Occurrence of angiotensin converting enzyme (ACE) in mammary gland and tongue taste epithelium was demonstrated for the first time. Six times higher ACE activity in lactating mammary gland, than non-lactating mammary gland, suggested pregnancy and lactation hormonal dependent expression of ACE in female mammals. ACE activity was highest in choroid plexus, less in spinal cord and moderate in cerebrum, medulla, cerebellum and pons. Distribution of ACE in different regions of skin, kidney and among other tissues was different. Presence of ACE in adrenal glands, pancreas, bone marrow and thyroid gland indicated functions other than blood pressure homeostasis for this enzyme.

**Keywords**: Angiotensin Converting enzyme, Body tissues, Sheep

Angiotensin converting enzyme (ACE, EC. 3.4.15.1) is a key enzyme of rennin-angiotensin-aldosterone system (RAS) that is involved in blood pressure and electrolyte homeostasis. It cleaves dipeptides from C-terminus of several peptide substrates. However, recent research has shown that this enzyme is involved in reproduction of male mammals and appears at puberty in testis, epididymis, prostate, seminal fluid under the influence of androgens and pituitary hormones. Even though this enzyme was identified in female reproductive organs like uterus, ovary, fallopian tubes, hormonal influence on expression of ACE in female mammals is unknown. Recently, gender differences in the expression of circulating as well as tissue RAS has been reported. In females, mammary glands are rudimentary before puberty and their development begins at puberty. Full development of mammary gland occurs during pregnancy and lactation under the influence of androgens and pituitary hormones. However, in male mammals mammary glands remain rudimentary.

ACE inhibitors are widely used in the treatment of hypertension, congestive heart failure, coronary artery disease and diabetic nephropathy. However, taste disturbance, skin rash, neutropenia are some of the side effects associated with use of ACE inhibitors. Taste disturbance includes absence of taste discrimination and metallic or sour taste. Different regions of tongue are responsible for various taste sensations due to presence of many types of epithelial cells. No information is available on ACE activity of epithelial cells of tongue from any animal species. Skin is the largest organ of the body and consists of epidermis and dermis, each with different functions and enzyme distributions. An unique feature of ACE is chloride dependent substrate specific hydrolysis. However, role of chloride on mammary and lingual ACE activity is unknown.

In this study ACE activity was measured in non-lactating lactating mammary gland of Ewe and mammary gland of Ram. ACE activity in different regions of tongue, skin, brain and other tissues was also measured. Influence of chloride on mammary and lingual ACE activity was determined. Permission from the ethic committee of the institute was sought to undertake the present study.

Tissues were obtained from local farm house and kept deep frozen at −70°C. Only parts of the tissues not involved in any disease process were used in the study. Lactating and non-lactating mammary glands were separated based on lactation and gender. Tongue was separated into anterior tongue, lateral tongue, central tongue and posterior tongue. Epithelial layer of each part of the tongue was obtained by incubating in (1 M) KCl for 5 h at 4°C and used for the preparations of tissue extract. Skin was removed from ear lobe. Dermis and epidermis were separated by treating with NH₄Cl (0.24 M) for 10 min at 4°C and used for preparation of tissue extract as described. For the preparation of tissue extracts of different parts of brain skull was opened exposing brain and spinal cord which were carefully excised and placed on ice. Different brain parts cerebrum, cerebellum, medulla, pons and choroid plexus were separated within 30 min and stored at −70°C. A part of tissue was excised from each of the thawed tissues and used for

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preparation of tissue extract. Renal regions were macroscopically separated into medulla and cortex as described\textsuperscript{16} and part of tissue was excised from each region and used for the preparation of tissue extract.

ACE was extracted from tissues as described\textsuperscript{17} using 0.1 M phosphate buffer (pH 8.3) with (50 mM) NaCl. The protein content of tissue extracts was measured by the method\textsuperscript{18} using bovine serum albumin (Sigma Chemical Company, USA) as standard.

**Determination of ACE activity in tissues**—Tissue ACE activity was measured with hippuryl-L-histidyl-L-leucine (HHL; Sigma Chemical Company, USA) as substrate by a modified method\textsuperscript{19}. The reaction mixture (0.175 ml) contained 0.1 ml of 5 mM HHL dissolved in 0.2 M phosphate buffer (pH 8.3) containing 0.6 M NaCl, tissue extract and distilled water. The tissue extract in a volume of 25 μl or less was used to initiate the reaction. After 30 min incubation at 37°C , the reaction was stopped by addition of 0.175 ml of 1 M HCl. Hippuric acid was extracted with ethyl acetate, dried and resuspended in ethanol and measured spectrophotometrically at 228 nm\textsuperscript{17}. The specificity of the reaction for ACE was tested by adding 50 μl of 10 μM captopril (Sigma Chemical Company, USA) in the incubation mixture. One unit of ACE was defined as the amount of enzyme catalyzing release of 1 n mole of hippuric acid from HHL per min at 37°C.

Effect of chloride on the ACE activity of mammary gland and tongue was studied by preparing tissue extracts in chloride free buffer and measuring ACE activity in the absence and in the presence of 300 mM NaCl. The Ethical Committee of the institute approved this study.

ACE activity has been shown in mammary gland, tongue and skin (Table 1). ACE activity was more in lactating mammary gland and less in non-lactating mammary gland and skin. ACE activity was not found in non-lactating mammary gland of Ram. In Ewe lactating mammary gland ACE activity was six times higher than non-lactating mammary gland. These results indicated that mammary ACE was subjected to hormonal regulation and appeared at puberty in female mammals. Further, mammary ACE seemed to be more sensitive to hormones related to pregnancy and lactation. Almost equal ACE activity was found in anterior, lateral and central tongue epithelial cells. However in posterior tongue epithelium, ACE activity was slightly less. This suggested specific function for ACE in tongue. To our knowledge mammary ACE and lingual ACE had not been determined in any animal species. ACE activity was same in both epidermis and dermis of the skin. Captopril caused an inhibition of above 97% of ACE activity in all the tissues.

ACE activity in different brain tissues are presented in Table 2. Captopril inhibited ACE activity in all brain tissues. ACE activity was high in choroid plexus and low in spinal cord. In cerebrum, medulla, cerebellum and pons ACE activity was moderate. High ACE level in choroid plexus strongly suggested role for ACE in cerebrospinal fluid of brain.

<table>
<thead>
<tr>
<th>Table 1— Angiotensin converting enzyme activity in sheep mammary gland, tongue epithelium and skin</th>
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<tr>
<td><strong>Tissue</strong></td>
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<tr>
<td>Ewe mammary gland</td>
</tr>
<tr>
<td>Non-lactating</td>
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<tr>
<td>Lactating</td>
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<tr>
<td>Ram mammary gland</td>
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<tr>
<td>Non-lactating</td>
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<tr>
<td>Anterior tongue taste epithelium</td>
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<tr>
<td>Lateral tongue taste epithelium</td>
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<td>Central tongue taste epithelium</td>
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<td>Posterior tongue taste epithelium</td>
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<td>Whole skin</td>
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<tr>
<td>Epidermis</td>
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<td>Dermis</td>
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ND—not detected
ACE activities of brush border epithelial cells, vascular endothelial cells and other tissue cells are presented in Table 3. ACE activity was more concentrated in renal cortical epithelial cells than intestinal epithelial cells, vascular endothelial cells of pulmonary vein and aorta. However, in kidney ACE activity was ten-folds higher in cortex than medulla. Likewise, pulmonary vein ACE activity was twice than aortic ACE activity. Bone marrow, pancreas, adrenals and lymph nodes exhibited less ACE activity. Lowest ACE activity was detected in the thyroid gland. These results indicated that distribution of ACE varies among tissues and within tissues of sheep.

Chloride influenced hydrolytic activity of mammary ACE and lingual ACE. In presence of chloride lingual ACE activity and mammary ACE activity was enhanced by 4.2 and 6.6 times, respectively. This suggested a non-essential activator role for chloride in catalytic activity of ACE.

The present study demonstrated existence of ACE activity in mammary gland and tongue epithelium for the first time. Further, lack of ACE activity in Ram mammary gland, high ACE activity in the Ewe lactating mammary gland suggest that ACE activity is regulated in female mammals by androgens and by pituitary hormones, like testicular ACE and epididymal ACE, in male mammals. Moreover, six times higher ACE activity in lactating mammary gland than non-lactating mammary gland suggested that ACE expression increased several-folds in presence of hormones of pregnancy and lactation. To our knowledge, hormonal influence on female mammal’s ACE activity has not been reported in any one of the species. The mammary ACE may be important for uptake of amino acids by mammary epithelial cells for milk protein synthesis, blood flow and nutrient supply in lactating mammals. Since taste disturbance is associated with the use of ACE inhibitors, ACE activity found in tongue epithelium suggested involvement of ACE in taste signal transduction. Alternatively, ACE may be part of local RAS of lingual epithelial cells. Equal ACE activity in dermis and epidermis of sheep differs from that found in murine skin.

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Distribution of ACE in different regions of sheep brain is different from rat, man and pig. However, high ACE activity found in choroid plexus like by others in rat and man suggests that it may be source for ACE in cerebrospinal fluid where it hydrolyzes peptides other than angiotensin. Occurrence of ACE activity in cerebrum, medulla, pons and cerebellum indicates a role for ACE in angiotensin II mediated synaptic transmission in brain.

Regional distribution of ACE activity in kidney was similar to that found in man and rat. Further, distribution of ACE activity in sheep kidney and intestine was similar to that found in mouse, but different from rat and guinea pig kidney and intestine. High ACE activity in pulmonary vein than aorta was different from higher ACE activity found in arterial than cultured venous endothelial cells. This suggests that distribution of ACE differs among blood vessels endothelial cells. Presence of ACE in adrenals, pancreas and thyroid gland indicates that ACE may have role in angiotensin II dependent actions like hormone secretion, pro-inflammatory, anti-proliferation, cell growth etc. in these organs.
In contrast bone marrow ACE may regulate haematopoietic stem cell differentiation and proliferation by hydrolyzing peptides other than angiotensin 14. Since chloride partially activated mammary and lingual ACE, it can be concluded that ACE is involved in hydrolysis of peptides other than angiotensin I in these tissues 14.

References