Loss of Taste Buds in The Circumvallate Papillae of Rat Tongue after Ovariectomy

Ervin Rizali¹, Widurini Djaja Suminta¹, Budiharto Sudiroatmodjo², Nadhira Haifa Prabowo¹, Elza Ibrahim Auerkari¹,*

1. Department of Oral Biology, Faculty of Dentistry, University of Indonesia, Jakarta, Indonesia.
2. Department of Preventive and Public Health Dentistry, Faculty of Dentistry, University of Indonesia.

Abstract
The menopausal loss of estrogen levels can be linked to oral discomfort including dysgeusia. Dysgeusia could be caused by degeneration of taste buds in the papillae of the tongue.

This study aimed to investigate the effect of menopause on the number of taste buds in the circumvallate papillae of the tongue.

Menopausal conditioning was induced by ovariectomy of Wistar rats with similar weight, age, and partus just once. The number of taste buds per papilla was counted before and after ovariectomy. The proliferation activity of taste bud cells was determined by evaluating the fraction of taste bud cells with nucleolar organizer region activity (AgNOR staining) with respect to the total number of taste bud cells. Multi-way ANOVA was used for statistical analysis.

The results show that ovariectomy resulted in a significant decrease in the number of taste buds in the circumvallate papillae (p<0.001) and also in the proliferation activity of taste bud cells (p<0.01). The decreasing trend in the number of taste buds continued at least up to 100 days after ovariectomy. In contrast, the proliferation activity dropped to a lower level within 10 days after ovariectomy and remained practically constant thereafter.

The number and proliferative activity of the papillae circumvallate taste buds of rats are changed following the induction of menopause through ovariectomy.


Keywords: Taste buds, ovariectomy, proliferation activity.
Received date: 18 August 2017
Accept date: 20 September 2017

Introduction
The menopausal decrease in estrogen levels can result in oral discomfort including dryness, sensation of burning and dysgeusia. Also, taste thresholds are higher in the elderly, and these effects could at least partly reflect the reduced hormonal production. The sensing organ of taste, the taste buds occur in the mouth, pharynx and larynx but are particularly abundant in the tongue. The lingual taste buds are distributed on the surfaces of the mammalian tongue in the fungiform, foliate and circumvallate papillae, of which the latter are for sensing the bitter taste in the posterior portion of the tongue. Since the fungiform and foliate papillae are more sensitive to trauma, the circumvallate papillae have been considered particularly useful for studying the condition and possible degenerative effects on taste buds.¹,²

There are partly conflicting reports about the mechanisms and even on the extent of age-related changes of taste, and associated changes of the circumvallate taste buds in mammals.²-⁵. The potential changes in the taste buds and blood vessels have received attention since some time²,⁶-¹⁰, and while some reports claim no age-related change in the taste buds,¹⁰ other studies suggest significant taste bud deterioration or atrophy with age¹⁰-¹². The maintenance of the taste bud cells is closely related to the condition of the peripheral nerve, so that cutting of the peripheral nerve will result in degeneration and eventual death of the taste bud cells. Therefore, it has been hypothesized that the degeneration in taste acuity with age could be directly related to loss of taste buds. For example, dysgeusia could be caused by decreasing number of taste buds in the papillae of the tongue. The decreasing hormonal production with aging could be involved in this...
process, but few studies have addressed this specifically. Most studies on the age-related changes in the taste buds have included both male and female subjects, without determining the possibly associated hormonal status. With the suggestion that at least some taste bud receptors are sensitive to estrogen,\textsuperscript{1,5-7,12,13} it can be anticipated that major changes in the hormonal production such as loss of estrogen at menopause could affect the taste bud maintenance and function.

This study aimed to investigate the effect of menopause on the number and proliferation activity of taste buds in the circumvallate papillae of rats after induced menopausal conditioning through ovariectomy.

**Materials and methods**

Wistar rats with a weight of ±250 g, age of 3-4 months and with partus just once were used as an experimental model. Menopausal conditioning was induced by ovariectomy under ether anesthesia for 16 rats, and another 8 rats without treatment were used as control. The animals were sacrificed by decapitation after anesthesia with ether, and in case of ovariectomized rats this was done 10, 30, 50 and 100 days after ovariectomy, with 4 rats in each group. The tongues were extracted and the material fixed in 10% formalin, cut into transverse slices at the posterior portion of the tongue containing the circumvallate papillae. The slices were processed for paraffin histology. From these serial 4 μm sections were prepared and stained with hematoxyline and eosine (HE) and AgNOR.\textsuperscript{14} The number of taste buds per papilla was counted from the circumvallate papilla sections under light microscope (100x). From each lingual sample, 5 papillae were checked this way (20 papillae per experimental group). An example of the cross-section of papillae and taste buds is shown in Figure 1a.

The proliferation activity of taste bud cells was determined by counting the number of argyrophilic nuclear organizer regions (AgNORs) of taste bud cells. For this purpose, the taste buds from four rats were evaluated for each experimental group (10, 30, 50 and 100 days after ovariectomy, and control group). For AgNOR staining, paraffin sections were dewaxed in xylene and hydrated in deionized water. The base of 2% gelatin and 1% aqueous formic acid was mixed in 1:2 (vol) with 50% silver nitrate, and applied to the sample sections under dark room conditions, incubating for 45 minutes. The samples were finally washed under deionized water for 10 minutes, dehydrated to xylene and mounted for inspection under light microscope (1000x). Apart from the number of AgNORs, the number of cells in the corresponding taste buds was also counted. An example of the appearance of taste bud cells with AgNORs (dark spots) is shown in Figure 1b. Statistical analysis was carried out using multi-way ANOVA test, assuming p < 0.05 to imply significance.

**Figure 1.a**

Examples of a) circumvallate papillae and taste buds; b) AgNORs (black spots) in taste bud cells.
Results

The observed number of taste buds per circumvallate taste bud (n), number of AgNORs per taste bud (Nbd), number of taste bud cells per taste bud (N) and the ratio \( R = \frac{Nbd}{N} \) after ovariectomy (control at zero time) are shown 10, 30, 50 and 100 days after ovariectomy in Table 1, and in Figures 2 and 3.

<table>
<thead>
<tr>
<th>Days after ovariectomy</th>
<th>Number of taste buds</th>
<th>10 days</th>
<th>30 days</th>
<th>50 days</th>
<th>100 days</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.25 ± 1.02</td>
<td>18.00 ± 1.83*</td>
<td>16.75 ± 1.71*</td>
<td>13.50 ± 1.29</td>
<td>12.50 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>14.25 ± 1.02</td>
<td>18.00 ± 1.83*</td>
<td>16.75 ± 1.71*</td>
<td>13.50 ± 1.29</td>
<td>12.50 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>14.25 ± 1.02</td>
<td>18.00 ± 1.83*</td>
<td>16.75 ± 1.71*</td>
<td>13.50 ± 1.29</td>
<td>12.50 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>50 days</td>
<td>14.25 ± 1.02</td>
<td>18.00 ± 1.83*</td>
<td>16.75 ± 1.71*</td>
<td>13.50 ± 1.29</td>
<td>12.50 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>100 days</td>
<td>14.25 ± 1.02</td>
<td>18.00 ± 1.83*</td>
<td>16.75 ± 1.71*</td>
<td>13.50 ± 1.29</td>
<td>12.50 ± 1.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Observed number of taste buds per papilla (n), number of AgNORs per taste bud (Nbd), number of cells per taste bud (N), and the ratio \( R = \frac{Nbd}{N} \) after ovariectomy (mean ± SD).

The decreasing number of taste buds with time appears to continue at least to the end of the follow-up period (100 days), although apparently at a lower rate with increasing time. A particularly strong decrease in the number of taste buds appeared to occur between 10 days and 30 days after ovariectomy. The observed decrease in the number of taste buds was statistically significant (p<0.001). Also significant (p<0.01) was the decrease in the number of AgNORs per taste bud after ovariectomy, reflecting the proliferation activity of the taste bud cells. However, in case of the number of AgNORs, the decrease took place within 10 days after ovariectomy and remained at a constant level thereafter (Figure 4). The same pattern was seen in the ratio of the number of AgNORs and number of taste bud cells per taste bud, because the number of bud cells showed no significant change after ovariectomy.

Discussion

The results of the present work indicate a decrease in the number of circumvallate taste buds with time after ovariectomy, and suggest a hormonally mediated mechanism in the maintenance of taste bud function. The shortest time period of 10 days after ovariectomy in rat would approximately correspond to two months after (induced) menopause in human. The observed deterioration of taste buds was not reflected in the number of taste bud cells in the remaining taste buds. However, the proliferation...
activity of the taste bud cells was reduced to a lower constant level within 10 days after ovariectomy.

There are few direct studies on the effects of changes in estrogen or other hormonal production on taste bud condition. However, in a number of previous studies the effects of aging on the taste buds have been investigated, and since age will inevitably affect hormonal production, similar though more indirect effects could be expected. Nevertheless, various studies speculated that the age-related differences also in the human gustatory system would not be as substantial as previously suggested. This is in contrast to the earlier report of Zhang et al who found the number of circumvallate taste buds rapidly decreasing in old age. More recently also Boyce et al found significant decrease in bitter taste acuity with age of human subjects. They also reported considerable individual variation in the circumvallate papillae, so that size of papillae was inversely correlated with number of papillae and bitter taste acuity. In a number of studies the taste buds were not investigated, but the size, number and distribution of circumvallate papillae were not found to vary with age. Another study has reported that the total number of taste buds including those in the circumvallate papillae in rats reached maximum at an age of about two months and decreased thereafter. Boyce et al noted a decrease in the number of human (mostly in male subjects) circumvallate taste buds with age, but this was only significant in subjects more than 80 years old. The size of the papillae in terms of the cross-sectional length of the circumvallate wall was found to increase in the youngest subject group (up to 15 years old), but remained constant thereafter. The mean size of the taste buds increased slightly with age, but the average number of cells within the taste bud showed no significant change. Number of AgNORs was found to decrease particularly in the oldest group. Reduced number of taste buds has also been observed in Wistar rats as a result of perinatal undernutrition.

Most of the above studies on the age-related changes in the taste buds have included both male and female subjects. This could also possibly imply parallel hormonal effects on taste buds in males, although to the knowledge of the present authors this has not been investigated in detail. Also, in general the hormonal status of the subjects has not been determined when examining the histopathological condition of taste buds. In any case there is a consistent and significant general decrease in taste sensitivity with age, affecting human subjects of both genders. For tasting function, also the condition of the associated nerves is important in addition to the taste buds and their taste receptor cells. The papillary structure and the underlying control genes of the tongue epithelium are very well conserved in mammals. Therefore, rodent animal models can be expected to work relatively well for studies on other species including human.

Some studies have suggested that the PROP (6-n-propylthiouracil) receptors associated with the taste buds are sensitive to estrogen. It is conceivable that major changes in the hormonal production such as loss of estrogen at menopause could affect the condition and function of taste buds. The results of the present work appear to confirm such an association between the estrogen status and taste bud maintenance in rats. This association was found not only as a continuous deterioration in the number of taste buds but also as a stepwise decrease in the proliferation activity of the taste bud cells after menopausal condition induced by ovariectomy. The observed deterioration initiated within 10 days after ovariectomy and continued for 100 days or to the end of the follow-up period of the study.

Conclusions

In conclusion, the results of the present study showed significant reduction in the number and proliferation activity of taste buds in the circumvallate papillae of rats after induced menopausal conditioning through ovariectomy. The loss in proliferation activity was completed in 10 days, but the deterioration in the number of taste buds continued throughout the 100 days follow-up period of the study.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References


