Histol Histopath (1991) 6: 549-558

Histology and Histopathology

Identification of sensory neurons supplying receptors in lingual muscles of the rat: histochemical and retrograde labeling study with horseradish peroxidase

Mahmoud F. Sherif, Ali G.H. Al-Zuhair and Basem A. Kharbat
Department of Anatomy, Faculty of Medicine, Kuwait University, Kuwait

Summary. Sensory innervation of lingual musculature was studied in young adult Wistar rats using retrograde labeling by horseradish peroxidase (HRP) and combined silver impregnation and acetylcholinesterase (AchE) methods. Intra-lingual injection of HRP resulted in labeling of neuronal somata in the trigeminal, superior vagal, and second cervical spinal (C_2) ganglia. When HRP was directly applied to the proximal stump of severed hypoglossal nerve, labeling occurred only in the cervical and superior vagal ganglia. Morphometric analysis revealed that the labeled neurons were of the small-sized category in all ganglia. However, in the trigeminal and C_2 ganglia, labeling occurred also among the medium-sized neurons. Combined silver and AchE preparations from lingual muscles revealed the absence of typical muscle spindles. Instead, there were free and spiral nerve terminals in the interstitium, and epilemmal knob-like or bouton-like endings surrounding non-encapsulated muscle fibers. These terminals showed AchE -ve reaction in contrast to the motor ones. Few ganglionic cells were scattered along the hypoglossal nerve with uniform AchE +ve reaction in their perikarya. This indicates that medium-sized neurons in the trigeminal and C_2 ganglia, and probably sensory neurons along the hypoglossal nerve mediate lingual muscle sensibility perceived by atypical sensory terminals.

Key words: Proprioceptors, Lingual Muscles, Cervical Spinal ganglia, Hypoglossal nerve, Trigeminal ganglion, Superior Vagal ganglion

Introduction

The tongue is an organ capable of diverse and delicate movements. Its musculature would be assumed to possess an extensive innervation. Lingual proprioception is a complex problem because of the uncertainty of both the nature of proprioceptors and the manner of their connection with the brain. While some authors reported the existence of muscle spindles in lingual musculature of a variety of species including man (Langworthy, 1924; Tarkhan, 1936a; Nakayama, 1944; Walker and Rajagopal, 1959; Bowman, 1968; Kubota et al., 1975; Fitzgerald and Sachithanandan, 1979), others were not able to find them (Hewer, 1935; Carleton, 1937; Yee et al., 1939; Cooper, 1953; Law, 1954; Bloom, 1960). On the other hand, Weddell et al. (1940) and Boyd (1941) encountered only some atypical nerve terminals, probably sensory in nature, in lingual muscles of the rabbit and rat. Such controversy about the existence of muscle spindles in tongue musculature and the nature of the atypical nerve terminals led to several misconceptions regarding how lingual muscle activity is controlled.

Concerning the course of sensory fibers from the lingual muscles and the location of their cell bodies, some studies indicated that lingual proprioceptive fibers arise from ganglia along the course of the hypoglossal nerve (Tarkhan, 1936a,b; Tarkhan and Abd-El-Malek, 1950; Holliger, 1955; Wozniak and Young, 1968). However, the sensory nature of such ganglia has not been verified. Other workers concluded that neurons subserving lingual proprioception reside in the trigeminal mesencephalic nucleus or in the semilunar ganglion (Barron, 1936; Smith and Marcarian, 1968; Dault and Smith, 1969). On the other hand, afferent fibers in the hypoglossal nerve conveying lingual proprioception have been shown to originate from neurons in the upper cervical and vagal ganglia (Yee et al., 1939; Tarkhan and Abou-El-Naga, 1947; Fitzgerald and Sachithanandan, 1979; Neuhuber and Mysicka, 1980; Chibuzo and Cummings, 1981).

Based on the existing controversy about the nature

Offprint requests to: Dr. Mahmoud F. Sherif, Department of Anatomy, The George Washington University, Medical Ctr, 2300 I Street, N.W., Washington, D.C., 20037, USA

of lingual proprioceptors and the resulting ambiguity due to the possible implication of more than one nerve and ganglion in the afferent innervation of tongue muscles, the present work has been undertaken. It attempts to identify lingual proprioceptors and the source of afferent fibers innervating lingual muscles using a combination of axonal transport technique, histochemical and morphometric analysis.

Materials and methods

Two groups of 12 male Wistar rats (280-300 gm) were used in this study. The lingual muscles in 4 animals from the first group, were infiltrated by 25-40% horseradish peroxidase (HRP) (Sigma type VI) in Ringer solution through deep intra-lingual multiple injections of 1-2 µl at different sites. The rest of the animals of the first group were operated upon, unilaterally, under Sagatal anesthesia (pentobarbitone sodium 40-60 mg/Kg, injected intraperitoneally) to expose the hypoglossal nerve. The main trunk of the nerve was severed, proximal to its bifurcation and distal to the ramus descendans hypoglossi, in 3 rats. The terminal branches of the hypoglossal, supplying only tongue muscles, were also severed in the remaining 5 rats. Crystals of HRP were directly applied to the proximal stumps of the severed nerves for 30-45 minutes. After a survival period of 48-72 hours, animals were euthanized, under Sagatal anesthesia, by intracardiac perfusion with 2.5% glutaraldehyde solution in 0.2 M phosphate buffer (pH 7.4). The brain stems, trigeminal, vagal, and first 4 cervical spinal ganglia were dissected out carefully and kept over night in 20% sucrose solution in buffer at 4° C. Serial frozen sections (30-40 µm) were obtained from all specimens and processed for demonstration of HRP reaction product according to the method of Mesulam et al. (1980). In all cases, the enzyme uptake was checked by the presence of HRP reaction product in motoneurons of the hypoglossal nucleus. All unlabeled and the resulting labeled neurons in all ganglia examined were counted and subjected to morphometric analysis to obtain neuronals diameters using the «Bioquant» computer program (system IV, 1986 version). All counts obtained were corrected according to Rose and Rohrlich (1987).

From the second group of animals, 6 rats were operated upon and the hypoglossal and lingual nerves were ablated alternatively on one side. After 15-18 days, animals were euthanized by intracardiac perfusion with buffered formalin solution. The tongues with their extrinsic muscles, *en masse*, were dissected out and bisected in the midline. Specimens from the unoperated sides were used as controls. Serial frozen sections (15-20 µm) from each specimen were alternately subjected to acetylcholinesterase (AchE) reaction according to Karnovsky and Root method (1964) and the silver impregnation technique of Sevier and Munger (1965). Some sections with AchE reaction were superimpregnated with silver to differentiate between nerve terminals showing acetylcholinesterase positive (AchE +ve) reaction indicating their motor nature and other non motor ones with acethylcholinesterase negative (AchE -ve) reaction. Tongue specimens with the terminal portions of lingual and hypoglossal nerves intact were taken out from the remaining unoperated animals, fixed by immersion in 10% fuming nitric acid in absolute acetone, paraffin embedded, serially sectioned and impregnated with silver according to the method mentioned by Sherif et al. (1981).

Results

Retrograde labeling experiments

Following deep intralingual injections of HRP, labeled neurons were detected in the trigeminal, proximal vagal and second cervical spinal (C_2) ganglia (Figs. 1a,b,c). In the case where HRP was directly applied to the proximal stumps of the hypoglossal nerve trunk (proximal to its bifurcation and distal to the ramus descendans hypoglossi) as well as to its terminal branches, labeled neurons were found only in the proximal (jugular) vagal and upper cervical (C_2 and C_3) ganglia. Neuronal counts revealed that following direct application of HRP to the main trunk of the hypoglossal and to its terminal branches, 26-32.5% and 14-18.4% of C_2 neurons were labeled respectively. Very few labeled neurons were detected in C_3 in case of direct application of HRP to the proximal stump of the hypoglossal trunk. However, about 2.5% of C_2 neurons were labeled following intralingual injection of HRP. In all experiments few labeled neurons (11-17 cells) were found in the jugular vagal ganglion (Table 1). The trigeminal mesencephalic root cells failed to take the label in any of the experiments.

Few intercalated HRP reactive neurons were detected along the terminal portion of the hypoglossal nerve in some tongue specimens obtained from cases of intralingual injection of the enzyme tracer. Examination of serial sections from silver and AchE preparations obtained from control specimens, where lingual muscles and terminal portion of the hypoglossal nerve were dissected out *en masse*, revealed the presence of such intercalated ganglionic cells along the course of the nerve. These cells appeared globular, unipolar in shape with a definite satellite capsule. They showed uniform AchE +ve reaction product in their somata. They were detected along thick nerve fibers, and in some instances they were present amidst the nerve fascicles (Fig. 1d,e).

Morphometric analysis

Estimation of neuronal diameters in C_2, vagal and trigeminal ganglia was obtained through computer analysis of data resulting from tracing cell circumference by a Cursor on Hi-pad digitizer. Tracing

Table 1. The amount of labeled neurons in the vagal and second cervical spinal (C_2) ganglia of the rat following application of HRP at different sites.

Mode of HRP application	No. of cases	Mean number of labeled neurons (± SEM)		Mean %* of labeled neurons (± SEM) in C_2
		In vagal ganglion	In C_2	
To hypoglossal trunk	3	13.13 (±1.3)	119.95 (±4.6)	29.79 (±1.9)
To terminal branches of hypoglossal	5	13.36 (±1.2)	55.97 (±4.6)	13.9 (±1.5)
Intralingual injection	4	14.87 (±1.7)	9.65 (±.26)	2.4(±.06)

* The percentage of labeled neurons were calculated in relation to the mean of total number of neurons in rat C_2 which was 402.67 (±39.36) cells.

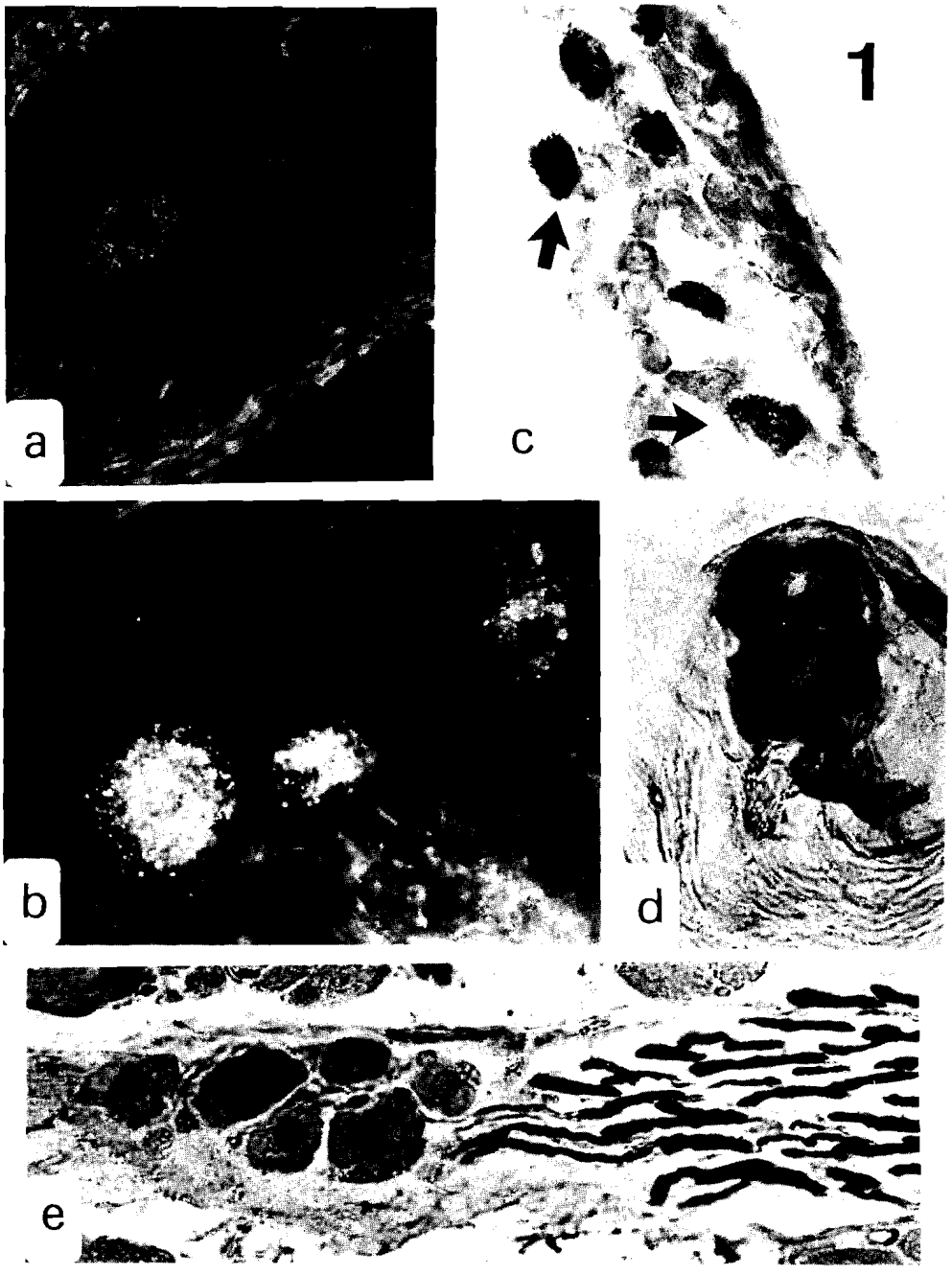

Fig. 1. Photomicrographs showing in **(a)** labeled neuron from the proximal (jugular) ganglion of the vagus and in **(b)** labeled neurons of C_2 ganglion, following intralingual injection of HRP and its application to the proximal stumps of the hypoglossal nerve. dark-field. × 600. **(c)** Labeled neurons in the trigeminal ganglion (arrows) counterstained with neutral red, following intralingual injection of HRP. bright-field. × 300. **(d)** Unipolar globular ganglionic cells amidst fascicles of the hypoglossal nerve, resembling dorsal root spinal ganglia cells, showing uniform AchE +ve reaction in their somata. × 600. **(e)** A group of globular ganglionic cells along the hypoglossal nerve showing definite satellite capsule. Nitric acetone silver impregnation. × 300

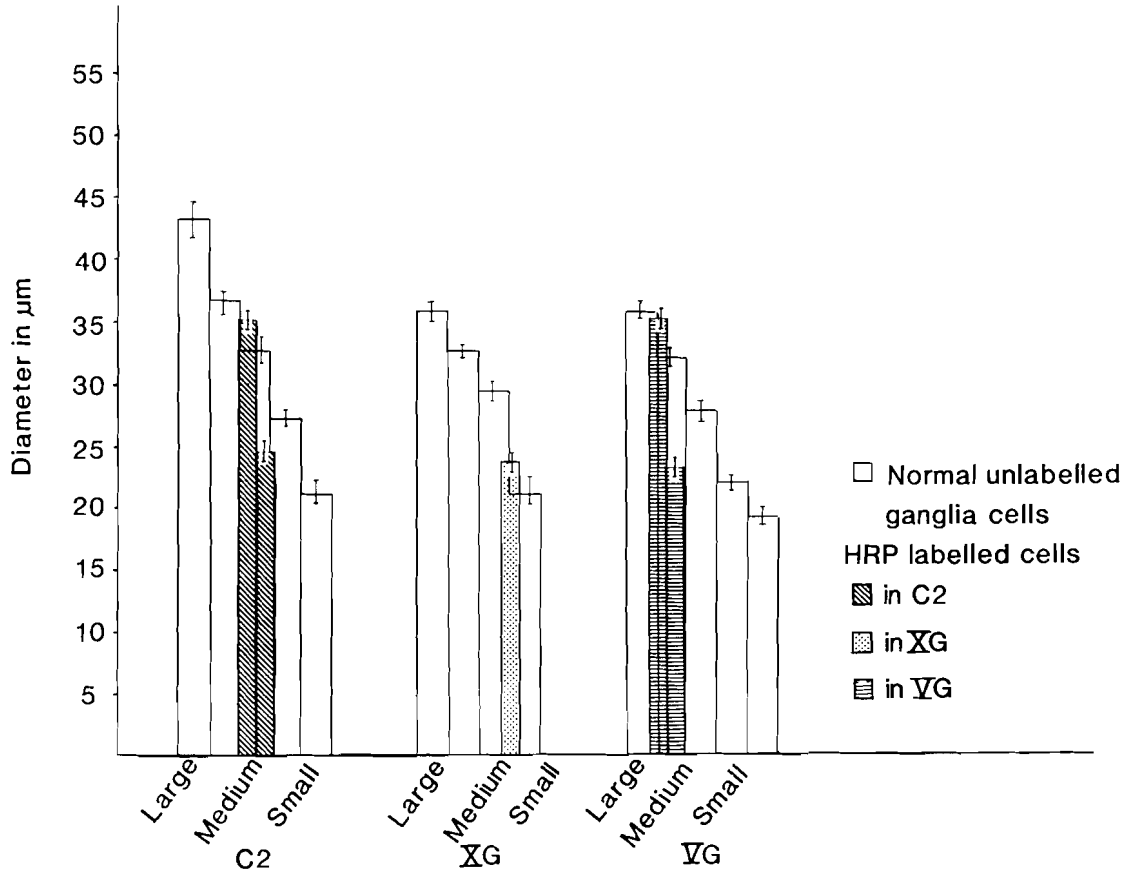

Fig. 2. Bar-chart indicating the mean diameters (± SEM) of the large, medium-sized and small neuronal groups of the second cervical spinal (C_2), superior vagal (XG) and trigeminal (VG) ganglia in the rat. The diameters of the labeled neurons in each ganglion are represented by super-imposed shaded columns for comparison.

included only neurons showing nuclei and nucleoli from the unlabeled ones, and those with maximum clear rounded central zone in the labeled neurons, indicating nuclear sites. This procedure was adopted to minimize errors which may result from tracing parts of the cells due to sectioning. The results revealed that neuronal populations in these ganglia could be categorized into large, medium-sized and small neurons according to cell diameter. The mean diameter (± SEM) of the large neurons in C_2 was 44.2 ± .1 µm, while that of the medium-sized and small ones was 36.7 ± .3 µm and 21.5 ± .3 µm respectively. On the contrary, the average diameter of the large neurons in both vagal and trigeminal ganglia ranged between 35.5 ± .3 and 35.9 ± .5 µm. The small neurons in the jugular ganglion of the vagus measured 21.7 ± .3 µm in diameter and those of the trigeminal ganglion reached 19.5 ± .3 µm. Measurements of the labeled neurons revealed that they were of two categories. One category the comprised neurons measuring 35.5 ± .6 µm in diameter in C_2 and 35.1 ± .7 µm in the trigeminal ganglion. No labeled neurons from this category were detected in the jugular ganglion of the vagus. The diameter of the other category of labeled neurons ranged between 23.9 ± .7 and 24.4 ± .6 µm. Labeled neurons from this category were present in all ganglia examined (Fig. 2). Comparison of the different neuronal sizes, using student t-test, revealed no statistically significant difference between the diameters of the large labeled neurons and both medium-sized ones in C_2 and the large cells of the trigeminal ganglion ($p < 0.05$). Also, the difference between the small-sized category of labeled neurons and the small cells in the three ganglia was insignificant.

Nerve terminals in the lingual musculature

Examination of serial sections from silver and combined silver and AchE preparations from the control sides of the tongue revealed that nerve fibers terminating in lingual muscles form a variety of endings. Some fibers terminate in a non-encapsulated spiral manner which lie freely in the interstitium (Fig. 3a). Others lose their myelin sheaths and terminate in epilemmal free knob-like endings or terminal boutons. These terminals showed some varicosities and were lacking sole-plates. In certain places they surrounded non-encapsulated ordinary muscle fibers with centrally located nuclei (Figs. 3b,c). Many fine unmyelinated free nerve terminals were observed in the intermuscular connective tissue as well (Fig. 3e).

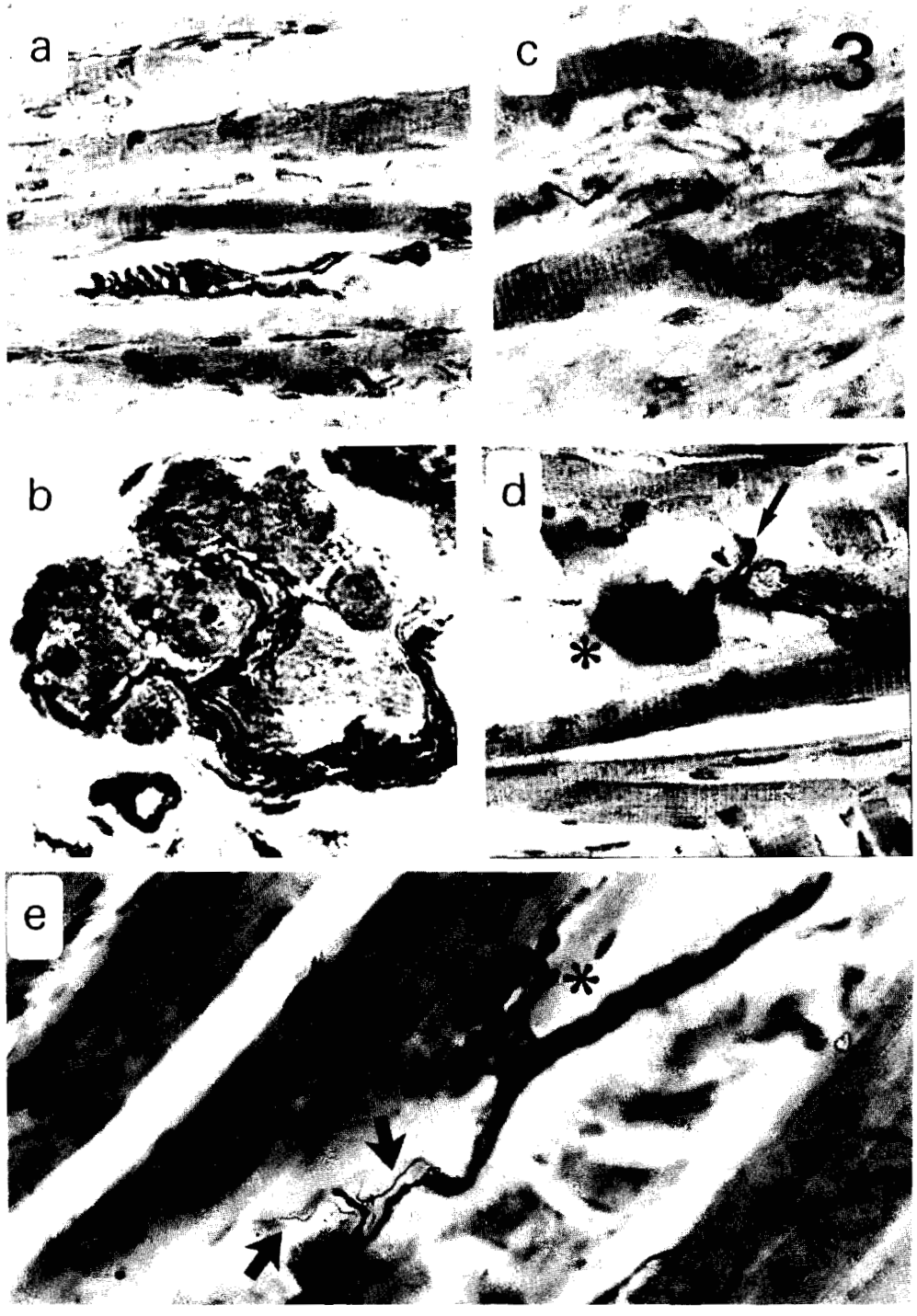

Fig. 3. Photomicrographs showing nerve terminals in the rat lingual muscles subjected to Sevier-Munger silver impregnation method; **(a)** unaffected spiral nerve terminal, 15 days after ablation of the hypoglossal nerve. × 300; **(b)** knob-like endings, lacking sole-plates, surround non-encapsulated muscle fibers with centrally located nuclei (from control material). × 600; **(c)** unaffected epilemmal knob-like terminals, 15 days after ablation of the hypoglossal nerve. × 600; **(d)** free knob-like terminal (arrow) and intense AchE +ve reaction at a terminal with sole-plate (asterisk) from control subject (combined silver and AchE preparation). × 600; **(e)** fine unmyelinated fibers (arrows) and foot-like terminals (asterisk) acquiring sole-plate in the form of a bed containing nuclei (from control subject; Sevier-Munger silver impregnation method). × 500

These unspecialized nerve terminals showed a negative AchE reaction. After 15-18 days following ablation of the hypoglossal nerve, atrophy of the tongue on the operated side was observed with abundat loose fibrous tissue separating individual muscle fibers. Most of the nerve terminals in the lingual musculature disappeared. However, some of the spiral, knob-like, and free nerve terminals still could be observed (Figs. 3a,c).

On the other hand, some thick foot-like nerve endings are located under the sarcolemma acquiring sole-plates in the form of sarcoplasmic bed containing a number of nuclei (Fig. 3e). These terminals showed intense AchE +ve reaction product confirming their motor nature (Fig. 3d) and they seemed unaffected following ablation of the lingual nerve. However, they disappeared after severing the hypoglossal nerve.

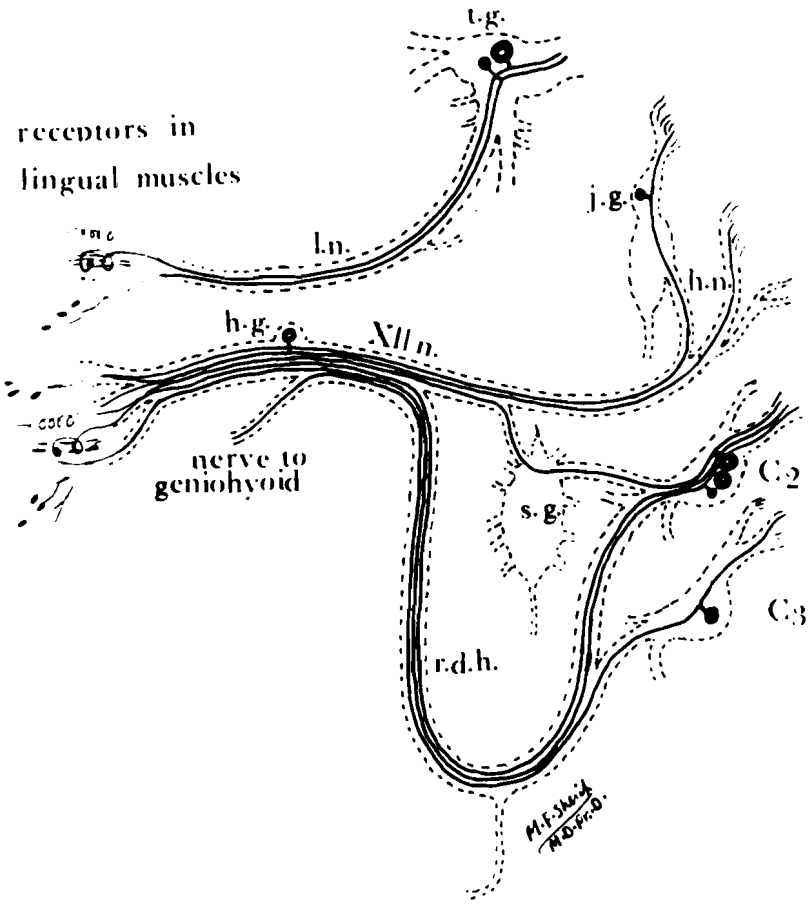

Fig. 4. Diagramatic illustration of the suggested peripheral pathways for lingual muscle sensibility via the lingual nerve (l.n.) to the trigeminal ganglion (t.g.) neurons, and via the hypoglossal nerve (XIIn) to the jugular ganglion of the vagus (j.g.) through the hypoglossonodose branch (h.n.), and to the second cervical spinal ganglion neurons (C_2) through both grey rami communicans of the superior cervical sympathetic ganglion (s.g.) and the ramus descendans hypoglossi (r.d.h.). The presence of hypoglossal ganglionic cells (h.g.) are shown as well.

Discussion

Retrograde labeling experiments of the present study indicate that sensory neurons innervating tongue musculature of the rat reside in the upper cervical spinal ganglia (C_2 and C_3) together with the proximal vagal and trigeminal ganglia. In our material, the first cervical nerve of the rat seems to lack dorsal root ganglion. This is in accord with what was mentioned by Neuhuber and Mysicka (1980). Labeling of neuronal somata in the cervical and vagal ganglia following direct application of HRP to the proximal stumps of the hypoglossal nerve confirms the presence of afferent fibers in the twelfth nerve and indentifies their mother neurons. The existence of afferent fibers in the hypoglossal nerve has been suggested through degeneration experiments (Langworthy, 1924; Corbin and Harrison, 1939; Yee et al., 1939; Zimny et al., 1970) and potential recording studies (Barron, 1936; Cooper, 1954; Bloom, 1960; Bowman and Combs, 1968; Morimoto and Kawamura, 1971; Zapata and Torrealba, 1988). The pathway of afferent fibers in the hypoglossal nerve to the cervical spinal ganglia has been shown to be through the ramus descendans hypoglossi (Neuhuber and Mysicka, 1980; Chibuzo and Cummings, 1981). For this reason the application of HRP in this study was almost always distal to the ramus descendans. Direct communication between the hypoglossal and second and third cervical nerves was mentioned in the rat and monkey (Weddell et al., 1940; Hedger and Webber, 1976; Fitzgerald and Sachithanandan, 1979). Another possible link between the hypoglossal and cervical nerves is through the superior cervical sympathetic ganglion. The passage of afferent fibers through sympathetic ganglia to their cells of origin in dorsal root ganglia has been demonstrated even in the grey rami communicans (Elfvin and Dalsgaard, 1977; Coggelshall and Galbraith, 1978; Oldfield and McLachlan, 1978). On the other hand, vagal hypoglossal connections have been reported in degeneration and neurophysiologic studies (Tarkhan and Abou-El-Naga, 1947; Sauerland and Mizuno, 1968; Tanaka, 1975; Zapata and Torrealba, 1988). These studies suggested that afferent hypoglossal fibers originate from the distal (nodose) ganglion of the vagus, since removal of this ganglion or sectioning of the hypoglossonodosal branch resulted in degeneration of fibers in the hypoglossal nerve and abolished responses evoked by hypoglossal stimulation in intact animals. However, in the present study,

neuronal labeling was found only in the proximal (jugular) ganglion of the vagus which mimics the findings of Neuhuber and Mysicka (1980) and Chibuzo and Cummings (1981). The discrepancy between these findings and those of extirpation studies can be explained be assuming that removal of the nodose ganglion or severing the hypoglossonodosal branch would have interrupted fibers originating from the proximal (jugular) vagal ganglion. Accordingly, it can be postulated that centripetal fibers from sensory neurons located in vagal and upper cervical ganglia convey afferent impulses from tongue muscles to the brain stem via the vagal rootlets and sensory roots of the cervical nerves. Nevertheless, Tarkhan and Abou-El-Naga (1947) failed to find degenerating fibers in the hypoglossal nerve after removal of the upper cervical spinal ganglia in the dog. Also, Nakamura et al. (1970) described persistence of the influence of centripetal afferent impulses in the hypoglossal nerve upon trigeminal monosynaptic masseteric reflexes after cutting the first and second cervical nerves and vagal roots or medullo-spinal junction in the cat. These studies suggest that some afferent fibers in the hypoglossal nerve reach the brain stem via the hypoglossal rootlets. Several authors reported the presence of ganglionic cells along the hypoglossal nerve in various species including man (Tarkhan and Abd-El-Malek, 1950; Holliger, 1955; Kubota et al., 1963; Wozniak and Young, 1968; Quayyum and Beg, 1975; Fitzgerald and Sachithanandan, 1979). However, the sensory nature of these ganglionic cells was questionable as they were believed by some authors to be autonomic in function. The findings of the present work using more recent silver and histochemical techniques showed that these cells are globular, mostly unipolar with intimate satellite capsule and uniform AchE +ve reaction, which stimulate primary sensory neurons in the trigeminal and dorsal root spinal ganglia. Further, they were located essentially on the nerve trunk and were labeled in HRP experiments, thus providing evidence that these cells are most probably subserving a sensory function. Accordingly, they may be representing the mother neurons of afferent fibers confined to the hypoglossal nerve itself (Fig. 4).

Neuronal labeling in the trigeminal ganglion following deep intralingual injection of HRP suggests the implication of the lingual nerve in sensory innervation of tongue muscles. In spite of the possible diffusion of the enzyme tracer to the lingual and gingival mucosa in the present study, Chibuzo and Cummings (1981) were able to obtain similar results following HRP injection in the individual extrinsic tongue muscles of the dog. The possiblity of lingual nerve implication in controlling tongue muscle activity was admitted in earlier studies on humans, since signs of lingual ataxia and difficulty of articulation were manifested following anesthesia of the lingual nerve (Carleton, 1937; Rowbotham, 1939). The results of degeneration experiments in the present study support this possibility, on account of the persistence of some sensory terminals in tongue muscles following ablation of the hypoglossal nerve. This finding is consistent with the observations of Fitzgerald and Sachithanandan (1979) who found unaffected sensory terminals including muscle spindles following interruption of the terminal portion of the hypoglossal nerve in the monkey. On the other hand, no labeling was found among the trigeminal mesencephalic root cells, in our experiments of direct application of HRP to the proximal stumps of the hypoglossal nerve, and after intralingual injection of the enzyme tracer. This eliminates any possible direct projection from the trigeminal mesencephalic nucleus to lingual musculature neither via the lingual nor the hypoglossal nerves as previously suggested by Dault and Smith (1969).

The quantitative analysis of this study revealed that no labeled neurons were detected in C_3 when HRP was applied to the terminal branches of the hypoglossal nerve. Also, there was a decrease in the percentage of labeled neurons in C_2 than when HRP was applied to the main trunk of the hypoglossal nerve. This can be attributed to the involvement of the branch to geniohyoid muscle in case of direct application of the enzyme tracer to the nerve trunk, since this branch usually leaves the hypoglossal nerve distal to the ramus descendans and proximal to its terminal branches. Similarly, the more pronounced decrease in the percentage of labeled neurons in C_2 following deep intralingual injection of HRP can be explained by the possible escape of the extrinsic lingual muscles from uptaking the enzyme tracer. On the other hand, there was no change in the number of labeled neurons in proximal (jugular) ganglion of the vagus in all experiments. It seems, therefore, that only the intrinsic tongue muscles of the rat have sensory representation in the vagal and trigeminal ganglia and minimal representation in C_2, while the extrinsic muscles have their representation in C_2 and the geniohyoid muscle in C_3 (Fig. 4). These results mimic those obtained by Neuhuber and Mysicka (1980). Further, comparing the sizes of the different neuronal populations in C_2, vagal and trigeminal ganglia revealed that the majority of the labeled neurons were of the small-sized category (23.9 ± .7 - 24.4 ± .6 μm in diameter). However, the larger labeled neurons (35.6 ± .6 - 35.1 ± .7 μm in diameter) correspond to those of the medium-sized ones in C_2 and to the large-sized group of the trigeminal ganglion. Also, the sizes of labeled neurons in the present study are comparable with those of the trigeminal mesencephalic nucleus. This nucleus is known to represent primary sensory neurons residing in the brain stem concerned with proprioception from the masticatory muscles. Previous studies showed that there is a unimodal distribution of its neuronal somata (14-40 μm) with a peak at 20-26 μm in the rat (Sivanandasingham and Warwich, 1976; Limwongse and De Santis, 1977). This parallelism between the sizes of the trigeminal mesencephalic root cells and

the labeled neurons in C_2 and trigeminal ganglia resulted from our experiments suggests that they are probably concerned with proprioception. According to Ranson and Clark (1959), Le Gros Clark (1965) and Brodal (1969), large afferent fibers conveying proprioception from muscles, joints and tendon organs belong to the large spinal ganglia cells, whereas fine fibers belong to the small cells and represent nociceptive and tactile afferents. Thus, the results of the present morphometric analysis can be correlated to previous studies of fiber spectra of the hypoglossal nerve. These studies revealed that its afferent component are mostly of the small caliber axons in the cat, rabbit and rat (Yeet et al., 1939; Blom, 1960; Lodge et al., 1973). Meanwhile, an explanation for the significance of the unspecialized morphologic appearance of nerve terminals in lingual muscles can be thought about.

In the present study, more recent specific silver impregnation methods for nerve fibers and terminals, combined whith histochemical demonstration of AchE activity to differentiate between motor and sensory terminals, have been used. The results confirmed the absence of typical muscle spindles from lingual musculature of the rat. Instead, there are free, epilemmal bouton or knob-like and spiral nerve terminals. Some of them surround individual non-encapsulated muscle fibers with centrally located nuclei. These terminals were AchE –ve indicating their sensory nature, unlike the motor ones with sole-plates which showed intense AchE +ve reaction. Such findings are in accord with most of the previous studies (Boecke, 1927, mouse, rat; Yee et al., 1939, cat, rabbit, rat; Cooper, 1953, cat; Prakash and Rao, 1980, buffalo). However, other investigators claimed that they could see muscle spindles in lingual muscles of these animals (Langworthy, 1924, cat, dog, rat; Tarkhan, 1936a, rabbit). This discrepancy can be attributed to different techniques used as well as the difference of interpretation of nerve terminal forms, especially the spiral ones. On the contrary, muscle spindles have been documented in lingual muscles of the monkey and man (Nakayama, 1944; Cooper, 1953; Walker and Rajagopal, 1959; Bowman, 1968; Kubota et al., 1975; Fitzgerald and Sachithanandan, 1979; Maiboroda and Mokin, 1983). It is known that there is species difference which indicates that capsulated sensory terminals are absent from lingual muscles of lower mammals. However, in higher mammals they are abundant, including muscle spindles, with some sort of transition between the species. Accordingly, it seems that along the phylogenetical ascent, the tongue of higher mammals including man acquired more diverse functions which may necessitate the presence of muscle spindles. On the contrary, in lower mammals simpler forms of receptors took over the role of lingual muscle sensibility. According to Mathews (1967) and Werner and Whitsel (1973), muscle spindles are more important for subconscious nervous control of muscular contraction and they have been regarded as being concerned with the control of posture and movements but not with perception. Unlike most skeletal muscles, the tongue does not act upon any joint but depends upon the differential contractions of its muscular components for the changes of its position and shape. Lingual tactile receptors may indicate tongue position by recognition of the structures in the oral cavity. Therefore, it can be assumed that spiral terminals and those surroundings muscle fibers with centrally located nuclei probably represent proprioceptors which are supplied by the medium-sized neurons in the cervical spinal ganglia and their corresponding large ones in the trigeminal ganglion. On the other hand, the free nerve fibers and some of the knob-like terminals are probably of the nociceptive and tactile variety which are innervated by the small-sized neurons in cervical spinal, vagal and trigeminal ganglia.

Acknowledgements. Supported by Kuwait University Research council Grant MA018.

References

Barron D.H. (1936). A note on the course of the proprioceptor fibers from the tongue. Anat. Rec. 66, 11-16.

Blom S. (1960). Afferent influences on tongue muscle activity. Acta Physiol. Scand. 49, (Suppl. 170) 1-97.

Boecke J. (1927). Die morphologische Grundlage der sympathischen Innervation der quergestreiften Muskelfasern. Z.F. Mikro. Anat. Forsch. 8, 561-639.

Bowman J.P. and Combs C.M. (1968). Discharge patterns of lingual spindle afferent fibers in the hypoglossal nerve of the rhesus monkey. Exp. Neurol. 21, 105-119.

Bowman J.P. (1968). Muscle spindles in the intrinsic and extrinsic muscles of the rhesus monkey *(Macaca Mulatta)* tongue. Anat. Rec. 161, 483-488.

Boyd J.D. (1941). The sensory component of the hypoglossal nerve in the rabbit. J. Anat. 75, 330-345.

Brodal A. (1969). Neurological anatomy in relation to clinical medicine. 2nd edition. Oxford University Press, London, New York, Toronto. pp 43-49.

Carleton A. (1937). Observations on the problem of the proprioceptive innervation of the tongue. J. Anat. 72, 502-507.

Chibuzo G.A. and Cummings J.F. (1981). The origins of the afferent fibers to the lingual muscles of the dog, a retrograde labeling study with horseradish peroxidase. Anat. Rec. 200, 95-101.

Coggelshall R.E. and Galbraith S.L. (1978). Categories of axons in mammalian rami communicants, part II, J. comp. Neurol. 191, 349-360.

Cooper S. (1953). Muscle spindles in the intrinsic muscles of the human tongue. J. Physiol. 122, 193-202.

Cooper S. (1954). Afferent impulses in the hypoglossal nerve on stretching the cat's tongue. J. Physiol. 126, 32.

Corbin K.B. and Harrison F. (1939). The sensory innervation of the spinal accessory and tongue musculature in the rhesus monkey. Brain 62, 191-197.

Dault S.Y.H. and Smith R.D. (1969). A quantitative study of the

nucleus of the mesencephalic tract of the trigeminal nerve of the cat. Anat. Rec. 165, 79-88.
Elfvin L.G. and Dalsgaard C.J. (1977). Retrograde axonal transport of horseradish peroxidase in afferent fibers of the inferior mesenteric ganglion of the guinea pig. Identification of the cells of origin in dorsal root ganglia. Brain Res. 126, 149-153.
Fitzgerald M.J.T. and Sachithanandan S.R. (1979). The structure and source of lingual proprioceptors in the monkey. J. Anat. 128, 523-552.
Hedger J.H. and Webber R.H. (1976). Anatomical study of the cervical sympathetic trunk and ganglia in the albino rat *(Mus norvegicus albinus)*. Acta. Anat. 96, 206-217.
Hewer E.E. (1935). The development of nerve endings in the human foetus. J. Anat. 69, 369-379.
Holliger A. (1955). Contribution à l'étude de la constitution de l'anse du nerfe hypoglosse; étude d'anatomie comparêe. Archives Anat. Histol. Embryol. 38, 4-46.
Karnovsky M.J. and Root L. (1964). A direct coloring thiocholine method for cholinesterases. J. Histochem. Cytochem. 12, 219.
Kubota K., Kubota J., Fukuda N., Asakura S., Nakagawa S. and Masui M. (1963). Comparative anatomical and neurohistochemical observations on the tongue of marsupials. Anat. Rec. 147, 337-353.
Kubota K., Negishi T. and Nasegi T. (1975). Topological distribution of muscle spindles in the human tongue and its significance in proprioception. Bull. Tokyo Med. Dent. Univ. 22, 235-242.
Langworthy O.R. (1924). A study of the innervation of the tongue musculature with particular reference to the proprioceptive mechanism. J. Comp. Neurol. 36, 273-297.
Law M.E. (1954). Lingual proprioception in pig, dog and cat. Nature 174, 1107-1108.
Le Gros Clark W.E. (1965). The tissues of the body. 5th edition, Oxford University Press, Ely House. London. pp 383.
Limwongse V. and De Santis M. (1977). Cell body locations and axonal pathways of neurons innervating muscles of mastication in the rat. J. Anat. 149, 477-488.
Lodge D., Duggan A.W., Biscoe T.J. and Caddy K.W.T. (1973). Concerning recurrent collaterals and afferent fibers in the hypoglossal nerve of the rat. Exp. Neurol. 41, 63-75.
Maiboroda I. and Mokin I. (1983). Structure of the neuromuscular spindles in the tongue of human fetuses. Arch. Anat. Histol. Embriol. 85, 39-45.
Mathews P.B.C. (1967). Muscle spindles and their motor control. Physiol. Rev. 44, 219-288.
Mesulam M.M., Hegarty E., Barbas H., Carson K.A., Gower E.C., Knapp A.G., Moss M.B. and Mufson E.J. (1980). Additional factors influencing sensitivity in the tetramethyl benzidine method for horseradish peroxidase neurohistochemistry. J. Histochem. Cytochem. 28, 1255-1259.
Morimoto T. and Kawamura Y. (1971). Discharge patterns of hypoglossal afferents in the cat. Brain Res. 35, 539-542.
Nakamura Y., Goldberg L.J., Mizuno N. and Clemente C.D. (1970). Masseteric reflex inhibition induced by afferent impulses in the hypoglossal nerve. Brain Res. 18, 241-255.
Nakayama M. (1944). Nerve terminations in the muscle spindle of the human lingual muscles. Tohoku Med. J. 34, 367-377.
Neuhuber W. and Mysicka A. (1980). Afferent neurons of the hypoglossal nerve of the rat as demonstrated by horseradish peroxidase tracing. Anat. Embryol. 158, 349-360.
Oldfield B.J. and McLachlan E.M. (1978). Localization of sensory neurons traversing the stellate ganglion of the cat. J. Comp. Neurol. 182, 915-922.
Prakash P. and Rao G.S. (1980). Anatomical and neurohistological studies on the tongue of the indian buffalo *(Bubalus bubalis)*. Acta Anat. 107, 373-383.
Quayyum M.A. and Beg M.A. (1975). Anatomical and neurohistological observations on the tongue of the indian goat *(Capra Aegagrus)*. Acta. Anat. 93, 554-567.
Ranson S.W. and Clark S.L. (1959). The anatomy of the nervous system, its development and function. 10th edition. W.B. Saunders Company. Philadelphia and London. pp 135-136.
Rose R.O. and Rohrlich D. (1987). Counting sectioned cells via mathematical reconstruction. J. Comp. Neurol. 263, 365-386.
Rowbotham G.F. (1939). Observations on the effect of trigeminal denervation. Brain 62, 364-380.
Sauerland E.K. and Mizuno N. (1968). Hypoglossal nerve afferents: elucidation of a polysynaptic hypoglosso-laryngeal reflex. Brain Res. 10, 256-258.
Sevier E.A. and Munger B.L. (1965). A silver method for paraffin sections of neural tissues. J. Neuropathol. Exp. Neurol. 24, 130-135.
Sherif M.F., Papadopoulos N.J. and Albert E.N. (1981). Fiber contribution from the mesencephalic nucleus of the trigeminal nerve to the trochlear nerve in the cat: A histological quantitative study. Anat. Rec. 201, 669-678.
Sivanandasingham P. and Warwich R. (1976). A comparative numerical study of the trigeminal mesencephalic nucleus. Acta. Anat. 96, 449-458.
Smith R.D. and Marcarian H.O. (1968). Centripetal localization of tooth and tongue tension receptors. J. Dent. Res. 47, 616-621.
Tanaka T. (1975). Afferent projections in the hypoglossal nerve to the facial neurons of the cat. Brain Res. 99, 140-144.
Tarkhan A.A. (1936a). Ein experimenteller Beitrag zur Kenntnis der proprioceptiven innervation der zunge. Z. Anat. U. Entw. Gesch. 105, 348-358.
Tarkhan A.A. (1936b). Üeber das vorhandensein afferenter Fasern im Nervus hypoglossus. Arch. Psychiat. U. Nervenkr. 105, 475-483.
Tarkhan A.A. and Abou-El-Naga I. (1947). Sensory fibers in the hypoglossal nerve. J. Anat. 81, 23-32.
Tarkhan A.A. and Abd-El-Malek S. (1950). On the presence of sensory nerve cells on the hypoglossal nerve. J. Comp. Neurol. 93, 219-228.
Walker L.B. and Rajagopal M.D. (1959). Neuromuscular spindles in the human tongue. Anat. Rec. 133, 438.
Weddell G., Harpman J.A., Lambley D.G. and Young L. (1940). The innervation of the musculature of the tongue. J. Anat. 74, 255-267.
Werner G. and Whitsel B.L. (1973). Functional organization by the somatosensory cortex. In: Handbook of sensory physiology. Vol. 2, Iggo A. (ed). Springer-Verlag.

Berlin, Heidelberg, New York. pp 621-700.

Wozniak W. and Young P.A. (1968). Nerve cells in the extracranial portion of the hypoglossal nerve in human fetuses. Anat. Rec. 162, 517-522.

Yee J., Harrison F. and Corbin K.B. (1939). The sensory innervation of the spinal accessory and tongue musculature in the rabbit. J. Comp. Neurol. 70, 305-314.

Zapata P. and Torrealba G. (1988). Reflex effects evoked by stimulation of hypoglossal afferent fibers. Brain Res. 445, 19-29.

Zimny R., Sobusiak T. and Matlosz Z. (1970). The afferent components of the hypoglossal nerve. J. Hirnforsch. 12, 83-100.

Accepted May 14, 1991