SPECTROSCOPIC STUDIES OF INHIBITION OF CALMODULIN ACTIVITY BY SOME DRUGS

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Abstract — The effect of four inhibitors on calmodulin (CaM) were studied by a fluorescence and ultraviolet techniques. four compounds [N-(6-aminohexyl) 5-chloro - 1 - napthalenesulphonamide] (W-7), 1 - [bis - (4 - chlorophenyl) methyl] - 3 - [2, 4-dichloro - β - (2,4 - dichlorobenzyloxyl) phenethyl] imidazolium chloride (R24571), trifluoperazine (TFP), thiodiphenylamide chloride (TDPAC) showed inhibitory effect on bovine brain phosphodiesterase (PDE) induced by CaM. The concentration of inhibitors producing 50% inhibition of Ca^{2+} / CaM activity (IC_{50}) and the Hill coefficient were correlating closely between the methods, K_i's and thermodynamic parameters for these interactions were estimated.
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INTRODUCTION

Ca^{2+} ion, as a second messenger in several different cell processes plays an important role in cell function (1). Evidences suggest that proteins such as calmodulin (CaM) can act as Ca^{2+} receptor in such processes (2-5). Some Ca^{2+} ion functions include, Ca^{2+} ability to regulate tropomyosin - troponin contractile system. Vascular smooth muscle contraction is also CaM dependent phosphorylation of myosin light chain kinase (6). CaM is a protein with a molecular wieght of 16500 dalton, and is ubiquitos in all eukaryotic cells (7). Many drugs of CaM inhibitory action were studied among which, are Ca^{2+} chelating agents, (8), smooth muscle relaxant (9), local anesthetics (10) neuropeptides and proteins (11-13). Many of these drugs contain hydrophobic region and a positive charge at physiological pH (12,13), which appear crucial in producing a direct complexation with CaM, although some other CaM antagonists, also inhibit protein kinase Competitively with its phospholipid cofactor (14). The modes of Ca^{2+} bindings to CaM and the sites of Ca^{2+} bindings are well established (15). It is suggested that upon such bindings conformational changes occur with large incrases in α - helical content of the protein (16,17). As a result of such bindings, hydrophobic binding sites that are capable of bindings to other proteins or inhibitors are exposed (18,19). A mechanism for the modes of activation and inhibition is described by Mettzer and coworkers (20).

CaM inhibitors have important pharmacological significance in that they provide both an understanding towards the mechanism by which drugs alter CaM action and also to explore further the physiological role of the calcium binding protein, i.e. the antipsychotic action associated with phenotiazine drugs, or the compound R24571 (Calmidazolium) is known to have antimycotic action. Other characteristics such as hypotensive effect is also found with some CaM inhibitors. To investigate further the modes of action and the relative effect of some CaM inhibitory drugs, we studied the inhibitory effect of compounds such as TFP. W-7, TDPAC, R24571 by fluorescence and UV, using direct method and also by using CaM activation of bovine brain phosphodiesterase inhibition and aquired kinetic and thermodynamic data which could reveal some mechanistic aspect of drug action as well as insight into the nature of bindings.

MATERIALS AND METHODS

Calmodulin purified from bovine brain with a 95-97% purity according to the method described previously (15,21). The degree of its purity was measured on the basis of its phosphodiesterase activation properties and electrophoretic migration in polyacrylamide gels, in the presence of sodium dodecyl sulfate (SDS) (22). Bovine brain cyclic 3', 5' nucleotide phosphodiesterase was prepared according to the method of Wallace and coworkers (23). W-7. phenothiazines, snake venom and buffer reagents such as mops, and tris, were obtained from Sigma. Calmidazolium (R24571) was obtained from Seikagaku Kogyo Co. (Tokyo, Japan). All other reagents were of analytical grade or the highest purity available. CaM concentrations were determined by measuring its phosphodiesterase activation as described previously (24). Extinction coefficient for the purified CaM ($\varepsilon^{1\%}_{220}$ = 3.3) was also used for such assays. Protein concentration was determined by Lowry method using serum albumin as standard (25). SDS gel electrophoresis