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"CALCIUM AS A POSSIBLE SECOND MESSENGER OF EXTERNAL SIGNALS IN LEISHMANIA DONAVANI". CO-9

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Leishmania donovani, a protozoan pathogen of human, is an obligate intracellular parasite that grows within lysosomes of macrophages and in alimentary tract od sandflies. The identification of the host by the parasite and its subsequent adaptive mechanisms to its new environment might involve the transduction of signals from the host to the parasite. The possibility that calcium plays a role in mediating such signals was investigated. By using the fluorescent CA^{2+} indicator FURA-2, we show that the concentration of free calcium in the cytoplasm of Leishmania donovani promastigotes is maintained at very low levels (73.5+/-10 - 94+/-8 NM AT (CA^{2+})0 range of 0 - 1MM). The maintenance of low (CA^{2+})I is energy dependent as it is disrupted by KCN, H+/-ATPASE inhibitors, and ionophores KCN, nigericin and DCCD increase cytosolic free calcium by mobilizing calcium from intracellular pools. Monensin and oligomycin increase (CA2+)I by allowing influx of calcium from the external medium through the plasma membrane but have no effect on intracellular pools. Intracellular traffic of calcium was examined by measuring the transport of $45Ca^{2+}$ in Digitonin permeabilized promastigotes. Two transport sytems for calcium were identified in these cells. One is respiration dependent, suggesting a mitochondrial localization. A second system is respiration indepent but requires either endogenous or externally added ATP. The ATP-dependent CA^{2+} transport is optimal at pH 7.1, has high affinity for calcium (KM=92 NM, VMAX=1 NMOLES/MIN/MG protein) and is sensitive to orthovanadate.

These properties suggest the presence of a CA2+/-ATPASE similar to that of mammalian endoplasmic reticulum. In preliminary experiments we found that GTP and inositol 1,4,5-triphosphate, caused a release of calcium from the endoplasmic reticulum of digitonin permeabilized promastigotes. Taken together, our results indicate that (CA2+)I in Leishmania donavani promastigotes is regulated at low concentration by mechanisms similar to those found in higher eukayotic cells.