

A Possible Signal Transduction Pathway for Amphotericin B-Induced IL-1 β Expression in Human Monocytic Cells

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INTRODUCTION

Amphotericin B formulations (AmB) remain the treatment of choice for severe mycotic infections.

AmB usage is hindered by adverse reactions (ADRs) such as:

Chronic: nephrotoxicity, anemia, hypokalemia, and hypomagnesemia.

Acute: fever, chills, hypotension, and cardiac arrhythmias.

AmB has been shown to increase transcription, translation, and subsequent expression of IL-1 β in human monocytes.

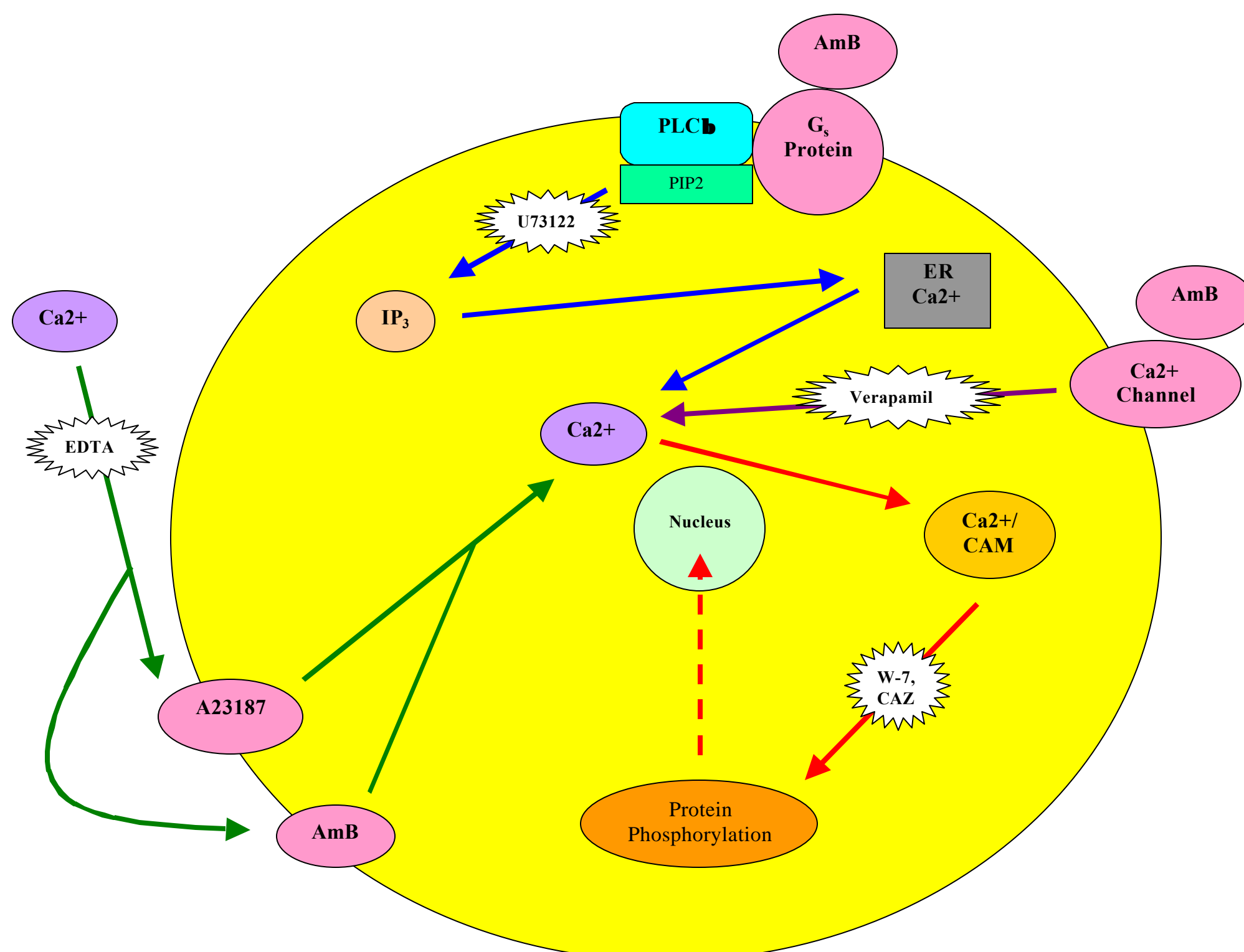
IL-1 β is believed to play a role in AmB associated ADRs.

Hypotheses:

- **I:** AmB increases intracellular calcium concentrations ($[Ca^{++}]_i$) by acting as a calcium ionophore.
- **II:** AmB utilizes calcium in its signal transduction pathway for IL-1 β in human monocytic cells.

Figure 1

Proposed Roles of Calcium in the Signal Transduction Pathway of AmB-Induced IL-1 β in Human Monocytic Cells and Specific Pharmacologic Inhibitors



METHODS

Pharmacologic Inhibition Studies:

Human monocytic cells (THP-1) were suspended at 1×10^6 cells/mL
One milliliter aliquots were distributed in 24-well culture plates
Cells were equilibrated at 37° in 5% CO₂ for 24 hours in supplemented media
Cells were exposed to experimental agents for 2 hours
Protein (IL-1 β) expression measured from supernatants via ELISA
Cell viability was evaluated by erythrosin B exclusion

Experimental agents (Refer to Figure 1):

- AmB 2.7 - 5.4 μ M (2.5 - 5 μ g/mL) alone
- A23187 2.5 - 40 μ M alone (Calcium ionophore)
- EDTA 10 μ M alone (Extracellular calcium chelator)
- EDTA 10 μ M + AmB 2.7 - 5.4 μ M
- Calmodulin (CAL) 1.2 μ M alone
- Calmodulin 1.2 μ M + AmB 2.7 - 5.4 μ M
- Calmidazolium (CAZ) 0.001 μ M alone (CAL antagonist)
- W7 0.1 μ M alone (CAL antagonist)
- Calmidazolium (CAZ) 0.001 μ M + 2.7 - 5.4 μ M
- W7 0.1 μ M + 2.7 - 5.4 μ M
- U73122 0.001 μ M alone (Phospholipase C inhibitor)
- U73122 0.001 μ M + 2.7 - 5.4 μ M

Intracellular Calcium Concentration Studies:

THP-1 monolayers adhering to glass coverslips were incubated with 2 μ M of the fura-2 AM for 45 min.

The cells were then placed into a 1 mL flow-through cuvette housed in a spectrofluorometer fitted with a water-jacketed cuvette holder maintained at 37°C and superfused with HBSS/15 mM HEPES buffer at a rate of about 3 mL/min.

Fura-2 emission fluorescence at 510 nm was monitored continuously as the excitation wavelength alternated between 340 and 380nm.

The fluorescence at each excitation wavelength was corrected for cell autofluorescence and the ratio of fluorescences was used to estimate $[Ca^{++}]_i$.

Appropriate corrections were made for the effects of AmB on autofluorescences.

RESULTS

Figure 2

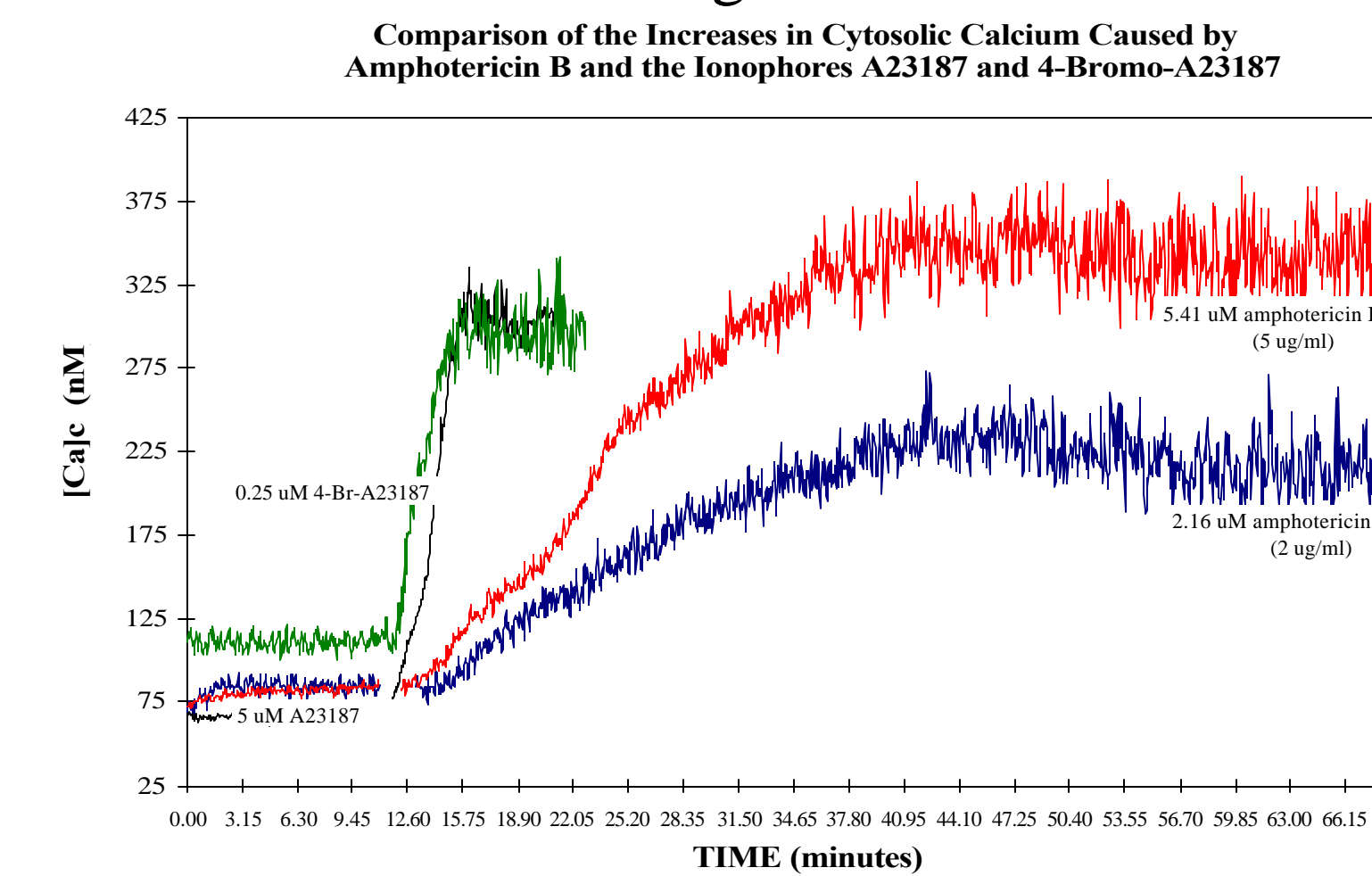


Figure 3

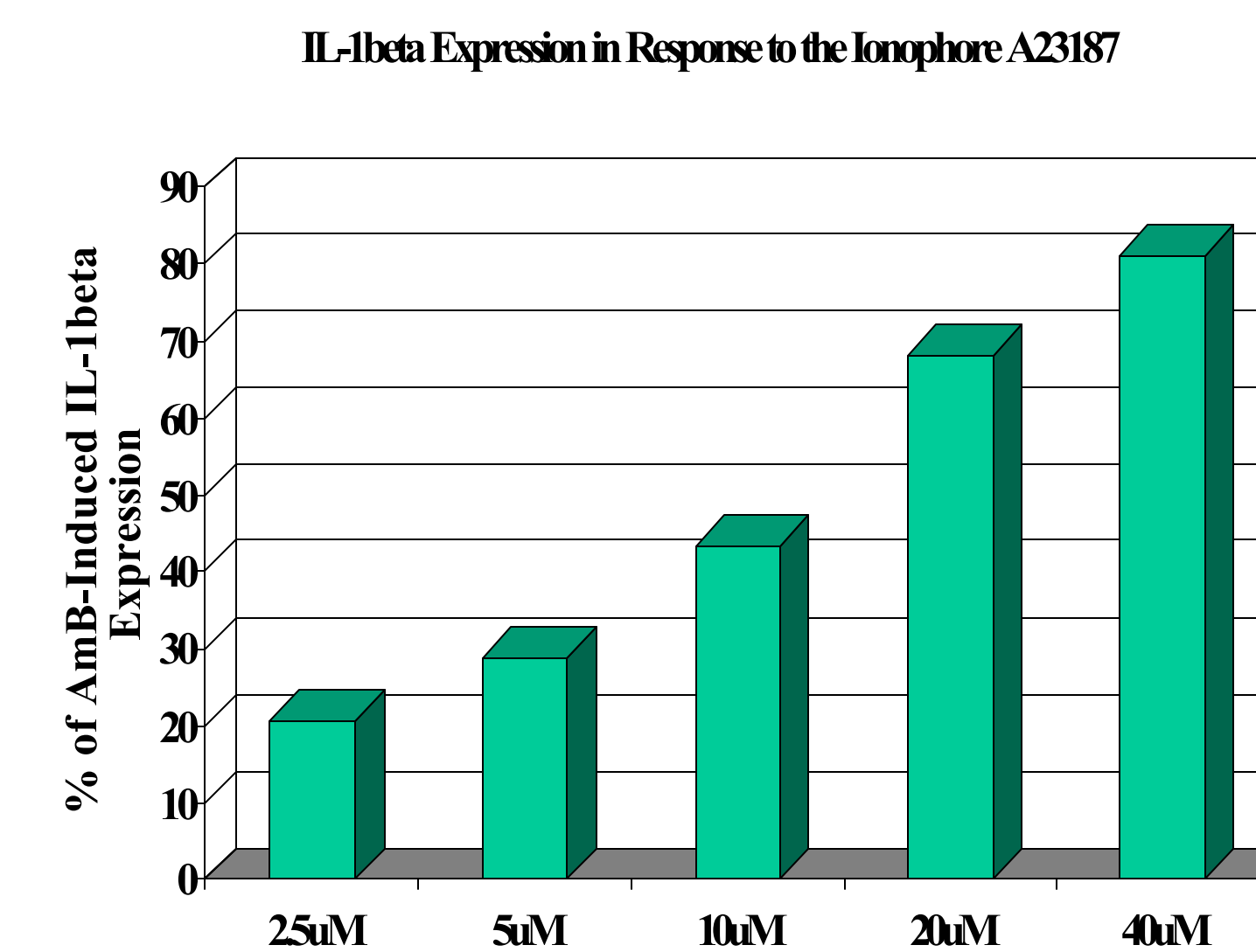


Figure 4

Extracellular Calcium Modulation

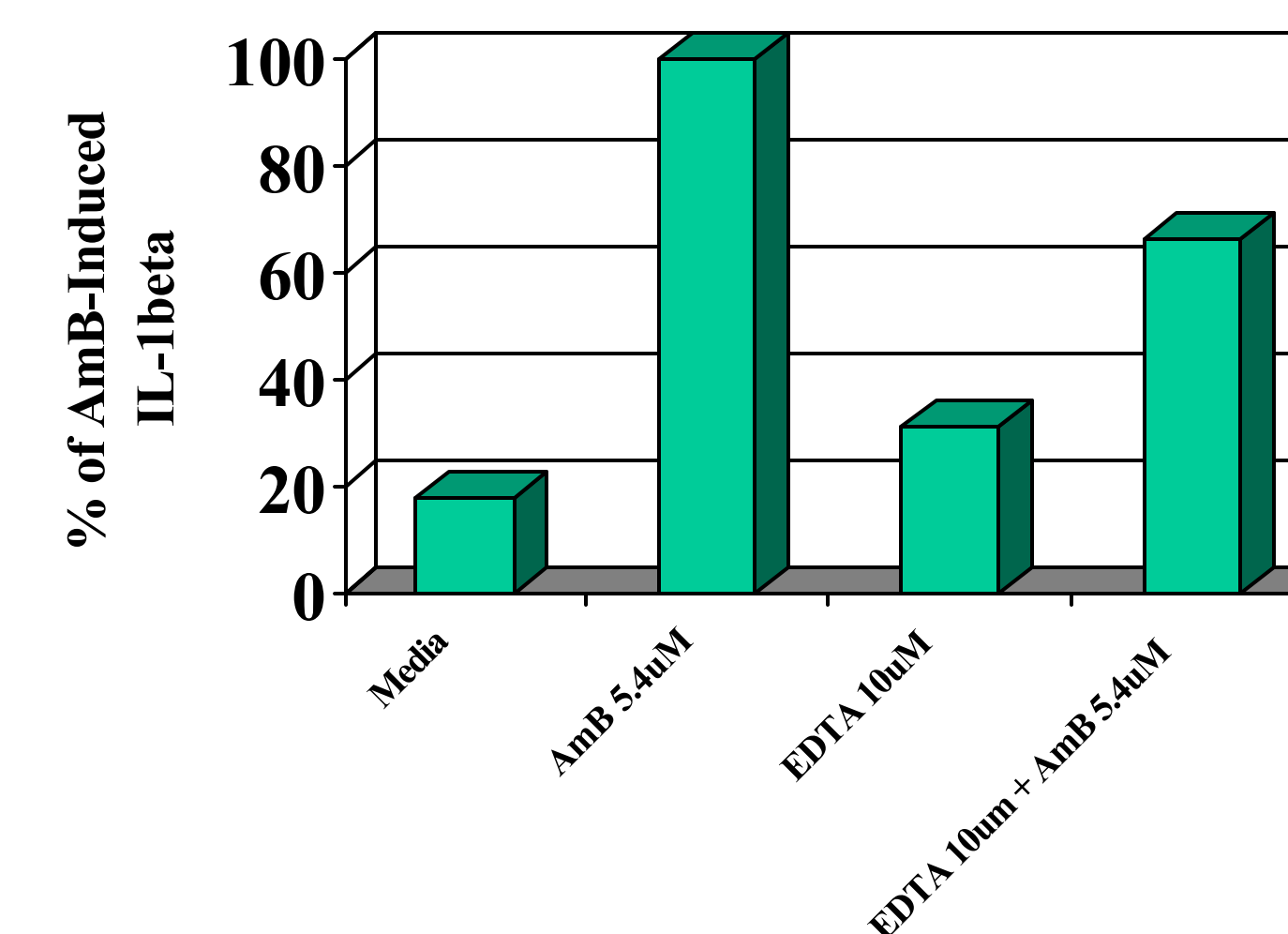
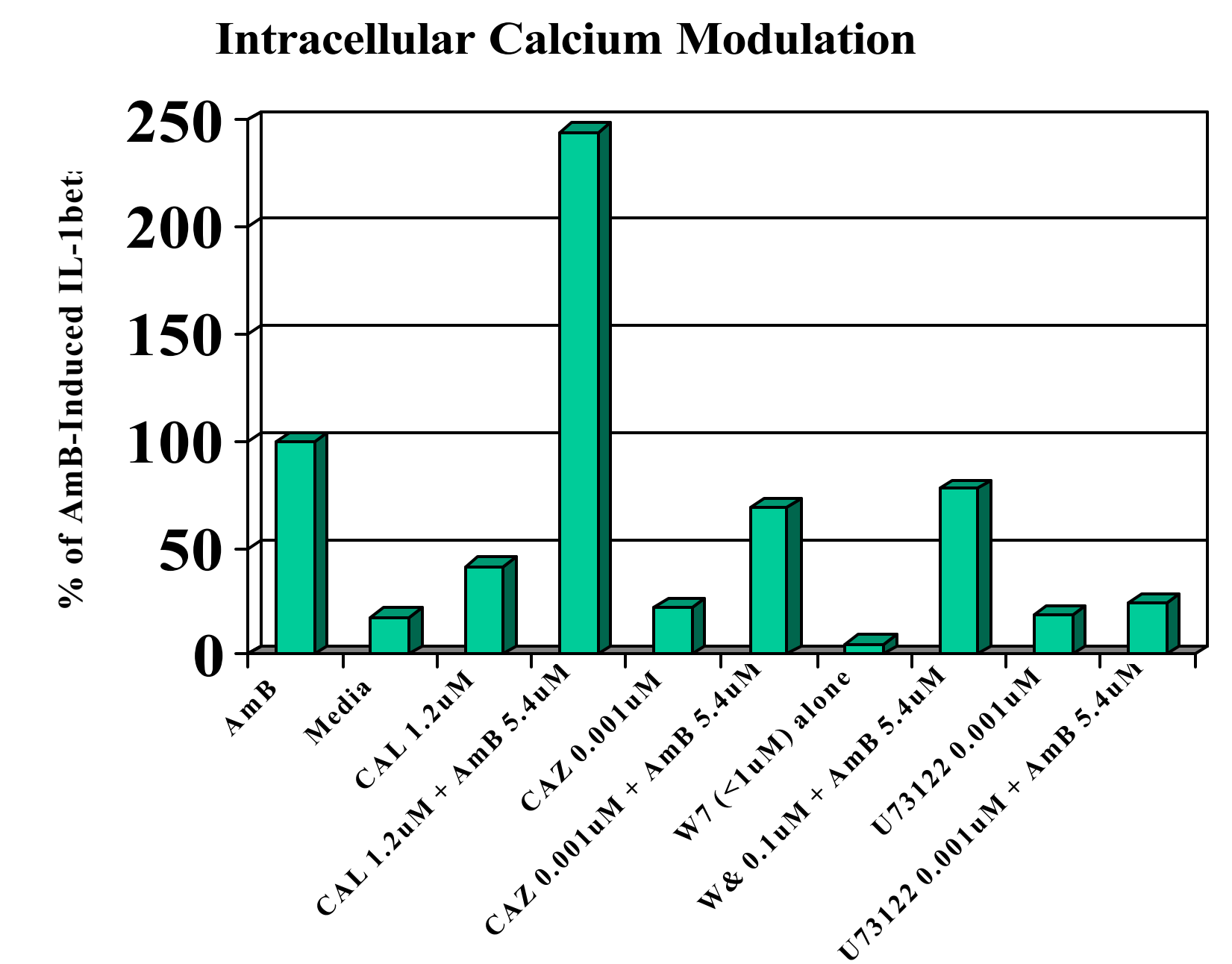


Figure 5



DISCUSSION

The rapid increase in $[Ca^{++}]_i$, as well as the dose dependent increase in IL-1 β in response to A23187 suggests that elevated $[Ca^{++}]_i$ is sufficient to cause expression of IL-1 β in these cells (Figures 2&3).

The observation that AmB causes elevations in $[Ca^{++}]_i$ similar in magnitude to those observed with A23187 (Figure 2) suggests that AmB acts as a calcium ionophore to initiate the calcium mediated signal transduction of IL-1 β . This is supported by the observation that the addition of EDTA attenuates the IL-1 β response to AmB (Figure 4).

The inhibition of AmB-induced IL-1 β by CAZ and W7, as well as the enhancement on AmB-induced IL-1 β expression, suggests a role for calcium/calmodulin in AmB-induced IL-1 β signal transduction (Figure 5).

The inhibition of AmB-induced IL-1 β by U73122 suggests a role for phospholipase C in this process (Figure 5).

CONCLUSION

AmB appears to increase $[Ca^{++}]_i$ thus acting as a calcium ionophore.

AmB utilizes calcium/calmodulin in its signal transduction of IL-1 β in human monocytic cells.

Further evaluation of the role of intracellular calcium stores and the mechanism for increased intracellular calcium is warranted.