

# HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY ON THE EFFECT OF GLOSSOPHAR- YNGEAL NERVE CRUSHING ON THE ACTIVITY AND ROLE OF SUBSTANCE P IN RAT VON EBNER'S GLAND

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## **ABSTRACT**

Substance P (SP) is a neuropeptide widely distributed in the central and peripheral nervous system as trophic factor. It was detected also in the Von Ebner's (VE) gland of the tongue. In this study, the formative effect of (SP) was investigated by causing temporary denervation of VE gland.

Twelve adult albino rats were used in this study. They were divided into three groups. In each rat, the left glossopharyngeal nerve was crushed on the left side and the right side was used as control. After two, four and eight weeks, specimens from the posterior third of the tongue were ex-

amined for histological structure, PAS reaction and the immunohistochemical reaction of (SP) and the results were recorded.

It was observed that the integrity and normal structure and the excretory function of the gland depends on intact innervation that supplies a neuropeptide trophic substance e.g. substance P.

## **INTRODUCTION**

Von Ebner's (VE) glands are sub-epithelial serous glands located in the vicinity of the tongue papillae (Miller et al., 1964 and Davies and Coubland, 1967). Functionally, their watery secretion helps to dissolve, dis-

tribute and wash away the substance to be tasted (Copelhaver et al., 1971, Roy and Weiss, 1973 and Suat and Robert, 1988). The parasympathetic (secretomotor) innervation of the glands is derived from the inferior salivatory nucleus via the glossopharyngeal nerve fibres. These fibres relay in small ganglia in the mucous membrane of the tongue (Arey 1954 and Witt and Reutter, 1998).

El-Eishi and State, (1974) and State et al., (1982) concluded that the integrity of VE glands was dependent upon intact nerve supply to either vallate or foliate papillae. Gabr and El-Mohandes (1990) stated that the parasympathetic innervation was capable of maintaining the histological and histochemical structure of VE glands of dogs. They suggested the presence of a neurotrophic substance having a formative influence upon VE gland. Lunderberg et al., (1979) suggested the possibility of the presence of substance P (SP) as a trophic factor. Substance P positive fibers have been detected in the developing lingual papillae as well as in the regenerating nerve fibers in rats ( Nishimoto et al., 1985). Robert et al., (1991)

and Ueba and Uchihashi (1991) stated that stimulation of substance P receptors resulted in degranulation of acinar cells in VE gland of the rat. Oomori et al., (1995) and Matsuda et al., (1997) studied the immunoreactivity of substance P in VE glands of rats. They observed immunoreactive ganglion cells among intra-lingual muscles, at the base of the vallate papillae and near the VE gland. They also described numerous substance P-immunoreactive varicose nerve fibers running closely associated with the serous cells and excretory duct cells, and were seen to run along the blood vessels in the gland. They stated that this substance may have an effect on the secretory activity of the serous cells and duct cells, and on the vasodilation of blood vessels of the VE gland. The present work aims to study the effect of denervation and re-innervation on the reaction of substance P in VE gland and the possible role of this substance in maintaining the structure and function of this gland.

## MATERIAL AND METHODS

Twelve healthy adult albino rats were used in this study. In each rat

and under Phenobarbital anaesthesia, skin incision was made parallel to the ramus and body of the mandible on the left side. By careful dissection, the left submandibular gland was retracted to expose the posterior belly of digastric and the stylohyoid muscle. By retracting these two muscles, the stylopharyngeus muscle and the glossopharyngeal nerve were brought into view. The left glossopharyngeal nerve (supplying the left VE gland) was exposed and crushed by fine artery forceps. The right VE gland was used as control. Careful suturing of the wound and postoperative care were done and each rat was put in a separate cage.

The operated animals were divided into 3 groups (4 animals each). Members of each group were sacrificed 2, 4 and 8 weeks after the operation. The part of the posterior third of the tongue containing the central circumvallate papilla and its surrounding VE glands was excised, fixed in neutral formalin and embedded in paraffin. Serial sections at 6 microns were cut for H&E and PAS stains and at 4 microns for immunohistochemical study using immunolabelling tech-

nique with anti-substance P serum.

## RESULTS

*1- Control ( right ) side* : Histologically, VE gland is formed of serous alveoli and ducts separated by interlobular septa ( fig. 1, a & b). In PAS stained sections the alveolar cells showed normal cytoplasmic reaction (fig. 2). Immunohistochemically, numerous substance P immunoreactive varicose nerve fibers were found closely associated with the serous cells and excretory duct cells ( fig.3 ). Numerous immunoreactive nerve fibers were seen to run along blood vessels in VE gland ( figs.4 ).

### *2- Operated ( left ) side :*

Two weeks after the operation: Histologically, many of the alveoli of VE gland appeared smaller in size and many of the alveolar cells showed scanty cytoplasm and pyknotic nuclei. The ducts were thick walled and surrounded by lymphocytic infiltration (fig.5). In PAS stained sections, the majority of the alveolar cells showed moderate cytoplasmic reaction ( fig.6 ). Immunohistochemically, Sections examined for substance P showed

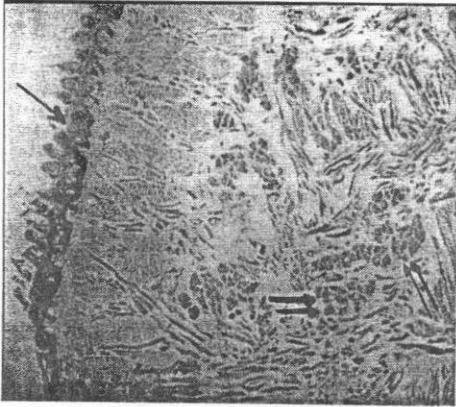
mild reduction in the immunoreactivity in most of the nerve fibers all over the gland ( fig.7 ).

*Four weeks after the operation :*  
most of the alveoli of VE gland showed degenerative changes, appeared smaller in size, most of the alveolar cells showed scanty cytoplasm and deeply stained small nuclei (fig. 8). In PAS stained sections, most of the remaining alveolar cells exhibited weak cytoplasmic reaction. There were some alveoli showing normal reaction. ( fig. 9 ).

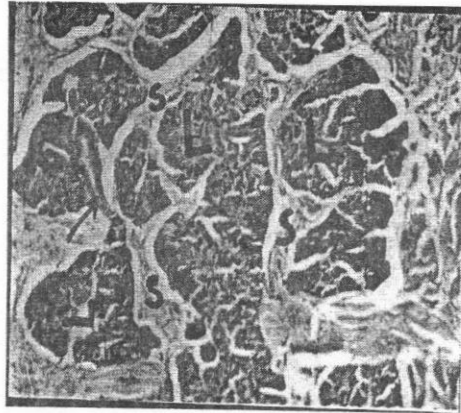
Most of the nerve fibers showed

marked reduction in substance P immunoreactivity. some nerve fibers were still having normal immune reaction ( fig.10 ).

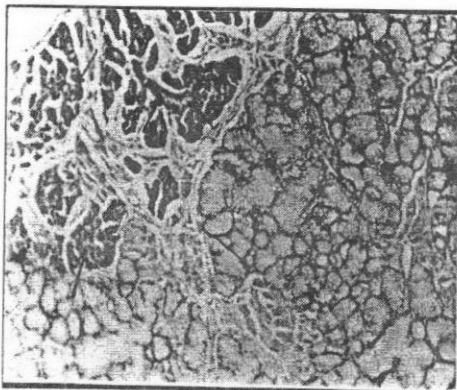
*Eight weeks after the operation :*  
The histological structure of VE gland on the operated side appeared nearly similar to the control with serous alveoli and ducts separated by interlobular septa (fig. 11)). In PAS stained sections, the alveolar cells showed moderate cytoplasmic reaction (fig.12). Most of the nerve fibers showed apparently normal immune reaction for substance P ( fig. 13 ).



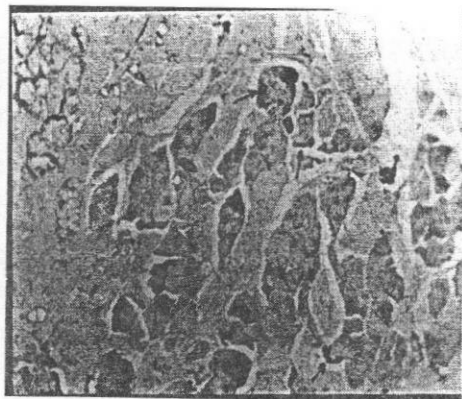
**Figure 1 , a :** A transverse section in the tongue of albino rat showing the tongue papillae (arrows) and the underlying VE gland (double arrows). (H&E X 40 ).



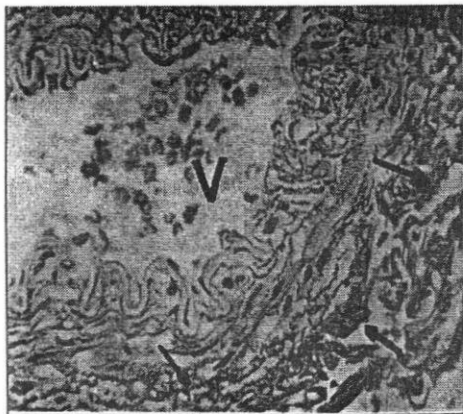
**Figure 1, b :** A photomicrograph of a section of VE gland of the control side showing lobules of serous alveoli (L) and interlobular duct (arrow) separated by interlobular septa (S). (H & E ; X 100 ).



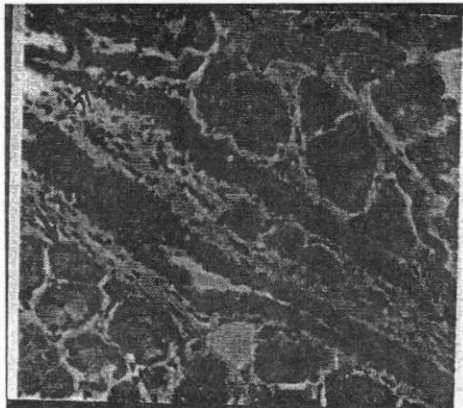
**Figure 2 :** A photomicrograph of a section in VE gland of the control side showing normal PAS reaction in the alveolar and duct cells (arrows). (PAS X 400 ).



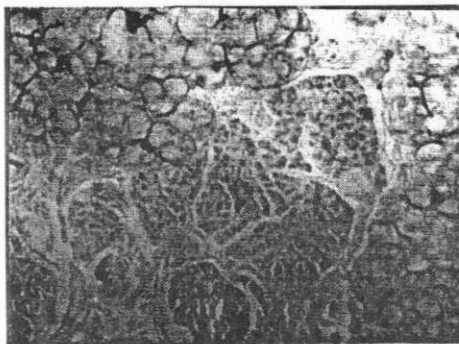
**Figure 3 :** A photomicrograph of a section of VE gland of the control side showing immunoreactive nerve fibers around the alveoli (arrows). (Immunohistochemical stain X 100 ).



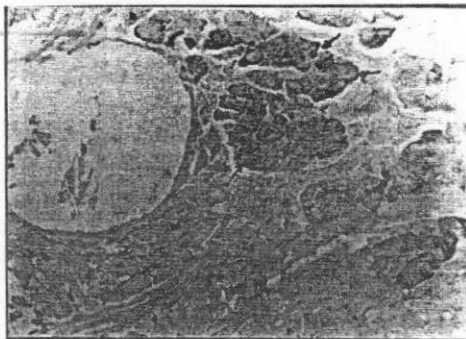
**Figure 4 :** A photomicrograph of a section in VE of the control side showing blood vessel (V) surrounded by numerous immunoreactive nerve fibers (arrows), (Immunohistochemical stain X 1000 ).



**Figure 5 :** A photomicrograph of a section of VE gland two weeks after the operation showing alveolar cells with scanty cytoplasm and piknotic nuclei (arrows). The ducts of the gland are thick and surrounded by lymphocytes (arrow heads ). ( H&E X1000 ).

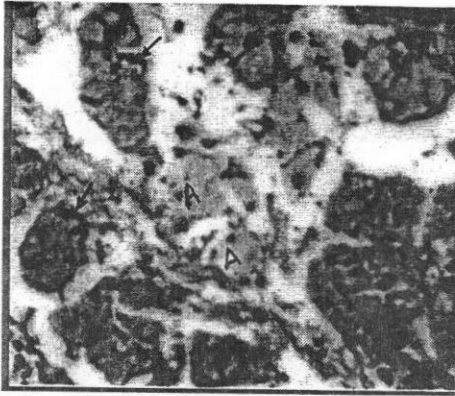


**Figure 6 :** A photomicrograph of a section of VE gland two weeks after the operation showing moderate cytoplasmic PAS reaction in most of the alveolar cells. (PAS X 100 ).

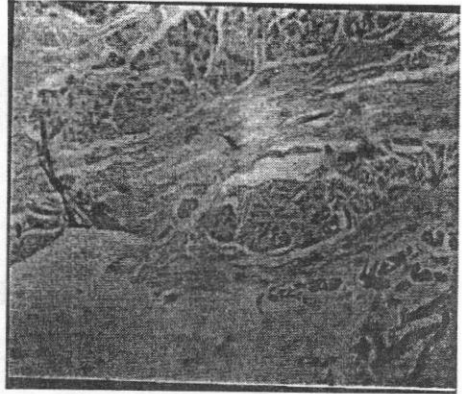


**Figure 7 :** A photomicrograph of a section of VE gland two weeks after the operation showing mild reduction in substance P immunoreaction in the nerve fibers all over the gland (arrows) (Immunohistochemical stain X100).

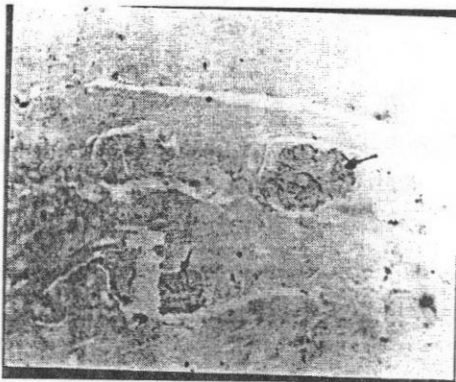




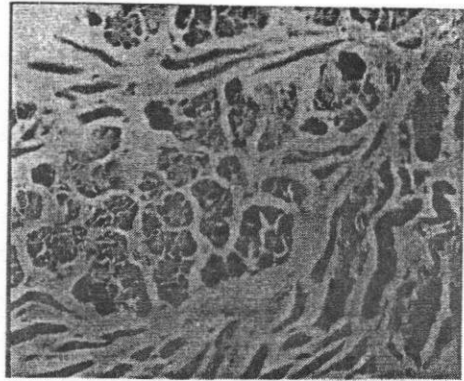
**Figure 8 :** A photomicrograph of a section of VE gland four weeks after the operation showing smaller alveolar cells with deeply stained nuclei (arrows ). Many degenerated alveoli are noticed (A). (H&EX1000).



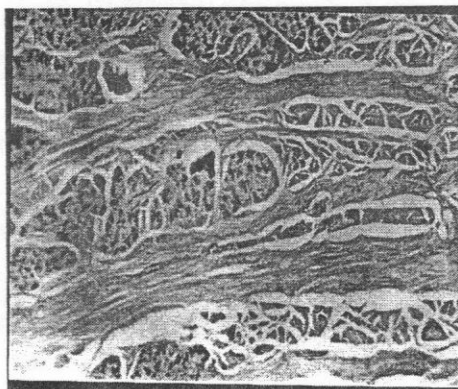
**Figure 9 :** A photomicrograph of a section of VE gland four weeks after the operation showing mild reaction for PAS stain. ( PAS X 100 ).



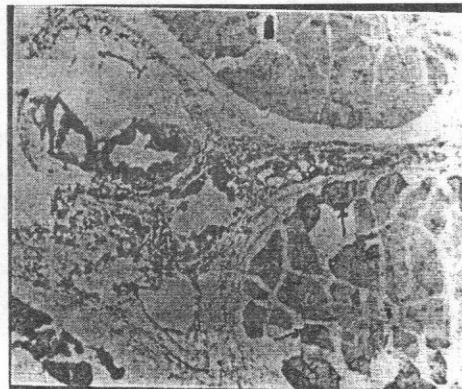
**Figure 10 :** A photomicrograph of a section of VE gland four weeks after the operation showing marked reduction in substance P immunoreactivity (arrows). (Immunohistochemical stain X100 ).



**Figure 11 :** A photomicrograph of a section of VE gland eight weeks after the operation showing most of the alveolar cells apparently similar to the control side. (H&E X 100 ).



**Figure 12 :** A photomicrograph of a section of VE gland eight weeks after the operation showing apparently normal PAS reaction in most of the alveolar cells. ( PAS X 100 ).



**Figure 13 :** A photomicrograph of a section of VE gland eight weeks after the operation showing apparently normal substance P immune reaction in most of the nerve fibers (arrows). (Immunohistochemical stain X 100).

## DISCUSSION

The previous studies upon denervation and reinnervation of taste buds and VE glands were done by many authors on dogs and rabbits using ordinary histological methods. Those authors concluded that, intact innervation was essential for maintaining the structure and function of VE gland (Guth, 1967, El Eishi and State, 1974, State and Bowden 1974)

Gabr and Elmohandes (1990), in  
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their study of VE glands of dogs using acetyl-cholinesterase activity, suggested that the nerve fibers may release a neurotrophic substance having a formative influence upon VE glands. Lunderberg et al., (1979) suggested the presence of substance P (SP), a peptide widely distributed in the central and peripheral nervous system, as trophic factor. Moreover, SP -positive nerve fibers have been detected in the developing lingual papillae as well as in the regenerating



nerves in rats (Nishimoto et al., 1985).). However, Witt and Reutter (1998) stated that autonomic and somatosensory nerves seem not to play a key role in formation and maintenance of early human taste buds.

Substance P and other neuropeptides were studied and described in the normal rats by Ueba and Uchihashi (1991) and Ringstedt et al., (1999) using physiological methods and Robert et al., (1991), Oomori et al., (1995) and Matsuda et al., (1997) using immunohistochemical techniques

In the present study, it has been shown that unilateral crushing of the glossopharyngeal nerve led to temporary disappearance of most of substance P immunoreactive nerve fibers in the VE gland of the same side accompanied by degenerative changes and marked reduction of secretory activity in that gland. The persistence of normal alveoli in the operated side may result from incomplete denervation (crushing) or the gland may have some nerve fibers from the opposite side. Reinnervation of the gland resulted in gradual reappearance of

substance P immunoreactive nerve fibers and regeneration of the glandular alveoli and reappearance of cytoplasmic secretory activities as evidenced by positive PAS reaction within eight weeks. From these findings, it can be concluded that the integrity and function of the VE gland depends on the presence of certain neuropeptides e. g . substance P. The presence of these neuropeptides depends on intact innervation.

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## دراسة هستولوجية ومناعية هستوكيميائية على تأثير هرس العصب اللسان بلعومى على نشاط ودور المادة ب فى غدة الفون إبىرفى لسان الفأر الأبيض

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تقع غدة الفون إبىرتحت وفي محيط حلمات التذوق فى اللسان وتلعب إفرازاتها دورا هاما فى إذابة وتوزيع والتخلص من المواد المتذوقة وتحصل هذه الغدة على تغذيتها العصبية من العصب اللسان بلعومى.

تعتبر المادة ب من الببتيدات العصبية الواسعة الإنتشار فى الجهاز العصبى المركزى والطرفى كما أنها موجودة ايضا فى غدة الفون إبىرفى اللسان . دلت الدراسات السابقة على أن التغذية العصبية فى حد ذاتها هى المسئولة عن الحفاظ على التركيب الهستولوجى والوظيفى لغدة الفون إبىر وذلك عن طريق إفراز مادة الأسيتيل كولين.

أثبتت الدراسات الحديثة وجود مواد وسيطة تسمى الببتيدات العصبية من ضمنها المادة ب وهذه المواد هى المسئولة عن المحافظة على التركيب والوظيفة لهذه الغدة.

تم فى هذا البحث دراسة تأثير المنع المؤقت للتغذية العصبية على وجود ونشاط المادة ب وتأثير ذلك على التركيب الهستولوجى والنشاط الفسيولوجى لغدة الفون إبىر وذلك باستخدام الصبغات المناعية الهستوكيميائية لتلك المادة. أجرى هذا البحث على إثنى عشر فأرا بالغاً قسمت إلى ثلاثة مجموعات ( اربعة فئران فى كل مجموعة).

تم فى هذا البحث إجراء عمليات هرس للعصب اللسان بلعومى الأيسر وذلك تحت تأثير التخدير الكلى للفئران .

وضعت هذه الفئران فى أقفاص منفصلة وتمت العناية بتغذيتها وعلاجها .

تمت التضحية بالفئران بعد اسبوعين وأربعة أسابيع وثمانية أسابيع من إجراء العملية وتم أخذ الجزء الخلفى للسان ووضعها فى الفورمالين المتعادل .  
تم عمل قطاعات شمعية للعينات بسمك ٦ ميكرونات وصباغتها بالهيماتوكسيلين والإيوسين وصبغة ال ب ا س وقطاعات بسمك ٤ ميكرونات للصبغة المناعية الهستوكيميائية للمادة ب .  
إتضح من دراسة هذه العينات ان نشاط المادة ب يقل تدريجيا بعد قطع الإمداد العصبى ويتزامن مع ظهور علامات ضمور ونقص فى عدد الخلايا وانخفاض فى نشاط هذه الخلايا .  
اتضح أيضا أن عودة ظهور نشاط المادة ب يتزامن مع عودة الشكل والنشاط الطبيعى لخلايا غدة الفون إبرنر.  
نستنتج مما سبق أن المادة ب مسئولة عن الحفاظ على الشكل والوظيفة فى غدة الفون إبرنر فى لسان الفأر الأبيض.

