Indian J.Sci.Res.3(1) : 13-19, 2012

FIBRE COMPONENTS OF THE EXTERNAL LARYNGEAL NERVE: A MORPHOLOGICAL AND HISTOCHEMICAL ANALYSIS

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ABSTRACT

The external laryngeal nerve (ELN) is a slender branch of the superior laryngeal nerve (SLN) which primarily innervates the cricothyroid and inferior pharyngeal constrictor muscles. However the exact nature of fibers which are contained in this nerve has not been ascertained yet. To this purpose, gross dissection was carried out on 50 (100 sides) human cadavers, to isolate the nerve and identify its course and branches. 10 dog (*Canis lupus familiaris*) cadavers were also dissected to isolate the ELN and to obtain specimens for histo-chemical analysis. In dogs the ganglion nodosum of vagus nerve and the Superior Cervical Sympathetic Ganglion (SCSG) were completely or partially fused, and the SCSG gave off 1 or more communicating twigs, that connected to the ganglion nodosum. In human beings the SCSG communicated with the SLN and its branches. In most cases the SCSG connected with the ELN (86 %). Hence, statistically, the ELN is a looped nerve. In both dog and human dissections every one of the ELN/ELN loops without exception gave off a branch to the thyroid gland. The Acetylcholinesterase (AChE) staining showed that the dog ELN contained AChE-positive myelinated, AchE-negative myelinated and AChE-positive unmyelinated nerve fibers indicating the presence of somatic motor fibers, somatic sensory fibers and post-ganglionic sympathetic fibers. This proves that the ELN is a mixed nerve. Thus, damage to the nerve during thyroid surgeries could result in functional impairment to not only voice, but also gland function and sensation in the region.

KEY WORDS: Ganglion nodosum, cervical sympathetic chain, acetylcholinesterase, laryngeal nerve loop

Grossly, the external laryngeal nerve (ELN) is described as a small, linear branch of the superior laryngeal nerve (SLN) that innervates the cricothyroid muscle, the inferior pharyngeal constrictor, contributes to the pharyngeal plexus and has connections with the superior cardiac nerve, cervical sympathetic ganglion and the thyroid gland (Gray and Williams ,1995). However, in recent times the ELN has become a focus of study, due to the fact that it is no longer recognized as a purely somato-motor nerve nor is it considered to be a linear nerve (Sun and Chang,1991; Sun and Dong,1997; Furlan, 2002). The various adverse effects observed in cases of surgical trauma to the nerve (Clouse and Flynn,1985; Kark et al., 1984; Kochilas et al, 2008; Moosman and Weese,1968; Moran and Castro; 1951)have prompted a closer look at the ELN. Numerous prior studies (Andrew,1956; Furlan, 2002; Hisa,1982; Hishida et al.,1997;Maranillo et al., 2003; Pulakunta, 2009; Sun and Chang,1991; Sun and Dong,1997) imply that ELN might contain somato-motor, postganglionic sympathetic and sensory fibers. However, no study has been conducted to histo-chemically verify and establish the exact fiber composition of the ELN. Our study intends to establish the nature of the fibers present in the nerve that is of such significance surgically.

MATERIALS AND METHODS

a- Dissection of Neck in Fresh Dog Cadavers

10 fresh dog(*Canis lupus familiaris*) cadavers (5 male and 5 female) aged between 2-4 years old, were obtained from the animal department of Chongqing medical university. Dissection of the neck was carried out under sterile precautions. The laryngeal nerves and their branches and also the superior cervical sympathetic ganglion (SCSG) and the ganglion nodosum were exposed. The communications between the cervical sympathetic trunk (CSC) and the superior laryngeal nerve were identified and photographed. Samples were also taken for histo-chemical analysis.

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b- Dissection of Neck in Human Cadavers

50 randomly chosen adult cadavers (100 sides) were fixed in formalin and dissected for this study. The ganglion nodosum, the SCSG, SLN, ELN, ILN, the thyroid arteries, the cricothyroid muscle and the inferior pharyngeal constrictors and the surrounding structures were exposed by careful blunt dissection and examined. The patterns of communication between the ELN and CSC were determined and photographed.

c-Fixation and Staining Procedure

Blocks of tissue were obtained from the fresh dog cadavers from the junction of ELN and a branch of the CSC. The tissues were fixed by Holt's (Holt et al.,1997) method and stained using the "direct coloring" thiocholine method for cholinesterase activity, described by Karnovsky (Karnovsky and Roots,1964) as follows:

i) The tissue blocks were fixed overnight in cold 10% formalin containing 1% CaCl2 and were then kept in 0.88M sucrose 1% gum arabic. ii) The blocks were then washed in distilled water for five minutes, blotted dry and quick frozen in iso-pentane-acetone-dry ice. iii) They were then cut into sections in a cryostat. iv) The cut sections were mounted on gelatinized slides. v) The slides were incubated with the medium which was prepared by dissolving 5mg of acetylthiocholine iodide in 6.5 ml 0.1M sodium hydrogen maleate buffer. The slides were washed with the running buffer. And then the following were added in order, with stirring between each addition: 0.5 ml 0.1M sodium citrate, 1 ml 30mMCuSo4, 1 ml water, and 1ml 5 mM potassium ferricyanide. After dehydration, the slides were sealed and examined.

d-Fiber identification

The nature of the nerve fibers was identified according to the Adams (Adams, et al, 1967) technique. Adams has, in his experiment, proved that AChE-positive myelinated fibers, AChE-negative myelinated fibres and AChE-positive unmyelinated fibers are somato-motor, somato-sensory and post-ganglionic sympathetic nerve fibers, respectively.

RESULTS

1. Of the 20 sides of dog's necks, all presented a clear communication between the SCSG and nodosum ganglion. The SLN was also found to originate close to the point of origin of the vagus nerve from the nodose ganglion and passed close to the CSC thus forming a Vago Sympathetic trunk. Fusion of the upper parts of the SCSG and Nodose ganglion was also observed in 10 specimens. This fusion made it virtually impossible to distinguish the points of origin of the SLN in some cases. The ELN was also observed giving off a branch to the upper pole of the thyroid gland and cricothyroid muscle, respectively in the 20 sides dissected (Fig. 1, 2).

2. 98 of the 100 sides of neck dissected in the human cadavers contained the thyroid branch of the ELN (external laryngeal nerve). The branching patterns of the nerve were in accordance with the SLN nerve loop types and sub-types described by Sun and Dong (Sun and Dong,1997)and later by Furlan (Furlan, 2002), but in majority of case, we observed a branch that terminated in the upper pole of the thyroid gland. The classic pattern was that of the ELN branching to supply the cricothyroid muscle and then giving off another branch to the upper pole of the thyroid gland. In almost all cases one or more communicating branches existed between the SLN and the CSC, thus completing the nerve loop. The details of our findings are as follows: the SCSG communicated with the ELN in 82 sides (82/100) (Fig. 3); the SCSG communicated with the SLN in 9 sides (9/100); the SCSG communicated with both the ELN and SLN in 2 sides (2/100); the SCSG communicated with both the ELN and ILN (Internal laryngeal nerve) in 2 sides (2/100) in table-1.

3. Under the microscope, a bundle of the AChE-positive unmyelinated fibers from SCSG passing through the nodosum was observed (Fig.4). AChE-positive myelinated, AChE-negative myelinated and AChE-positive unmyelinated fibers were identified in the midst of the nerve bundle of dog ELN (Fig. 5). Especially, the nerve fibers of thyroid gland were AChE-positive unmyelinated fibers.

DISCUSSION

Traditional gross anatomy had always believed that the ELN was a purely somatomotor nerve based on its supply to cricothyroid and inferior pharyngeal constrictor muscles. Through anatomical and functional investigations, recently a school of thought emerged that the ELN was not composed of purely somatomotor fibres. This shift in componential description of the ELN was prompted by a series of studies conducted worldwide on the morphology of the ELN and its branches. Early studies in this line conducted by Nonidez (Nonides,1931) concluded that the thyroid gland of dog received innervations from the SCSG and SLN. Action potential studies on SLN of rat reported detection of a variety of sensory fibers contained in the SLN (Andrew,1956). Presence of small twigs that originated from the ELN and coursed downwards towards the thyroid gland along with branches of the thyroid artery to reach the thyroid gland was noticed (Moosman and Weese, 1968). They appeared to be Parasympathetic in function (Moosman and Weese, 1968). A fluorescence histo-chemical study demonstrated the presence of noradrenergic nerve fibers carried by the SLN and ELN (Hisa, 1982). More recently the existence of an anastomotic loop connecting the CSC and the SLN together with its branches, ELN and ILN was reported (Sun and Chang, 1991). In the afore mentioned exhaustive study, they dissected 60 cadavers (120 sides) and reported the existence of the nerve loop in 98.3% of the cadavers. The overwhelming presence of the ELN loop in the majority of cadavers(78.3 %) proved that it is the norm and that the ELN is not a linear nerve. They classified the SLN loop into 3 categories, 5 types and 17 subtypes. The 3 categories i.e. V-shaped, U-shaped and mixed, were first described in this study. Later the ELN loop presence in 60 cadavers was reverified in applied anatomical study (Sun and Dong, 1997). Sun and Chang's study was the first meticulous anatomic investigation that included a classification for anastamoses between the CSC/SCSG and the SLN (Furlan, 2002). Following that Furlan repeated Sun's work and confirmed the Sun and Chang findings and also to an extent excluded the possibility of racial differences in the ELN loop. Furlan also stresses upon the need to preserve the communications between the SLN and the SCSG/CSC during radical surgeries of the neck. Presence of sensory fibers was noticed in the ELN (Maranillo et al., 2003). A special connection between the ELN and the Sympathetic trunk has been noticed (Kochilas et al., 2008). A recent case report mentions that the ELN was found to give off branches that pierced the upper pole of the thyroid gland (Pulakunta et al., 2009). Additionally, the post operative consequences of damage to this small but vital branch of the SLN attain importance in light of the fact that the ELN is not a purely motor nerve. The above-mentioned papers give us a clue that ELN not only carries pure somatomotor nerve fibers, but also carries post-ganglionic sympathetic fibers derived from a communicating branch from the SCSG/ CSC (Sun and Chang, 1991; Sun and Dong, 1997; Furlan, 2002). In addition, ELN might carry sensory fibers (Maranillo, 2003), and even carry parasympathetic nerve fibers (Andrew,1956). Thus ELN may not be a purely somatic motor nerve, but a mixed nerve. However no histo chemical verification of the nature of the components of the ELN has been published to date. Our exhaustive study intended to verify the exact nature of the types of fibers contained in the ELN. The experiment was designed to contain gross anatomical verification by dissection of dog cadavers and human cadavers, and histo-chemical verification by collection of samples cadavers and staining for AChE for microscopic examination.

Comparison Between Human and Dog Vagus Nerve (Cervical Part) and Cervical Sympathetic Chain

According to our observation, in dog the ganglion nodosum and the SCSG are fused or at least partially fused thus creating a direct communication between the ganglia (Fig.1, 2). The vagus nerve (cervical part) and the CSC are also partly or completely fused resulting in the formation of a vago-sympathetic trunk (Fig.1, 2, 4). In human being the ganglion nodosum of vagus nerve and the SCSG are separated. The SCSG communicated with the SLN and its branches (Table 1, Fig. 3). In most cases the SCSG connected with the ELN (86 %). The results are consistent with Sun and Furlan's results (Sun and Chang, 1991; Sun and Dong, 1997; Furlan, 2002), in which the percentage of SCSG-ELN connection is 78.3%, 92.5% and 78%, respectively. From a statistical point of view, the ELN is a looped nerve, and non linear in course. From classical textbook (Gray and Williams, 1995), the communicating twigs from SCSG may contain postganglionic sympathetic nerve fibers and sensory nerve fibers. As for the innervations of the thyroid gland, Morris Human Anatomy says that the vasomotor and secretory nerve supply of the thyroid gland consists of unmyelinated fibres from the middle and inferior cervical ganglia of the sympathetic system. In our experimental groups, both dog and human cadaver dissections demonstrated the presence of one or more branches to the upper pole of the thyroid gland given off by the ELN. All the ELN/ELN loops observed without exception gave off one or more branches to the thyroid gland.The results prove that the ELN is one of the origins of thyroid innervations and must carry post-ganglionic sympathetic fibers too.

Nerve Fiber Identification of Dog ELN

The results of our histo-chemical staining and observation of the dog ELN demonstrated the presence of 3 different types of fibers in the ELN, AChE-positive myelinated fibers, AChE-negative myelinated fibers and AChE-positive unmyelinated fibers. It is well known that peripheral nerve shows AchE activity. Adams (Adams et al.,1967) verified that AChE-positive myelinated fibers, AChE-negative myelinated fibers and AChE-positive unmyelinated fibers represents somato-motor, somatosensory and postganglionic sympathetic nerve fiber, respectively. Thus the ELN contains somatic motor, somatic sensory and post-ganglionic sympathetic fibers. Our results prove that the ELN is a mixed nerve and also prove that the thyroid glandular branch is postganglionic sympathetic in nature, which may play an important function in vasomotor and secretory functions of thyroid gland.

CONCLUDING REMARKS

Our findings have established ELN is looped in shape as a mixed nerve the ELN carries somato-motor fibers to the cricothyroid and inferior pharyngeal constrictor muscles and also carries post-ganglionic sympathetic fibers to the thyroid gland. A few sensory fibers were also identified in the histo-chemical study which could probably originate from the cricothyroid joint (Kochilas et al., 2008). This fact assumes significance in thyroidectomies and other such surgeries of the neck during which it is common to encounter the ELN. Many methods to safeguard the ELN were proposed by surgeons to preserve the ELN during surgery (Clouse and Flynn, 1985; Holt et al., 1977; Kark et al., 1984; Lordland,1950; Moosman and Wees,1968 and Moran and Castro,1951). Our findings suggested that the ELN is a mixed nerve carrying even a few sensory fibers lays further emphasis on the need to protect the nerve during thyroidectomies and other surgeries of the neck.

Table 1: Connections between CSC and SLN and/or its branches

Structure connected with the CSC	SLN	ELN	ILN	SLN & ELN	ELN & ILN	No Communication	Total Number
Number of Sides	9	82	3	2	2	2	100

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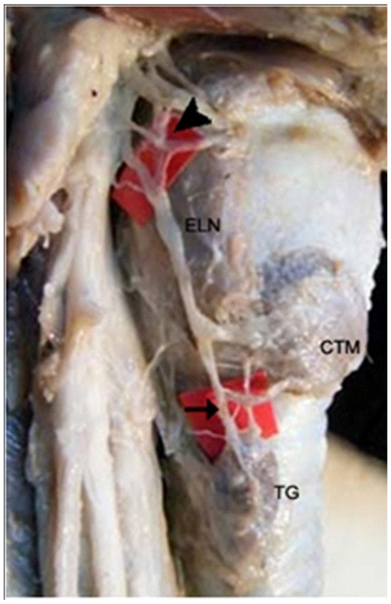

Fig. 1(a)

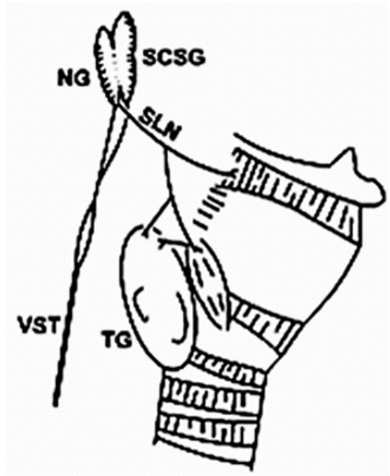

Fig. 1(b)

Fig. 1a & b: Showing sketch of the dissected right side of neck of the dog depicting the origin, course and termination of the ELN. The SLN loop (➤) and the ELN loop (➞) are marked ELN: External Laryngeal Nerve, CTM: Cricothyroid muscle, TG: Thyroid Gland

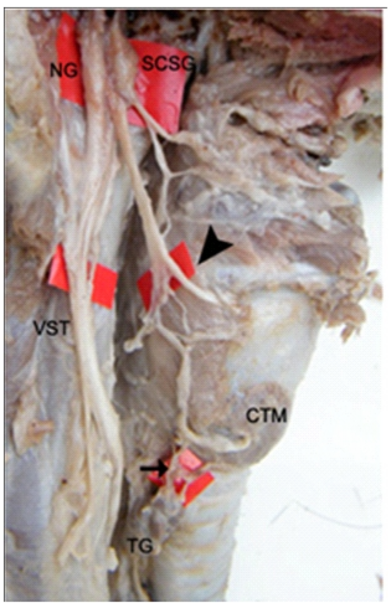

Fig. 2(a)

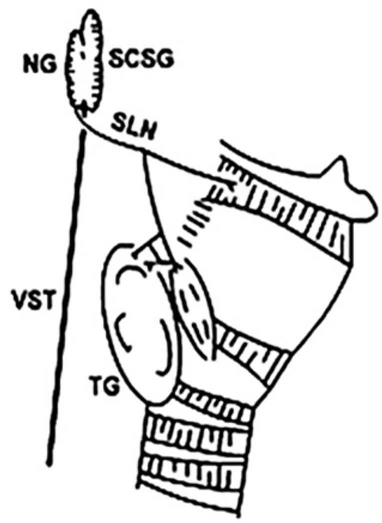

Fig. 2(b)

Fig. 2 a & b: Showing sketch of the dissected right side of neck of dog. The Nodose Ganglion (NG) and the SCSG connected to each other and also the Vagosympathetic trunk (VST) can be observed. Both the SLN loop (➤) and the ELN loop (➞) are also marked in this dissection CTM. Cricothyroid muscle, RG: Thyroid gland

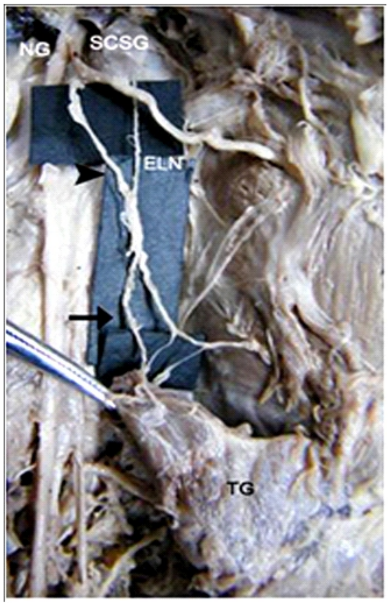

Fig. 3(a)

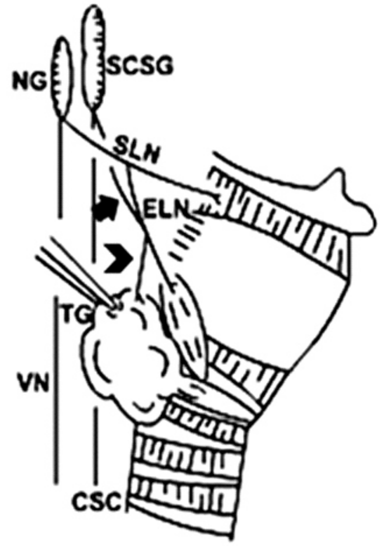

Fig. 3(b)

Fig. 3 a & b: Showing sketch of the most commonly observed pattern of communication, wherein, the ELN itself receives a communicating branch from the SCSG, and then gives off numerous glandular branches to supply the upper pole of the thyroid gland post and a muscular branch to the Cricothyroid muscle

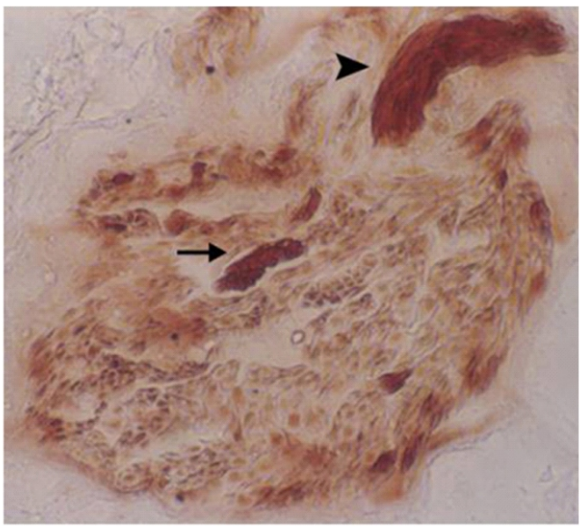

Fig. 4: Stained cross section of the dog nodosum showing a bundle (➤,➔) of AChE- positive unmyelinated fibers from SCSG passing through the ganglion nodosum

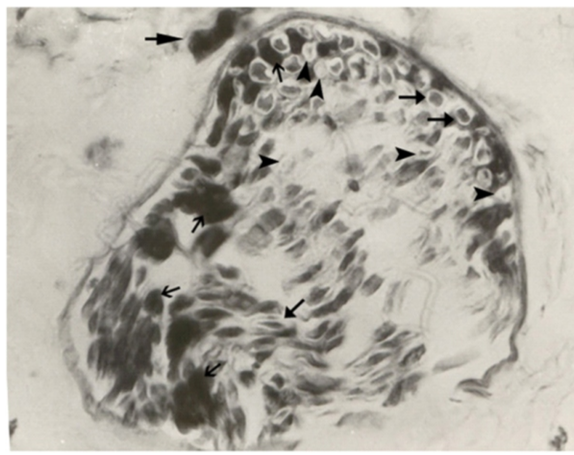

Fig. 5: AChE staining showing that in the cross section of dog ELN AChE-positive myelinated axon (➙), AChE-negative myelinated axons (➤), and AChE-positive unmyelinated axons (➙) are observed. Some obliquely cut nerve fibers containing numerous AChE-positive unmyelinated axons and some AChE-positive myelinated axons are also present. The thyroidbranch of the ELN is also seen at the upper left side of the picture (➙)

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