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Effects of Some Hormones on Enzyme Activities of Carbonic Anhydrase from Brain *in vitro*

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Hormones are 3 classes as steroid hormones, peptide and proteohormones and derived hormones from amino acids. The influence of PTH, E_2, T_3, T_4, TSH and cortizole hormones has been investigated on bovine brain carbonic anhydrase *in vitro*. The effect of each hormone on carbonic anhydrase was investigated by Wilbur-Andersen method modified by Rickly and coworkers. All of the hormones were determined to have inhibition effect, on bovine brain carbonic anhydrase isoenzyme. The I_{50} values of hormones caused inhibition were determined by means of activity percentage [I] diagrams. The values were PTH, E_2, T_3, T_4, TSH and cortizole were I_{50}: 1.54×10^{-14} M, 1.72×10^{-11} M, 3.6×10^{-8} M, 4.48×10^{-13} M, 5.73×10^{-9} M, 1.22×10^{-11} M for bovine brain carbonic anhydrase, respectively.

Keywords: Hormones, Carbonic Anhydrase, Brain, Zn^{2+}.

INTRODUCTION

Carbonic anhydrase (CA, EC 4.2.1.1) isozymes are a family of zinc metalloenzymes, which catalyze the interconversion of CO_2 and HCO_3^- [1]. The enzyme discovered about long times years ago [2-4], is abundantly present in mammalian red blood cells and to a lesser extent in different types of tissues and secretory organs [5,6]. In addition, carbonic anhydrase from plant, yeast and bacteria have been reported and partially characterized [5-11]. The important roles of the enzymes in various cell types have been extensively reviewed [5,6]. At present, approximate 14 isozymes (I-XIV) are reported and found to be distributed throughout the living organisms [5,6,11].

The endocrine (or hormonal) system controls many crucial aspects of the working of the body, for example development of sexual characteristics and development of the brain. Hormones generally carry fairly long-lasting messages, in contrast to the rapid signalling of the nervous system.

Carbonic anhydrase was a critical enzyme, which can be thought to be affected by hormones, which are widely consumed. This *in vitro* study planned in order to investigate the affect of hormones with or without on the enzyme, which is important with respect to human health.

EXPERIMENTAL

Purification of carbonic anhydrase from bovine brain tissue: Brain tissue from bovine brain membrane was kept in physiological saline and then washed with 0.09 % NaCl solvent until erythrocyte was completely removed from the medium. Carbonic anhydrases isoenzyme from bovine brain was purified by means of affinity column having a structure of Sepharose 4B-L-tyrosine-sulfonyamide [12] and the study was carried out with these enzymes. The elutes were plotted by doing protein determination at 280 nm and CO_2-hydratase activity [13] and the purification was controlled with SDS-PAGE [14].

Determination of carbonic anhydrase activity and effect of hormones on isoenzymes: Carbonic anhydrase activity and effect of hormones was assayed by hydration of CO_2 was measured by the method of Rickli *et al*. [13] and Wilbur-Anderson with bromothymol blue as indicator. CO_2-Hydratase activity as enzyme unit (EU) was calculated by the equation $(t_o\text{-}t_c/t_c)$ where t_o and t_c are the times for pH change of the non enzymatic (buffer) and the enzymatic reaction, respectively.

Determination of I_{50} values: The values of I_{50} (hormone concentration reduced the enzyme activity by 50 %) have been determined graphically using seven different hormone concentration. For the hormones shown inhibition effect, the values of I_{50} with diagram of activity per cent [I] were calculated.

RESULTS AND DISCUSSION

Many chemicals at relatively low dosage affect the metabolism of biota by altering normal enzyme activity, particularly inhibition of a specific enzyme [15]. The effects can be dramatic and systemic [16]. Indeed, carbonic anhydrase isoenzymes are important enzymes for body metabolism because they regulate pH in most tissue. Therefore, in the

present study, investigation of effects of certain hormone on bovine brain carbonic anhydrase was proposed. Parathormone (PTH), estradiol (E_2), thyroid hormone (T_3), thyroid hormone (T_4), thyroid stimulating hormone (TSH) and cortizol (COR), as hormones were chosen for the investigation of inhibition or activation effects.

For the hormones exhibitimg effect, the inhibitor concentrations causing up to 50 % inhibition (I_{50} values) were determined from the regression analysis graph. I_{50} values obtained for of bovine brain carbonic anhydrase are shown in Table-1.

TABLE-1
VALUES OF I_{50} TERMS OF MOLARITY OF THE TEST HORMONES CAUSING A 50 % REDUCTION OF BOVINE BRAIN CARBONIC ANHYDRASE ISOENZYME ACTIVITY

Hormones	Brain carbonic anhydrase
Parathormone (PTH)	1.54×10^{-14} M
Estradiol (E_2)	1.72×10^{-11} M
Thyroid hormone (T_3)	3.60×10^{-8} M
Thyroid hormone (T_4)	4.48×10^{-13} M
Thyroid stimulating hormone (TSH)	5.73×10^{-9} M
Cortizol (COR)	1.22×10^{-11} M

To show inhibition effects, activity % values of carbonic anhydrase for five different concentrations of each hormones were determined (Figs. 1-6). All of the hormones which we were investigated their effects in our study in different levels effected carbonic anhydrase. The results show that the inhibition effects of PTH, E_2, T_3, T_4, TSH and cortizole were I_{50}: 1.54×10^{-14} M, 1.72×10^{-11} M, 3.6×10^{-8} M, 4.48×10^{-13} M, 5.73×10^{-9} M, 1.22×10^{-11} M for bovine brain carbonic anhydrase, respectively.

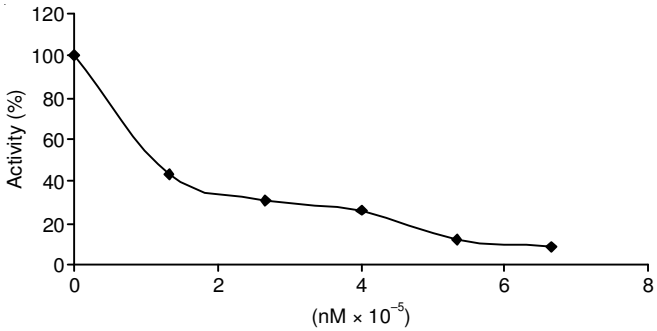

Fig. 1. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of parathormone (PTH) for five different concentrations

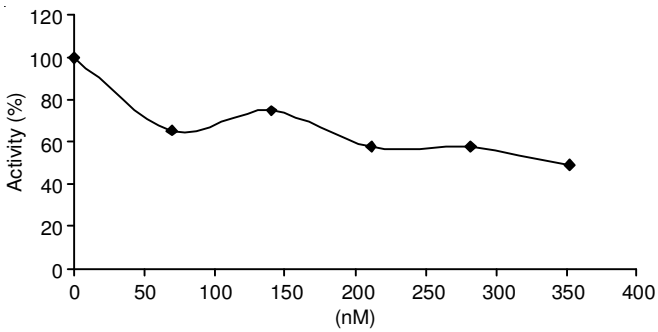

Fig. 2. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of thyroid hormone (T_3) for five different concentrations

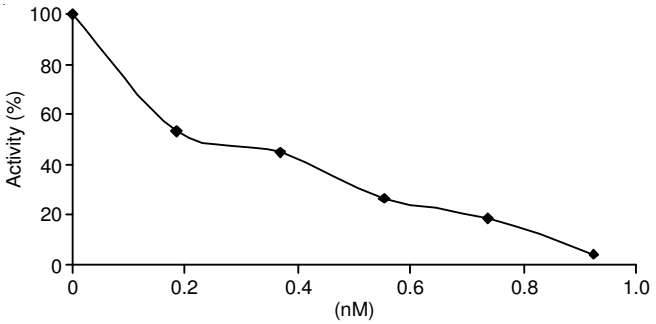

Fig. 3. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of estradiol (E_2) for five different concentrations

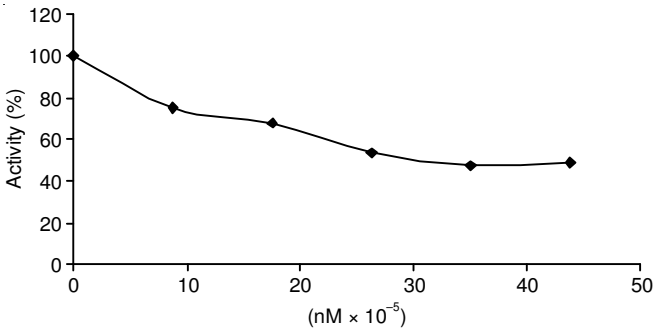

Fig. 4. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of thyroid hormone (T_4) for five different concentrations

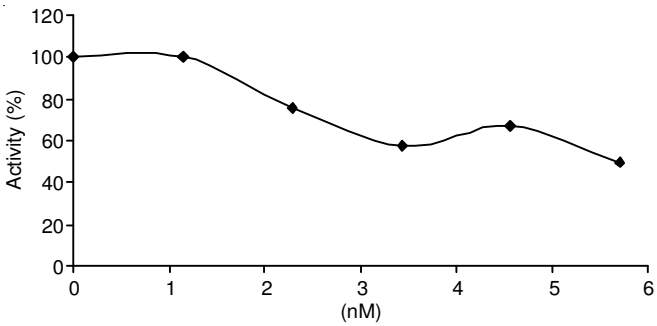

Fig. 5. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of thyroid stimulating hormone (TSH) for five different concentrations

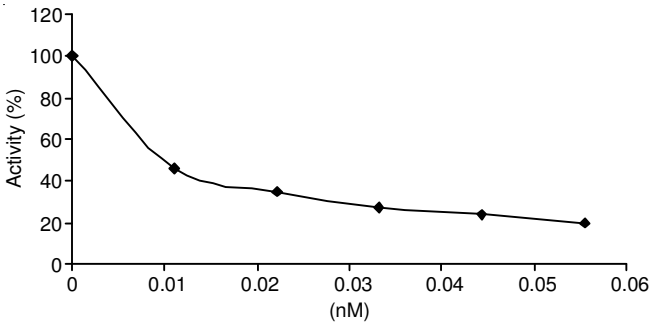

Fig. 6. Activity % *vs.* [hormone] regression analysis graphs for brain carbonic anhydrase in presence of cortisol (COR) for five different concentrations

It is generally recognized that carbonic anhydrase controls the bulk of carbon dioxide exchange between blood and tissues as well as the regulation of proton and other ion movements between cells and extracellular fluids. All of the carbonic

anhydrase isoenzymes are also deeply involved in a great number of secretory activities including fluid movements [17]. Since carbonic anhydrase is a very important enzyme for the body, the inhibition effects of these hormones should be considered for not only bovine brain carbonic anhydrase isoenzyme but also all carbonic anhydrase isoenzymes.

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