REGULATION OF MULTIPLE FORMS OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE FROM BOVINE HYPOTHALAMUS: New Factors Modulating Enzyme Activity*

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Studies of bovine hypothalamic cyclic nucleotide phosphodiesterase (PDE) indicate the presence of several peaks of PDE activity, distinguishable by DEAE-cellulose column chromatography, displaying different substrate specificities, kinetic behavior, and regulatory properties. Evidence is presented that chromatographically separated forms of PDE activity are subject to control by Ca$^{2+}$-calmodulin, cyclic nucleotides, limited proteolysis, reagents affecting sulfhydryl groups, and neurohormone "C"—one of several new cardioactive compounds isolated from hypothalamic magnocellular nuclei of animals—in a complex substrate-specific and concentration-dependent manner. Of particular interest is the finding that each of the forms of cGMP PDE, being Ca$^{2+}$/calmodulin-dependent, possesses sensitivity to activation by cAMP, especially under conditions favoring the oxidation of thiol groups of PDE, resulting in a loss in responsiveness of the enzyme to the activation by calmodulin. This effect appears to be relatively stable but readily reversible by sulfhydryl reducing reagents, which restore both the cGMP PDE sensitivity to competitive inhibition by cAMP and the responsiveness of the enzyme to activation by calmodulin. A reinterpretation of the regulatory properties of multiple forms of PDE is proposed.

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INTRODUCTION

The role of cyclic nucleotides in central nervous system function, with cyclic adenosine 3',5'-monophosphate (cAMP) and perhaps cyclic guanosine 3',5'-monophosphate (cGMP) acting as second messengers for various neurotransmitters, neurohormones, drugs, and other biologically active compounds, is well established.

The cyclic nucleotide system comprises a complex group of distinct enzymes catalyzing the synthesis and degradation of cAMP and cGMP—adenylate cyclase, guanylate cyclase, and cyclic nucleotide phosphodiesterase (PDE).

PDE (EC 3.4.1.17) catalyzes the conversion of cAMP and/or cGMP to their respective nucleotide 5'-monophosphates and shares with adenylate cyclase and guanylate cyclase the important function of delicate control of the intracellular cyclic nucleotide levels and consequently their effects.

Multiple forms of PDE displaying different molecular size and charge, kinetic behavior, substrate specificities, regulatory properties, and cellular and subcellular distribution have been described in mammalian tissues (for review, see ref. 1). However, little is known of the structural and functional interrelationship between the multiple forms of the enzyme.

The present study was undertaken to further characterize the properties of cyclic nucleotide PDE from the hypothalamus—an organ which plays a most significant role in the regulation of neuroendocrine functions. In this paper we present evidence that chromatographically separated forms of PDE activity in the bovine hypothalamus are subject to control by Ca\(^{2+}\)-calmodulin (CaM), cyclic nucleotides, partial proteolysis, reagents affecting sulfhydryl groups, and neurohormone "C" (NC)—one of several new cardioactive compounds isolated by A. A. Galoyan from the hypothalamic magnocellular nuclei of animals (2)—in a complex substrate-specific and concentration-dependent manner.

A reinterpretation of the regulatory properties of multiple forms of PDE is proposed.

EXPERIMENTAL PROCEDURE

Materials. [8-\(^{3}\)H]cAMP (21 Ci/mmol) and [8-\(^{3}\)H]cGMP (19 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks. (U.K.). Cyclic AMP, cGMP, 5'AMP, 5'GMP, EGTA*, trypsin from bovine pancreas, soybean trypsin inhibitor, BSA, p-chlo-

* Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; DEAE, diethylaminoethyl; EGTA, ethylene glycol bis (β-aminoethyl ether)-N,N'-tetraacetic acid; PDE, phosphodiesterase; CaM, calmodulin; NC, neurohormone "C".