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Taste buds degeneration induced by lingual artery spasm in subarachnoid hemorrhage in rabbits

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Abstract

The role of the vasospasm induced by subarachnoid hemorrhage (SAH) in tongue taste bud degeneration is well established; however, the role of lingual artery spasm induced by SAH in that has not been efficiently studied. It was aimed to investigate the relationship between the taste buds degeneration and lingual artery spasm after SAH. Seventeen rabbits were randomizied into three groups: untouched control group (n=5), physiologic serum saline injected group (SHAM; n=6), and subarachnoid hemorrhage group (study; n=6). Experimental SAH was performed by injection 0.75 ml auricular arterial homologous blood was injected into cisterna magna three times in two weeks. After three weeks, all the rabbits were sacrificed and their tongues were removed under general anesthesia following intracardiac formalin injections. Normal and degenerated taste buds numbers and lingual artery vasospasm indexes were examined stereologically. Results were analyzed statistically with the Kruskal Wallis and the Mann Whitney U tests. Significance was accepted as $p<0.05$. The mean vasospasm index (VSI) values of lingual arteries and the numbers of degenerated taste buds per mm3 were: 1.52±0.13 / 2±1 in control; 1.99±0.21 / 5±2 in SHAM; and 2.64±0.78 / 13±4 in the study group. There was a linear relationship between the numbers of degenerated taste buds and VSI values of lingual arteries in control/SHAM ($p<0.05$); SHAM/study ($p<0.05$) and control/study ($p<0.01$). For the first time in the literature, it was found that the taste buds of the tongue may be affected by subarachnoid hemorrhage induced lingual artery vasospasm.

Keywords: Subarachnoid hemorrhage, lingual artery spasm, taste buds degeneration, experimental

Introduction

Cerebral ischemia induced by subarachnoid hemorrhage (SAH) can lead to disruptive neurological consequences due to the cerebral vasospasm. The blood supply to the tongue is provided by the external carotid artery (ECA). Degenerations of tongue structures induced by vasospasm of ECA are rarely reported in the literature.

The greater petrosal nerve originating from geniculate ganglion innervates taste buds on the palate and the anterior 2/3 of the tongue [1]. Trigeminal nerve fibers have responsible for the sense of tongue [2]. The chorda tympani, a branch of the facial nerve, carries taste sensations from the anterior two-third of the tongue and joins the lingual nerve [3]. Taste informations of the chorda tympani, glossopharyngeal and vagal nerves has been integrated by the insular cortex, claustrum [1] and insular cortex [2].

The mechanism of taste buds degeneration of tongue could result from ECA vasospasm induced facial nerve and lingual artery spasm [3]. Spasm of the stylomastoid artery and the petrosal artery may cause facial nerve or geniculate ganglion insult. Chorda tympani injuries [4] and geniculate ganglion lesions may cause fungiform papillae atrophy [5]. Insults of the chorda tympani, glossopharyngeal, vagal and also trigeminal nerve cause hypogeusia or ageusia [6].

The Cavalieri principle, a stereological method, is an effective method in volumetric measurements of biological structures. The Cavalieri principle provides numerical values expressing precise and unbiased quantitative measurements. In this principle, sections of an object that are parallel and of equal thickness are taken, and the volume of the object is calculated by the total number of section areas and thickness of the section.The histological sections have been used in the calculation of the volume [7].

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In this study, it was aimed to investigate the effect of lingual artery spasm, which is a branch of ECA, on taste buds in the tongue, in SAH model in rabbits.

Materials and Methods

Seventeen rabbits which were 2.5 years old and weighing 4.5±0.5 kg were used. Ethical approvals were obtained from the animal experimentation local ethics committee at Ataturk University. The rabbits were taken from Ataturk University, Medical Experimental Application and Research Center. The rabbits were put in standard cages with a bed of sawdust under controlled lighting and standard humidity. The rabbits were randomized and divided into three groups: untouched control group (n=5), physiologic serum saline injected group (SHAM; n=6), and subarachnoid hemorrhage (SAH) composed group (study; n=6).

To perform experimental SAH, homologous blood of rabbits was injected into the cisterna magna three times in two weeks. After two weeks, all rabbits were sacrified under general anesthesia after intracardiac formalin injection. For histological analysis, tongues, geniculate ganglia and bilateral facial nerve complexes were removed.

Vasospasm index (VSI) values of lingual arteries and degenerated taste buds numbers were examined by stereological methods. The data was analyzed with the Kruskal-Wallis and Mann-Whitney U tests. Significance was accepted as $p<0.05$.

Stereological methods

The objective of this study was to assess the practicality and ease of a design-based stereological method for volume estimation, the Cavalieri principle, and its use to estimate and compare the taste bud density (d=n/Plicae) and the total number of taste buds per all tongue rabbits compared to controls. We hypothesise that there is a relationship between the taste buds degeneration and lingual artery spasm after SAH these the taste bud density (d=n/Plicae) and the total number of taste buds per all tongue. Lingual artery vasospasm indexes were also analyzed with this method.

Histological examination

The tongues, facial nerves, and geniculate ganglia of all rabbits were removed and put into 10% buffered formalin solution for ten days for fixation. These samples were embedded in paraffin and later sectioned via Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). To determine and compare the histological architectures of the tongue taste buds, facial nerve roots and geniculate ganglia were examined together. The anterior one-third of all tongues was divided longitudinally into ten segments, and each segment was cut into tissue sections of 5 µm thickness using a microtome, thus making a total of about 20 sections.

Taste buds contain the taste receptor cells, which are also known as gustatory cells. The periferal projections of the bipolar neurons residing in the geniculate ganglion terminate on the gustatory cells of the taste buds. Histological preparations was done to compare to differentiation in normal, SHAM and study groups. Paraffin was melt at 60°C for 30 minutes and removed by keeping in device solution for 15 minutes, then rehydrated by keeping in 99% alcohol for 15 minutes. Samples were washed with distilled water, alcohol and xylol, then removed and covered with a thin lamella for microscope examination.

Sections were stained with hematoxylin eosin (H&E) dyes and examined under a light microscope. In order to estimate accurately the number of taste buds, the average surface diameter of each tongue was measured and surface values were estimated. Later, the taste bud density (d=n/Plicae) and the total number of taste buds per all tongue were estimated per mm^3. VSI values of information-carrying facial nerves and lingual arteries were estimated to compare the density of taste buds of tongue.

Results

Two animals dead in the experimental period. Fungiform papillae of an adult rabbit contained approximately 5 or 7 taste buds, although many contained zero buds. Several taste buds were lined up along the papillary lateral wall around each vallate papilla of the tongue. Taste buds are well delineated in tongual epithelium under the mucosae. They were observed a little more translucent and their cells were arranged in vertical laminas similar to slices of pine. Each taste bud opened the surface of tongue with a pore surrounded with microvilli of tongual surface. Each had central dominant nucleus. Long apical microvilli had dense cytoplasmic extensions surrounded by epithelial cells, resembling unsheathed unmyelinated nerve fibers. In some specimens, taste buds exhibit synaptic organization some nerve fibers at the basal lamina. The basal membrane was penetrated by small fasciculus of afferent nerve fibers and a spiral convolution around the cells was seen at the bottom of the taste buds. Facial nerve endings constitute dense plexuses at the tongue peripheral epithelium and sent fibers into the taste buds.

Figure 1 shows the lingual artery among lingual muscles/nerves and VSI value estimation methods in the magnified form are seen in a normal rabbit. Moderately constructed lingual artery and near the lingual nerve of a SHAM and, deformed endothelial cells and the convoluted inner elastic membrane is seen in a study section (Figure 2). Normal taste buds of a normal, moderately deformed in a SHAM and severely deformed taste buds in a study rabbit were shown in figüre 3.

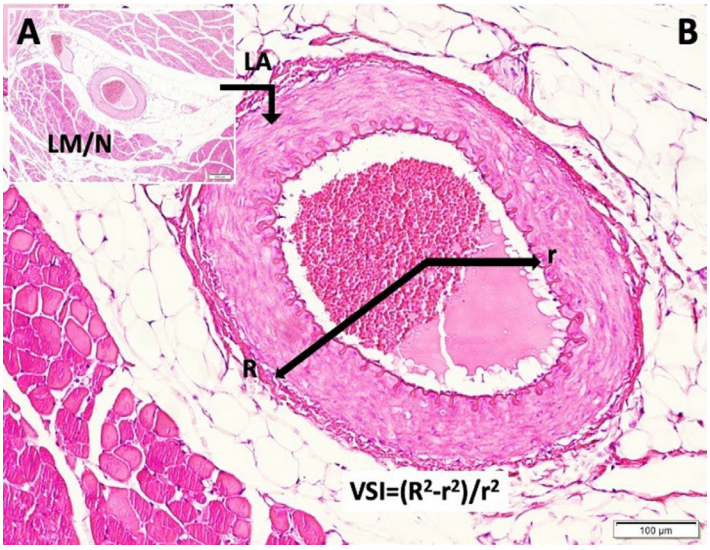

Figure 1. Lingual artery (LA) among lingual muscles/nerves (LM/N) (light microscope, hematoxylin eosin, A:x4), and vasospasm index (VSI) value estimation methods in a magnified form (light microscope, hematoxylin eosin, B:x10) are seen in a normal rabbit.

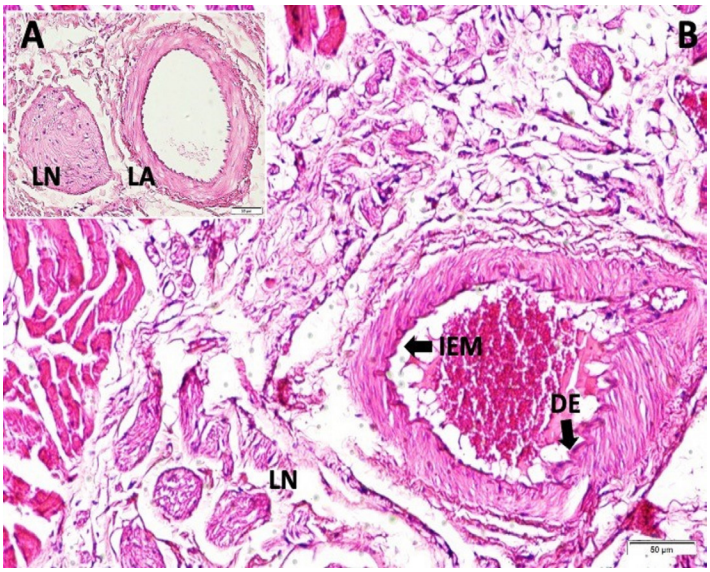

Figure 1. Moderately constructed lingual artery (LA) and near the lingual nerve (LN) (light microscope, hematoxylin eosin, A:x4; B:x20) of a SHAM; and deformed endothelial cells (DE) and convoluted inner elastic membrane (IEM) (light microscope, hematoxylin eosin, B:x20) are seen in a study rabbit.

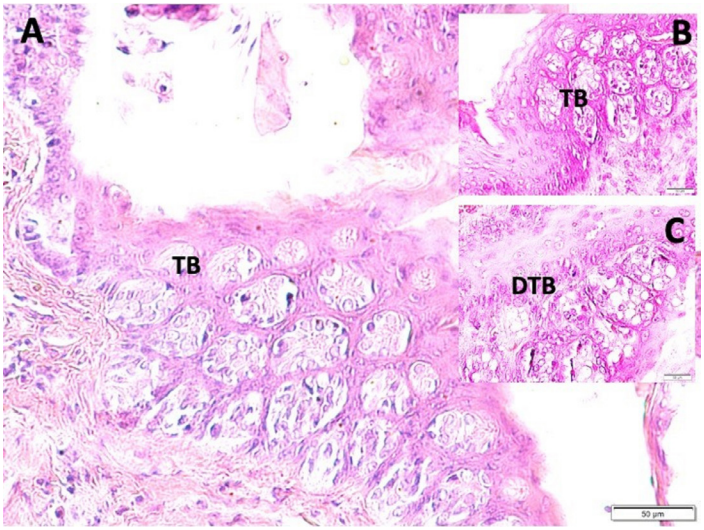

Figure 3. Taste buds (TB) of a normal (light microscope, hematoxylin eosin, A:x4; B:x20), moderately deformed in a SHAM (light microscope, hematoxylin eosin, Base:x10) and severely deformed taste buds (DTB) are seen in a study rabbit.

The mean VSI values of lingual arteries and the numbers of degenerated taste buds/mm3 were: 1.52±0.13 / 2±1 in control; 1.99±0.21 / 5±2 in SHAM and 2.64±0.78 / 13±4 in study group. There was a linear relationship between the numbers of degenerated taste buds and VSI values in control/SHAM ($p<0.05$); SHAM/study ($p<0.005$) and control/study ($p<0.0001$) (Table 1).

Table 1. The mean vasospasm index (VSI) values of lingual arteries and the numbers of degenerated taste buds per mm³

	VSI values	P	Taste Bud/ Vallate P/mm³	P
Control (n=5)	1.52±0.13		2±1	
SHAM (n=6)	1.99±0.21	<0.001	5±2	<0.001
Study (n=6)	2.64±0.78		13±4	

Discussion

Taste buds containing papillae of tongue supplied by rich vascular collaterals of dorsal, profound and superficial lingual arteries may origin from the facial artery innervated by a rich cervical sympathetic vasoconstrictor and facial cholinergic parasympathetic vasodilator fibers. The profound lingual artery gives vertical branches making anastomoses with dorsal, submucosal and subpapillary arteries to the upper surface of the tongue. Salivary glands and oral side of the gingiva is supplied by the sublingual arteries, a branch of the external carotid artery or superior thyroid artery. The middle meningeal artery with its petrosal branch is responsible for the facial nerve blood supply. The petrosal artery is damaged during surgical procedures, tumor or trauma procedures [8]. Although SAH induced vasospasm has been well known but lingual artery related tongue taste bud degeneration has been studied in insufficient studies. In this study, it was aimed to investigate the relationship between the taste buds degenerations and lingual artery spasm, in cases of SAHs.

The taste bud responsible for sensing and responding to the basic taste stimulants which is transferred by chorda tympani, glossopharyngeal and vagal nerves [9]. The taste sensing fibers originate from geniculate, petrosal and nodose ganglions [10] and reach the nucleus of the solitary tract. The geniculate ganglion originated chorda tympani innervates taste buds on the anterior tongue [1]. Taste fibers in the chorda tympani of the facial nerve run to the lingual nerve and innervate the anterior two-third of the tongue [11]. Taste bud cells in the oral cavity regulate glucose homeostasis causing by insulin secretion from pancreatic β cells. Taste information has been recorded in the insular cortex, claustrum [4] and anterior opercular-insular cortex [2]. Parabrachial taste signals are projected to the reticular formation and solitary nucleus. Trigeminal nerves are responsible for lingual somatosensation [12].

Cerebral vasospasm after SAH can induce cerebral ischemia, leading to a devastating neurological outcome [13]. Yet, the role of ECA vasospasm in SAH was rarely investigated in the literature [6]. In our findings, ECA vasospasm may be a predictive role in the serious degeneration of the tongue. ECA vasospasm facilitates ischemia of the dorsal root ganglion and parasympathetic ganglia of the lower cranial nerves [6] and results in the vasodilation failure of the lingual arteries.

Ischemia of the facial nerve is thought to be one of the most possible causes of idiopathic facial palsy. Interruption of the stylomastoid and the petrosal arteries can cause ischemic damage of the geniculate ganglion and the facial nerve. Peripheral facial palsy may also occur following embolization or ligation of the middle meningeal artery, maxillary or carotid arteries [14].

Bilateral sectioning of the glossopharyngeal nerves and/or chorda tympani cause hypogeusia or ageusia on the sweet taste in the mouse [14]. Chorda tympani crushing injuries cause taste sensing disturbances [15] secondary to decreased numbers of taste buds [16]. Interruption of geniculate ganglion functions causes taste buds atrophy [12]. Denervation of the lingual nerve – chorda tympani results in fungiform papillae atrophy [5]. Bilateral lesions of gustatory cortex considerably impair taste sensation. Our study showed that SAH induced vasospasm can cause ECA spasm

induced taste buds atrophy which was caused by facial nerve induced denervation atrophy and lingual artery spasm related ischemic degeneration [13].

Limitation

The cerebral ischemia secondary to vasospasm was not investigated in this study, because the aim of the study was to study the relationship between the taste buds degeneration and lingual artery spasm after SAH.

Conclusion

For the first time in the literature, it was found that the taste buds of the tongue might be affected and atrophied by subarachnoid hemorrhage induced lingual artery vasospasm.

Conflict of interests
The authors have no conflict of interest to declare

Financial Disclosure
All authors declare no financial support.

Ethical approval
Ethical approvals were obtained from the animal experimentation local ethics committee at Ataturk University

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