Functional Evaluation Of Angiotensin-Converting Enzyme (ACE) Changes Through Site-Directed Mutation

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Hypertension is a worldwide health problem considered to be one of the greatest public health challenges being a major risk factor for cardiovascular and renal disease. Currently, several studies have focused on the Renin-Angiotensin System, an endocrine system where renin cleaves angiotensinogen in the Leu10-Val11 bond and releases the decapeptide Angiotensin I (AI), which is converted into Angiotensin II (AII) by the action of Angiotensin Converting Enzyme I (ACE). The ACE has a large physiological importance, being a target enzyme of several therapeutic studies. This way the objective of this work is investigate the role of amino acids Asp140, Gln259, Ala332, Ser 333, Gln 355, Thr 358, Phe435 and Arg 500, strong candidates for interaction with the ACE inhibitor lisinopril. An analysis of sequence homology to the ACEs of *Xenopus laevis*, mouse rat and human, the human isoforma N-domain (ND) and isoforms of 65 and 90 kDa in rat and after alignment of sequences verify the conservation of amino acid residues proposed to be mutated. Primers were synthesized complementary chains containing the mutation, we used a vector containing the cDNA with the human ACE gene (pACE) followed by PCR. The products obtained from PCR were digested with the enzyme DpnI which is specific for DNA methylated and hemi-methylated, and cloned in the DH5 alpha bacteria. Finally, in the sequencing were confirmed the presence of the mutations in the all DNAs and we performed the alignment.

Word Keys: ACE, Site-Directed Mutation, Hypertension
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