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

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**INTRODUCTION**

Carbonic anhydrase (CA, EC 4.2.1.1) isozymes are a family of zinc metalloenzymes, which catalyze the interconversion of CO₂ and HCO₃⁻ [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 effect 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 anhydrase 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 CO₂-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 CO₂ was measured by the method of Rickli et al. [13] and Wilbur-Anderson with bromothymol blue as indicator. CO₂-Hydratase activity as enzyme unit (EU) was calculated by the equation (tₙ₀-tₙ/tₙ₀) where tₙ₀ and tₙ are the times for pH change of the non enzymatic (buffer) and the enzymatic reaction, respectively.

**Determination of I₅₀ values**: The values of I₅₀ (hormone concentration reduced the enzyme activity by 50%) have been determined graphically using seven different hormone concentrations: PTH, E₂, T₃, T₄, TSH and cortizole were I₅₀: 1.54 × 10⁻⁴ M, 1.72 × 10⁻³ M, 3.6 × 10⁻⁴ M, 4.48 × 10⁻⁵ M, 5.73 × 10⁻⁶ M, 1.22 × 10⁻⁷ M for bovine brain carbonic anhydrase, respectively.

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
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 exhibiting 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>
<thead>
<tr>
<th>Hormones</th>
<th>Brain carbonic anhydrase</th>
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</thead>
<tbody>
<tr>
<td>Parathormone (PTH)</td>
<td>$1.54 \times 10^{-14}$ M</td>
</tr>
<tr>
<td>Estradiol (E$_2$)</td>
<td>$1.72 \times 10^{-11}$ M</td>
</tr>
<tr>
<td>Thyroid hormone (T$_3$)</td>
<td>$3.60 \times 10^{-8}$ M</td>
</tr>
<tr>
<td>Thyroid hormone (T$_4$)</td>
<td>$4.48 \times 10^{-13}$ M</td>
</tr>
<tr>
<td>Thyroid stimulating hormone (TSH)</td>
<td>$5.73 \times 10^{-9}$ M</td>
</tr>
<tr>
<td>Cortizol (COR)</td>
<td>$1.22 \times 10^{-11}$ M</td>
</tr>
</tbody>
</table>

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 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 cortizol were I$_{50}$: $1.54 \times 10^{-14}$ M, $1.72 \times 10^{-11}$ M, $3.6 \times 10^{-8}$ M, $4.48 \times 10^{-13}$ M, $5.73 \times 10^{-9}$ M, $1.22 \times 10^{-11}$ M for bovine brain carbonic anhydrase, respectively.

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.

REFERENCES

7. F.P. Vetch and L.C. Blankenship, Nature, 197, 76 (1963); https://doi.org/10.1038/197076a0.