3$ was ineffective in stimulating a release of Ca$^{2+}$ from non-microsomal tissue (Figure 10).
Figure 1. Method for determining release of calcium from tick salivary glands with the use of a microelectrode. Changes in free medium levels of calcium from fourteen permeabilized tick salivary glands (0.1 mM digitonin) or non-permeabilized microsomes prepared from sixty glands were monitored with a Ca^{2+} sensitive microelectrode in response to adding IP_1, IP_2, IP_3 and H_2O.
Ca$^{2+}$ MICROELECTRODE AND REFERENCE

10 µl IP$_3$, IP$_2$, IP$_1$
or H$_2$O

1 ml SUPPORT MEDIUM W/PERM.
S.G. OR MICROSOMES

STIRRER

AMPLIFIER

RECORDER
Figure 2. A representative temporal trace of changes in free medium calcium after adding 20 $\mu$M IP$_1$, IP$_2$, IP$_3$ and H$_2$O to tick salivary gland microsomes. The times of IP$_1$, IP$_2$, IP$_3$ and H$_2$O additions are indicated at arrows and experiments were performed sequentially, left to right. A 10 mm recorder deflection corresponded to a 5 nmole change in bathing medium Ca$^{2+}$. 
Figure 3. Changes in free medium \( \text{Ca}^{2+} \) (see Figures 1 and 2) after adding \( \text{IP}_1 (\square) \), \( \text{IP}_2 (\bigcirc) \) and \( \text{IP}_3 (\triangle) \) to whole permeabilized tick salivary glands. Additions of the three inositol phosphate analogs were monitored in separate experiments and compared with a \( \text{H}_2\text{O} \) control. An analysis of variance was used to determine significant differences at the \( p < 0.05 \) level. The effects of \( \text{IP}_1 \) and \( \text{IP}_2 \) were significantly different from all other additions after 2 minutes. The effect of \( \text{IP}_3 \) was significantly different from the effects of \( \text{IP}_1 \) and \( \text{IP}_2 \) at all times. Values represent the mean ± S.E.M.
Figure 4. Changes in free medium Ca$^{2+}$ (see Figures 1 and 2) after adding IP$_2$ (○) and IP$_3$ (△) to permeabilized tick salivary glands. IP$_3$, H$_2$O, IP$_2$ and H$_2$O; respectively, were added to the medium at 4 minute intervals. TMB-8 (75 μM) was used to inhibit IP$_3$-sensitive calcium release. An analysis of variance was used to determine significant differences at the p < 0.05 level. Effects of IP$_3$ were significantly different with and without TMB-8 after 1 minute. Values represent the mean ± S.E.M.
The image contains a graph showing the change in Ca\(^{2+}\) concentration (nmoles/ml) over time (min). The graph is divided into two panels:

**Panel a:**
- **Y-axis:** Ca\(^{2+}\) Change (nmoles/ml)
- **X-axis:** TIME (min)
- The graph shows the change in Ca\(^{2+}\) concentration over time for IP\(_3\) and IP\(_2\) with and without water (H\(_2\)O).
- IP\(_3\) without water shows a significant decrease in Ca\(^{2+}\) concentration, while IP\(_2\) and IP\(_3\) with water show minimal change.

**Panel b:**
- **Y-axis:** Ca\(^{2+}\) Change (nmoles/ml)
- **X-axis:** TIME (min)
- The graph shows the change in Ca\(^{2+}\) concentration over time for IP\(_3\) and IP\(_2\) with TMB-8.
- IP\(_3\) with TMB-8 shows a slight decrease in Ca\(^{2+}\) concentration, while IP\(_2\) with TMB-8 shows a slight increase.
Figure 5. Changes in free medium Ca$^{2+}$ (see Figures 1 and 2) after adding IP$_2$ (○) and IP$_3$ (△) to permeabilized tick salivary glands. IP$_2$, H$_2$O, IP$_3$ and H$_2$O; respectively, were added to the medium at 4 minute intervals as illustrated in Figure 2. An analysis of variance was used to determine significant differences at the $p < 0.05$ level. This order of addition produced a significant difference with the IP$_2$ when added before IP$_3$. Values represent the mean ± S.E.M.
Figure 6. Electron micrograph of the 100,000 g pellet from feeding lone star tick salivary gland tissue. A large number of free or attached ribosomes (R) and membrane-bound vesicles (➡️) were present in the total 100,000 g pellet. Figure B represents an enlargement of the indicated portion in Figure A (X19,268).
Figure 7. Changes in free medium Ca\(^{2+}\) (see Figures 1 and 2) after adding IP\(_1\) (□), IP\(_2\) (○) and IP\(_3\) (△) to tick salivary gland microsomes. IP\(_3\), H\(_2\)O, IP\(_2\), H\(_2\)O, IP\(_1\) and H\(_2\)O; respectively, were added at 4 minute intervals as illustrated in Figure 2. An analysis of variance was used to determine significant differences at the p < 0.05 level. The effect of IP\(_3\) was significantly different from the effects of IP\(_1\) and IP\(_2\) after 2 minutes. Values represent the mean ± S.E.M.
The diagram shows the change in Ca\(^{2+}\) concentration (nmoles/ml) over time (min) for the following treatments:

- IP\(_3\) - H\(_2\)O
- IP\(_2\) - H\(_2\)O
- IP\(_1\) - H\(_2\)O

The y-axis represents Ca\(^{2+}\) concentration, and the x-axis represents time (min). The graph indicates a decrease in Ca\(^{2+}\) concentration with time, with markers for IP\(_1\), IP\(_2\), and IP\(_3\) treatments.
Figure 8. Changes in free medium Ca\textsuperscript{2+} (see Figures 1 and 2) after adding IP\textsubscript{1} (□), IP\textsubscript{2} (○) and IP\textsubscript{3} (△) to non-microsomal (900 g and 11,500 g combined pellets) tick salivary gland tissue. Water, IP\textsubscript{3}, H\textsubscript{2}O, IP\textsubscript{2}, H\textsubscript{2}O, and IP\textsubscript{1}; respectively, were added at 4 minute intervals as illustrated in Figure 2. Values represent the mean ± S.E.M.
Ca²⁺ Change (nmol/ml)

TIME (min)

H₂O - IP₃ - H₂O - IP₂ - H₂O - IP₁
Figure 9. Dose response changes in free medium Ca$^{2+}$ (see Figures 1 and 2) after adding IP$_3$ to tick salivary gland microsomes. The IP$_3$ concentrations of 1, 2, 5, 10 and 20 µM; respectively, were added sequentially at 4 minute intervals as illustrated in Figure 2. An analysis of variance was used to determine significant differences at the p < 0.05 level. The effect of 1 µM IP$_3$ was significantly different from the H$_2$O control. Values represent the mean ± S.E.M.
Figure 10. Comparison of changes in free medium Ca\textsuperscript{2+} (see Figures 1 and 2) after adding IP\textsubscript{3} to microsomal and non-microsomal tick salivary gland tissue. The IP\textsubscript{3} and H\textsubscript{2}O were added at 4 minute intervals as illustrated in Figure 2. An analysis of variance was used to determine significant differences at the p < 0.05 level. A comparison of tissue fractions showed a significant difference in the effects with 20 µM additions of IP\textsubscript{3}. Values represent the mean ± S.E.M.
NON-MICROSOMAL TISSUE W/20µM IP₃

MICROSOMAL TISSUE
W/1µM IP₃

MICROSOMAL TISSUE
W/20µM IP₃

Ca²⁺ Change (nmoles/ml)

TIME (min)

IP₃ - H₂O

4 min  4 min
DISCUSSION

A peptidergic factor from tick brain increases inositol phosphates in isolated whole salivary glands providing evidence that plasma membrane associated phosphoinositides are metabolized in response to an agonist in tick salivary glands (McSwain et al., 1989). The final physiological consequence of this neuropeptide's action is unknown at this time. Inositol 1,4,5-trisphosphate (IP₃), an immediate product of agonist induced metabolism of membrane associated phosphatidylinositol 4,5-bisphosphate, mobilizes Ca²⁺ from intracellular stores, possibly the endoplasmic reticulum, in many cells (Berridge, 1987). Physiological levels of Ca²⁺ affect the activities of several key regulatory enzymes in tick salivary glands (Sauer et al., 1989). We sought to test the hypothesis that intracellular Ca²⁺ is mobilized by inositol phosphates in tick salivary glands.

Digitonin selectively binds to membrane cholesterol which produces micelle channels allowing substances to move freely through the plasma membrane (Peppers and Holz, 1986; Tsien et al., 1982). Since the salivary glands of ticks are relatively small, Ca²⁺ released from whole salivary glands can be monitored in a support medium with the use of a Ca²⁺-sensitive microelectrode. Data indicate that release of Ca²⁺ takes place in response to IP₃ but not to IP₁ or IP₂ when added to whole salivary glands and microsomes isolated from rapid feeding adult female ticks. TMB-8, a specific
inhibitor of IP₃ stimulated release of Ca²⁺ from intracellular stores in Dictyostelium discoideum (Lydan and O'Day, 1988), blocks IP₃-stimulated Ca²⁺ release from salivary gland microsomal tissue. Sequential dose response experiments indicate that 1 µM IP₃ can effect a significant release of Ca²⁺ from microsomes.

The activation of Ca²⁺ mobilizing receptors can rapidly increase the levels of cytoplasmic Ca²⁺ by both releasing stored Ca²⁺ and stimulating an uptake (Hughes and Putney, 1988). Berridge and Galione (1988) suggest that there are both receptor-controlled and second messenger oscillator mechanisms that effect Ca²⁺ uptake and Ca²⁺ release. In the presence of Ca²⁺ and ATP, the endoplasmic reticulum Ca²⁺ pumps form a 100 kDa phosphorylated intermediate which can be blocked by VO₄⁻ (Imamura and Schulz, 1985). Vanadate prevents non-mitochondrial Ca²⁺ uptake by inhibiting Ca-ATPase activity in pancreatic acinar cells and rabbit irisciliary body (Simons, 1979; Streb et al., 1983; Socci and Delamere, 1988). The uptake of mitochondrial calcium can be inhibited by sodium azide (Streb and Schulz, 1983). Although vanadate and azide were included in the medium in all experiments it was surprising that IP₁ and IP₂ stimulated an apparent uptake of Ca²⁺ into whole tick salivary glands. Of all the inositol phosphates identified in cells to date, only IP₃ has been clearly shown to have a "second messenger" function (Berridge, 1987). These results suggest a role for either or both IP₁ and IP₂ in stimulating an uptake of Ca²⁺.
into Ca$^{2+}$ sequestering pools unaffected by vanadate or azide. IP$_1$ and IP$_2$ did not stimulate an uptake of Ca$^{2+}$ into microsomal tissue. Clearly more research is needed to elucidate the physiological roles of IP$_1$ and IP$_2$.

The sequential additions of IP$_2$ and IP$_3$ to whole glands produced a significant difference only when IP$_2$ was added first. This could be due to unmetabolized IP$_3$ remaining in the medium which continues to stimulate a release of Ca$^{2+}$ when IP$_2$ is subsequently added. When IP$_1$ and IP$_2$ were added without prior addition of IP$_3$ to the bathing medium a significant uptake of Ca$^{2+}$ into permeabilized whole glands was observed. GTP and polyethylene glycol (PEG) were included in the bathing medium when testing the effects of inositol phosphates on Ca$^{2+}$ release in microsomal tissue. Preliminary data indicated that without their inclusion results were inconclusive (data not shown). Dawson et al. (1986) suggested that a protein, phosphorylated by GTP, binds to the microsomal membranes before IP$_3$ can stimulate Ca$^{2+}$ release and that the binding of this protein is favored by the presence of PEG, a fusogen that forms membrane bound vesicles. Thomas (1988) concluded that GTP increases the proportion of the sequestered Ca$^{2+}$ which is available for release by IP$_3$, either by unmasking latent IP$_3$-sensitive Ca$^{2+}$ release sites or by allowing direct Ca$^{2+}$ movement between IP$_3$-sensitive and IP$_3$-insensitive Ca$^{2+}$ storage pools.

The role of IP$_3$ in stimulating a release of Ca$^{2+}$ from feeding tick salivary glands has been clearly demonstrated.
Although the formation of inositol phosphates and the signal transduction pathway exists in tick salivary glands (McSwain et al., 1989), a possible physiological function for the breakdown products of IP$_3$ is of additional interest. Functions for inositol phosphates other than IP$_3$ are mostly unknown; however, because the enzymes which effect their formation are quite specific (Majerus, 1986), it is speculated that the various inositol phosphates should control or effect vital cellular functions. As discussed earlier, IP$_1$ and IP$_2$ appear to stimulate an uptake of Ca$^{2+}$ into vanadate and azide insensitive pools of permeabilized whole salivary glands. Many secretory cells use IP$_3$ to generate the calcium signal required to control either ion permeability or the release of granules by exocytosis (Berridge, 1986). Sequential events in the latter process are poorly understood. Tick salivary glands perform an array of functions during tick feeding (Kaufman, 1989). Many of its cells are filled with granules which later disappear and are likely secreted as the tick progresses through feeding. If IP$_1$ and IP$_2$ stimulate an uptake of Ca$^{2+}$ into the vesicular granules of tick salivary glands, one might speculate that there is a possible role for these molecules in granular secretion. Another possible explanation for the apparent uptake of Ca$^{2+}$ in response to IP$_1$ and IP$_2$ is the complexing of free Ca$^{2+}$ with phosphates derived from the metabolism of inositol phosphates. In this case the IP$_3$ stimulated release of free Ca$^{2+}$ would be much greater than observed. These
highly speculative proposals await further investigation to
determine the cellular functions of Ca$^{2+}$ in response to IP$_1$
and IP$_2$. 
REFERENCES


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Master of Science

Thesis: THE ROLE OF INOSITOL 1,4,5-TRISPHOSPHATE IN MOBILIZING CALCIUM FROM INTRACELLULAR STORES IN THE SALIVARY GLANDS OF AMBLIOOMMA AMERICANUM (L.)

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