The number and distribution of fungiform papillae and taste buds in the tongue of young and adult Akkaraman sheep

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SUMMARY

The purpose of this study was to investigate quantitatively the relation between the loss of taste sense and the aging in mammals. Various parameters such as the numbers and topographic distributions of fungiform papillae, the numbers and morphology of their taste buds, were studied comparatively for the tongues of young (6-9 months old) and aged (5-6 years old), female and male Akkaraman sheep. Also the length and area of the tongues were determined.

There was no significant difference between young and old animals in respect to the density and the bud numbers of the fungiform papillae. However, the diameters of the fungiform papillae and their taste buds, and the length and surface area of the tongues of old animals were statistically different from those of the young ones.

No significant difference was observed in the numbers of gustatory papillae and taste buds between the aged and the young animals. It is concluded that there is no relation between the loss of the sense of taste and the decrease in the number of papillae and their taste buds with age.

KEY-WORDS : Akkaraman sheep - fungiform papillae - taste buds - number-distribution - aging.

Introduction

The dorsal surface of tongue is rough and covered with raised structures called papillae. There are five classes of papillae: fungiform, circumvallate, foliate, filiform and lenticular. These papillae, generally, have similar characteristics in all animals. However, they exhibit large variations in form, arrangement and numbers depending on the particular life style of the animal. The fungiform, circumvallate and foliate papillae are gustatory papillae that contain sensory receptors for taste, the taste buds. The filiform and lenticular papillae are nongustatory, mechanical papillae important in mastication [4].

It is generally accepted that the sensory organs loose their efficiency, and do not function properly in older animals [24]. The partial loss in the taste sensivity of the mammals is attributed to genetical factors [15], diseases [32], and age [25]. Taste reception depends on the interaction between ions and molecules and the receptor cells contained of the gustatory papillae [2]. It has been reported that the stimulation threshold for taste reception is higher in older individuals than those of younger ones [14, 25]. There are some studies indicating that the intensity of taste reception is proportional to the number of gustatory papillae [31], the variation observed among the individuals in taste reception are due to the number of taste buds and to their arrangement on the tongue [18, 22], and the taste quality decreases with the decreasing number of taste buds [2, 8, 30].

ROBINSON and WINKLES [28] have concluded in their study that the observations of partial loss of taste buds on gustatory papillae with age is possibly due to the methods used in previous studies.
In this study, we utilize the method of ROBINSON and WINKLES [28] to investigate if there is a change in the sense of taste with age and if it is accompanied by a decrease in the number of taste buds. Tongues of young and old Akkaraman sheep have been investigated comparatively as far as the following parameters are concerned: morphology and topography of the fungiform papillae and their taste buds, tongue length, and tongue area.

**Materials and methods**

Thirty-two tongues were obtained from 16 young (8 males, 8 females) and 16 old (8 males, 8 females) Akkaraman sheep. Akkaraman is the dominant breed of sheep in Anatolia, Turkey. Sheep tongues were obtained from Konet Slaughter House owned by the municipal authority of Konya. The ages of the sheep were determined from the use and erosion ratio of the incisive teeth before the slaughter. 6-9 months old individuals were identified as young and 5-6 years old ones were identified as old. Following the slaughter of sheep the tongues were removed by cutting the posterior part of the tongue and they were inspected for gross lesions and anomalies.

The tongues were washed under running water and after the measurement of their length and width, the specimens were fixed in 4% formaldehyde in phosphate buffer with pH 7.4.

The location and arrangement of the fungiform papillae were determined by a stereomicroscope on each tongue and a map was constructed to show their topographical distribution (Figure 1). The total number of the fungiform papillae on each tongue was determined by a stereomicroscope.

Tissue pieces containing samples of fungiform papillae were dissected from rostral, intermediate and caudal parts of tongue. These specimens were rinsed in running water, dehydrated in graded ethanol and embedded in paraffin. In order to perform morphometric measurements of the papillae and taste buds, the blocks were cut into eight micrometers thick sections parallel to the tongue surface, and stained with hematoxylin and eosin (Figure 2).

In order to determine the density of the fungiform papillae, the tongues were sectioned into one cm pieces from rostral to caudal. Furthermore, the surface of each tongue was calculated and the number of the fungiform papillae and taste buds per one cm area counted.

**Results**

As a result of the counting of the fungiform papillae, no significant difference was observed in papillae density with respect to age and sex. It was observed that the density of the fungiform papillae were the same for the young and the old subjects (Table I).

The number of taste buds in different regions was obtained by direct counting of the taste buds on any section containing whole papillae samples under microscope. The samples of papillae containing more than one taste bud were recorded and their percentage was determined for each region. The results obtained for the numbers of investigated fungiform papillae and taste buds were applied to all papillae of this kind on the tongue.

The surface area of each one out of the 32 tongues used in this study was calculated by measuring the length (the distance between the apex and radix of the tongue) and multiplying it by the width measured at the level of the torus. Density of papillae, diameter of papillae, taste bud diameter and taste bud numbers for fungiform papillae, tongue length, tongue area, tongue width measurements were analysed by ANOVA test, in order to determine whether the means are similar for two age groups or not (p < 0.05).
observed that the fungiform papillae are generally distributed in the rostral part of the tongue (Table II).

The results of the diameter measurement of the fungiform papillae show differences with respect to age and sex for a given region. However, we note that the diameter of the papillae increases from the rostral region towards the caudal region (Table III). The results of the fungiform papillae/taste bud diameter measurements for the three regions of the tongue show significant differences with respect to age and sex (Table III). In addition, it is observed that the diameter of taste bud increased from rostral to the intermediate region, but decreases in the caudal region. The diameters of taste buds, generally, are larger in the older individuals than the younger ones.

There were no significant differences in the number of taste buds per papillae between the rostral, intermediate and caudal regions with respect to either age and or sex. From the observations we conclude that the rostral and caudal regions have more papillae than the intermediate region, and also each papilla in these regions contains higher number of taste buds than in the intermediate region (Table III).

The fungiform papillae contain generally 1, 2 or 3 taste buds in all individuals and in all regions of the tongue. However, the percentage of papillae with a single taste bud is lower in the caudal region than in the rostral and intermediate regions. Also, the fungiform papillae in the caudal region show a wider spectrum. In relation to the ratios of papillae containing more than one taste bud, in contrast, the papillae in the rostral and intermediate regions, generally, do not contain more than four taste buds (Table IV).

The total number of taste buds of fungiform papillae show no statistically significant difference with by age. Taste buds of fungiform papillae per cm² show significant differences with respect to age and sex. As a result of the increase of the length of the tongue (hence the surface of the tongue) by age, the number of taste bud of fungiform papillae per cm², decreases in old individuals (Table I).

Measurements of the surface of the tongue have shown statistical differences with respect to age and sex. It is noted that the increase in the tongue length in old subjects is, naturally, accompanied by an increase in the tongue surface (Table V).

**Discussion**

There are two types of gustatory papillae (fungiform and circumvallate) on the tongue of Akkaraman sheep. The fungiform papillae are symmetrically distributed in nearly equal numbers on both halves of the tongue. The fungiform papillae are mainly distributed in the rostral two-thirds of the tongue. Most of the papillae (70.3 %) are located within the first two centimeters of the tip of the tongue. The absolute number of fungiform papillae on the tongue of Akkaraman sheep is 434 ± 2.6 in average. The absolute number of the papillae obtained for Akkaraman sheep in this study is higher than those reported for Cynomolgus monkeys [5], bovine [9], hamster [17], rabbit [19], cat [29] and human [20]. It is observed that the number of fungiform papillae stays constant for young and old Akkaraman sheep. Similar observations were reported previously by ROBINSON and WINKLES [28] in for cats and BRADLEY et al. [4] in for monkeys.

The location and numbers of fungiform papillae in Akkaraman sheep, also, are comparable to those of many ruminants. For example, most of the fungiform papillae in Bactrian camel, Camelus bactrianus were found on the lateral margins of the rostral two-third of tongue [10]. The fungiform papillae in blackbuck, Antilope cervicapra, and Barbary sheep Ammotragus lervia are present as rounded bodies, and more densely distributed [11, 12]. The fungiform papillae in India goat, Capra aegagrus were reported as large and could easily be seen with naked eye, and scattered over the entire dorsum, being in abundance at the tip [26].

The diameter of the fungiform papillae increases from rostral to intermediate region of the tongue and this finding is in agreement with the results obtained by MISTRETTA and

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**Table I.** — Absolute numbers and densities of fungiform papillae and their taste buds in young and old Akkaraman sheep.

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>Absolute number of fungiform papillae Mean±SD</th>
<th>Absolute number of taste buds Mean±SD</th>
<th>Density of fungiform papillae (Papillae number per cm²) Mean±SD</th>
<th>Density of taste buds (Taste bud number per cm²) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young males</td>
<td>430.68±10.06</td>
<td>1185.65±71.40</td>
<td>13.26±0.20</td>
<td>36.50±2.20</td>
</tr>
<tr>
<td>Young females</td>
<td>442.76±7.30</td>
<td>1243.44±96.00</td>
<td>16.13±0.40</td>
<td>45.29±2.90</td>
</tr>
<tr>
<td>Old males</td>
<td>424.94±6.30</td>
<td>1256.34±75.00</td>
<td>9.24±1.00</td>
<td>26.88±1.60</td>
</tr>
<tr>
<td>Old females</td>
<td>437.76±8.40</td>
<td>1165.83±82.00</td>
<td>11.87±0.60</td>
<td>31.61±3.00</td>
</tr>
</tbody>
</table>

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**Table II.** — Distribution of the fungiform papillae in different portions, from the tip of the rostral region to caudal region of the tongue of young and old Akkaraman sheep.

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>Total length of the tongue (cm)</th>
<th>Distribution of the fungiform papillae (%) in different topographical regions of upper surface of the tongue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First region (0-2ND cm)</td>
<td>Second region (2-5TH cm)</td>
</tr>
<tr>
<td>Young males</td>
<td>11.37</td>
<td>0.65</td>
</tr>
<tr>
<td>Young females</td>
<td>10.43</td>
<td>0.71</td>
</tr>
<tr>
<td>Old males</td>
<td>13.35</td>
<td>8.72</td>
</tr>
<tr>
<td>Old females</td>
<td>14.15</td>
<td>0.67</td>
</tr>
</tbody>
</table>

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*Revue Méd. Vét., 2003, 154, 11, 709-714*
The mean diameter of the fungiform papillae and the taste buds increases from the rostral to intermediate and decreases from the intermediate to the caudal region of the tongue. The diameter of fungiform papillae and the taste buds is larger in the older animals than in the younger ones and this difference is statistically significant. Nevertheless, no changes were observed in the morphological structure of the papillae and taste buds between the old and the young animals.

The length of tongue has been observed to increase with age in bovine [9], in rats [23], in monkeys [4], in cats [28] and in human [18]. The measurements reported in this study reveal that the tongue length increases with age, resulting in an increase in the tongue area. As a result of this, the numbers of fungiform papillae and of the taste buds are lower in the old than those of the young subjects. MISTRETTA and BAUM [23] have pointed out that the increase in the surface of the tongue with age is not accompanied by an increase in the number of the papillae per surface unit. Particularly, they have stressed that this pitfall should be avoided when comparing the numbers of taste buds in the young to those in the old subjects.

Even though there are early studies on various species with various techniques reporting that fungiform papillae contain one [16,21] or more [9] taste buds, many studies have shown that there are fungiform papillae with no taste buds in human [1,18,22], in Rhesus monkey [4], in rats [23], in hamsters [17] and in Fischer rats [20] in various ratios.

The fungiform papillae and their taste buds are innervated by Chorda tympani nerve. The papillae exhibit very important morphological changes following the dissection of this nerve and therefore, various authors have suggested that fun-
Figs. 2A-F. — Histological sections of fungiform papillae from the tongue of 6-9 months-old Akkaraman sheep, stained with hematoxylin and eosin. 2A) Sagittal section of a fungiform papillae with its taste buds (arrows) in the surface epithelium, X50. 2B) A higher magnification of a taste bud with its pore opening into the oral surface, X500. C-D) Transversally sectioned fungiform papillae with single taste buds (X50 and X500 respectively). E) A fungiform papillae with multiple taste buds, X250. F) A higher magnification view of taste buds of a fungiform papilla with multiple taste buds, X 1250.
fungiform papillae without taste buds may not exist [6, 13, 27, 28]. ROBINSON and WINKLES [28] have discussed that the observations of fungiform papillae without taste buds are probably due to the method used in these studies. Furthermore, they have concluded that the series of sections should be cut parallel to the dorsal surface. The method proposed by ROBINSON and WINKLES [28] was used in this study and no fungiform papillae without taste buds were observed.

The results obtained from the counting of fungiform papillae and the number of taste buds per papillae in Akkaraman sheep are as follows: the number of taste buds per papillae ranges from 1 to 38; the mean numbers of taste buds per papillae for the young animals are 2.42 in the rostral region, 1.45 in the intermediate region and 2.46 in the caudal region; and these means for the old animals were 2.40, 1.41, 2.44, respectively. The means for the whole tongue are 2.11 in the young and 2.08 in the old subjects. The numbers of fungiform papillae and taste buds per papillae are higher than those in humans [1, 3, 7, 22] and in Rhesus monkey [4].

The number of the taste buds in each fungiform papilla shows a great variation with age and the region of the tongue. The ratio of papillae containing one, two, and three taste buds ranged within 19-84%, 13-36%, and 1-15%, respectively. These results are not in accordance with the results reported previously for Cynomolgus monkey by ARVIDSON et al. [3].

The diameter measurements performed in this study for the fungiform papillae show that the papillae become larger from rostral to caudal and the number of the taste buds of fungiform papillae increases with the size of the papillae. While this observation agrees with the results of DA VIES et al. [9] for bovine and ROBINSON and WINKLES [28] for cats, it is in contradiction with the result obtained by MILLER [18] and CHENG and ROBINSON [7] for humans.

There is a strong contradiction between the earlier studies on the numbers of gustatory papillae and the recent studies. Four main factors lead to these contradictory results: the size of tissue under investigation, the extent of the pathological changes in older specimen, the statistical methods used in the analysis of the data [23] and the use of improper methods in the studies. Reorganization of the studies by taking these factors into consideration, would probably remove the contradiction. Also, it seems very unlikely that a decrease with age could occur in the numbers of gustatory papillae and taste buds. In this study, a decrease with age is not observed in the numbers of the fungiform papillae and taste buds in Akkaraman sheep. It is concluded that the causes of the decline in the sense of taste should be searched elsewhere.

References