MASTER'S THESIS

The role of calcium in control of animal cell proliferation: ornithine decarboxylase induction by L-asparagine in Reuber H-35 hepatoma cell

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Date of Award:
1991

THE ROLE OF CALCIUM IN CONTROL OF ANIMAL CELL PROLIFERATION: ORNITHINE DECARBOXYLASE INDUCTION BY L-ASPARAGINE IN REUBER H-35 HEPATOMA CELLS

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A thesis submitted in partial fulfillment of the requirements for the degree of Postgraduate Studies Committee for the degree of Master of Philosophy

September 1991

Hong Kong Baptist College

ABSTRACT

Cellular ornithine decarboxylase (ODC, L-ornithine carboxyl-lysase; EC 4.1.1.17) can be induced by each of the transport system A, ASN or N amino acids, most potently asparagine. The involvement of calcium in various cellular processes including cell proliferation has been well established. ODC is the rate-limiting enzyme in the biosynthesis of polyamines which are required for cell proliferation.

In this study, the role of Ca^{2+} in asparagine-stimulated ODC induction was investigated in Reuber H-35 hepatoma cells. Dose response effect of extracellular Ca^{2+} on enhancing ODC induction was observed. Additions of $CaCl_2$ above 0.1 mM to the culture medium enhanced asparagine-elicited ODC activity to maximal level. When extracellular Ca^{2+} was removed, ODC activity decreased significantly. Moreover, asparagine-stimulated ODC activity was strongly antagonized by the addition of a calcium flux blocker Lanthanum ion (La^{3+}) or a Ca^{2+} chelator EGTA (ethylene glycol (β-aminoethyl ether)-N-N' tetra-acetic acid). Additional extracellular Ca^{2+} was able to reverse the EGTA-inhibited ODC induction. These observations indicate the requirement of extracellular Ca^{2+} for a favorable ODC induction. Using a Ca^{2+} ionophore, ionomycin, and a blocker of Ca^{2+} release from intracellular store, TMB-8 (8-(N,N-diethyl amino)octyl-3,4,5,-trimethoxybenzoate), it was found that depletion of intracellular stored Ca^{2+} or blockage of intracellular Ca^{2+} release could not inhibit asparagine-stimulated ODC activity. Accordingly, we are able to conclude that extracellular Ca^{2+}, but not intracellular Ca^{2+}, is important for ODC induction.

Several types of voltage dependent calcium channel (VDCC), namely L, N and T, that mediate Ca^{2+} entry across cell membrane, were investigated. Using VDCC blockers, $NiCl_2$, $CdCl_2$, verapamil and nifedipine, it was found that L and N type VDCC channels are probably involved in the process of ODC induction, probably by mediating influx of extracellular Ca^{2+}.

The involvement of calmodulin in a variety of Ca^{2+}-dependent cellular functions has been well established. The effect of the calmodulin antagonist W_7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide) on ODC induction was

investigated. It was shown that the enzyme induced by asparagine was potently blocked by W_7 with 50% inhibition at 23 μM, indicating that ODC induction is likely to be mediated by a Ca^{2+}-calmodulin regulatory process.

Initial investigation in the role of cyclic AMP and protein kinase C systems in ODC induction was conducted. Using cAMP modulators Rp- and Sp-cAMP, isomers of adenosine 3'-5'-monophosphorothioate, we found that Rp-cAMP, an antagonist of cAMP-dependent protein kinases, had no inhibition effect on asparagine-elicited ODC induction and that the Sp-cAMP, an agonist of cAMP-dependent protein kinases, could neither induce ODC activity by itself nor enhance asparagine effect on ODC induction. However, protein kinase C inhibitor H_7 (1-(5-isoquinolinesulfonamyl)-2-methylpiperazine), potently inhibited asparagine-induced ODC activity with 50% inhibition at 60 μM H_7. It can be concluded that protein kinase C, but not cAMP system, is involved in ODC induction by asparagine.

It appears that the involvement of calcium in ODC induction requires participation of calmodulin regulation which collaborates with protein kinase C other than cAMP dependent protein kinases.

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