The anatomy of cervical sympathetic ganglia in Saanen goats*

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Summary: Sympathetic ganglions located in the cervical region are important organs that make the final synapse of the sympathetic nerve fibers reached to the head, neck, and forelimbs. As far as we know, there are not any anatomical data about cervical sympathetic ganglia in Saanen goat. In this study, we determined the nerve branches separated from the ganglia and the location of the ganglia. We also determined the expression of some enzymes and proteins such as tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), neuropeptide Y (NPY) and substance P (SP) in ganglia. Ganglion cervicale craniale (GCC) was on the medial side of bulla tympanica. Mainly branches named as nn. carotici interni, n. jugularis and nn. carotici externi was found to be separated from this ganglion and thin branches joined to the nearby nerve. It was found that n. vertebralis, the two branches that constitute the ansa subclavian, and the thin nerve branches involved in the surrounding tissues and organs separated from ganglion cervicothoracicum (GCT) that located in the first intercostal space. A total of five ganglion cervicale medium (GCM) found at the junction of the two branches forming the ansa subclavia. Another ganglion was not found on where cervical part of truncus sympathicus in all dissections and histological examinations. DβH, TH, NPY and SP were revealed to be express in all ganglia. DβH and NPY in CCG, TH in MCG, DβH, NPY and TH in GCT were found to be more intense staining.

Keywords: Anatomy, immunohistochemistry, Saanen goat, sympathetic ganglia.

Introduction

In the sympathetic nervous system, two neurons function between the center and the effector organ. Synapses between these neurons occur in the ganglion (35). GCC and GCT are always present in domestic mammals, whereas the presence of GCM varies with species or even individuals (14, 16, 35). The presence of ganglion, also named to as ganglia intermedia, has been reported in some species (27).

The immunoreactivity of enzymes such as TH and DβH and proteins such as NPY and SP known to be expressed by sympathetic nerves in the ganglia trunci sympathici have been reported (2, 3, 5, 8, 13, 24). NPY that originated from the sympatho-adrenomedullary nervous system has a vasoconstrictive and mitogenic effect on blood vessels. It functions in blood pressure regulation and angiogenesis (23). SP which performs functions such as pain perception, emotional behavior, stress, smooth muscle contraction and saliva production

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has a wide spread in the body (9, 10, 11, 36). In addition, SP in the neuropeptide structure is also found in preganglionic neurons of sympathetic ganglia (12). Catecholamine neurons, such as dopamine, norepinephrine and epinephrine contain the TH enzyme. After this enzyme converts tyrosine to a composition called as dopa, dopa is also turned into dopamine neurotransmitter. In addition to TH, neurons that use norepinephrine as a neurotransmitter also have the DβH which converts dopamine to norepinephrine (1).

It is stated that cause dysfunctions in the head, neck and forelimb of pathological conditions that may occur in cervical sympathetic ganglia due to some metabolic diseases (21) or arterial insufficiency (28). One of them is Horner’s syndrome (29), the other is the suppression of melatonin release expressed in the pineal gland (26). Damages occurred in GCT for different reasons can lead to arrhythmias in the heart (22).

The literature on the anatomy of the cervical sympathetic ganglia that have important functions is not encountered in Saanen goats. The purpose of this study was to determine the location and size of cervical sympathetic ganglia, nerve branches separated from them, and also demonstration of the presence of sympathetic neurons via DβH, NYP, TH and SP antibodies known to be expressed by sympathetic neurons in the ganglia.

Materials and Methods

In this study, a total of 14 samples were evaluated by examining each half of 7 adult female Saanen goats separately. Our study was approved by our local Ondokuz Mayis University Animal Experiment Local Ethical Committee (Ethics committee number: 2012/28). After perfusion, all the materials were fixed with 10% formaldehyde solution. The dissections were performed under a stereomicroscope (Olympus SZ61 TRC). Photos were taken with a digital camera (Olympus C-5060). Measurements were measured with a digital caliper (Mitutoyo, Japan). These measurements were analyzed via ordinary least squares (OLS) technique. Nomina Anatomica Veterinaria (30) was used for anatomic denomination.

Four blocks from each of GCC, GCM and GCT were prepared for histological and immunohistochemical examinations. Tissue sections were in 5 μm thick. The prepared sections were stained with haematoxylin-eosin (H&E) and were processed for immunohistochemical investigation with primary antibodies Tyrosine Hydroxylase Polyclonal antibody (PA1-4679, Thermo Fisher Scientific, USA), anti-Neuropeptide Y antibody (PA5-19568, Thermo Fisher Scientific, USA), Substance P Polyclonal antibody (Bs-0065R, Bioss, USA) and Anti-Dopamine β Hydroxylase antibody (Millipore AB 1585, USA) by using standard streptavidin-biotin peroxidase complex method (SBPC) with a commercial kit (Zymed, USA). The reaction product was visualized by aminoethylcarbazole (AEC) chromogen (Zymed, USA) and counterstained with Mayer haematoxylin. Immunohistochemistry results were interpreted using a light microscope (Nikon Eclipse E600). For the quantification of the immunological staining in the tissue the analysis was initiated on the basis of the high intensity reaction fields. All of the sections were examined at a magnification of 400 X. The staining densities of cells that are positive in each area [(0) no reaction; (1) poor; (2) medium; (3) intense staining] was determined.

In addition, the blocks prepared for determination of whether or not any ganglions in the cervical part of the truncus sympathicus between GCC and GCM or GCT were examined by staining with H&E (25).

Results

GCC (Figure 1-a) and GCT (Figure 2-a) were found to be present in all examined Saanen goats. While GCM (Figure 2-b) existed in some materials, there was no another ganglion in the cervical part of the truncus sympathicus in all examined materials.

Ganglion cervicale craniale: GCC located in the ventral of art. atlantooccipitalis, at the medial and ventral of bulla tympanica and at the caudal of inn. retropharyngei mediales. The average length, width, and thickness of this ganglion, which was generally oval (10/14) and sometimes spindle (4/14) shaped, were determined as 9.35 ± 0.99 mm, 4.03 ± 0.44 mm and 3.21 ± 0.32 mm, respectively.

It was observed that two nerves separated from the cranial half of the GCC. One of these was nn. carotici interni (Figure 1-b), and the other was n. jugularis (Figure 1-c). It was determined that the n. jugularis joined to the n. glossoopharyngeus (Figure 1-d) and n. vagus (Figure 1-e). The nn. carotici interni usually established of two (11/14), very rarely one (2/14) or three (1/14) thin nerve branches. This nerve which accompanied the a. carotici internus after leaving the ganglion constituted the plexus caroticus internus around this artery. The nn. carotici interni ended in sinus cavernosus.

It was determined that generally (10/14) 3, sometimes (4/14) 4 nerve branches separated from the ventral half of the ganglion. One of these joined to ramus pharyngeus (Figure 1-e) which separated from n. vagus, the other one participated directly n. vagus. Nervus caroticus externus originated as a single nerve from the ventral side of the ganglion (Figure 1-f) was observed to separated two branches (4 samples) immediately after the distinction. In three samples, these branches formed plexus caroticus externus around the a. carotici externus the same named artery. In one sample, while one of the branches shaped the plexus, the other participated in the n. laryngeus cranialis (Fig. 1-f). The nerve branches directly participating in cervical nerves from GCC could not observed in this study.
Figure 1. Medial view of GCC in Saanen goat.
a) GCC, b) nn. carotici internii, c) n. jugularis,
d) n. glossopharyngeus, d’) the branch that separates from n. glossopharyngeus and extended to glomus caroticus, e) n. vagus, e’) ramus pharyngeus of n. vagus, e”’) n. laryngeus cranialis, f) n. caroticus externus, f’) the branch that separates from the n. caroticus externus and joins to the n. laryngeus cranialis, g) cervical part of tr. sympatheticus, h) branch that separates from CCG and joined to both n. vagus and the ramus pharyngeus of n. vagus, i) n. hypoglossus, ap) a. pharygea ascendes, acc) a. carotis communis, ace: a. carotis externa, aci) a. carotis interna, bar) 1 cm.

Şekil 1. Saanen keçisinde GCC’nin medial görünümü
a) CCG, b) nn. carotici internii, c) n. jugularis,
d) n. glossopharyngeus, d’) n. glossopharyngeus’tan ayrılan ve glomus caroticus’a uzanan kol, e) n. vagus, e’) n. vagus’un ramus pharyngeus’u, e”’) n. laryngeus cranialis, f) n. caroticus externus, f’) n. caroticus externus’tan ayrılan ve n. laryngeus cranialis’e katılan kol, g) tr. sympatheticus’un servikal bölümü, h) CCG’den ayrılan ve hem n. vagus’a hem de onun ramus pharyngeus’unu katılan kol, i) n. hypoglossus, ap) a. pharygea ascendes, acc) a. carotis communis, ace: a. carotis externa, aci) a. carotis interna, bar) 1 cm.

Figure 2. The view from left side of GCT and GCM in Saanen goat.
a) GCT, b) GCM, c) cranial branch of ansa sublavia, c’) caudal branch of ansa sublavia, d) branch that separated from GCM and accompanied to a. subclavia sinistra, e) n. vertebralis, f) the branch joined from GCT to T1 n. spinalis, g) the branch that separates from caudoventral of GCT and joined to plexus aorticus, h) the branch that separates from ventral of GCT and dispersing to heart at the level of the left auricula, i) the branch that separated from ventral of GCT and dispersed to heart, j) the branch that separates from GCT and extended a long with a. sublavia sinistra, k) branch that dispersed on m. longus colli, acc) a. carotis communis sinistra, ass) a. subclavia sinistra, np) n. phrenicus, nv) n. vagus, ts) cervical part of the truncus sympatheticus, C6, C7, C8) ventral branches of the 6th, 7th, and 8th nn. cervicalis spinalis, T1, T2, T3) ventral branches of the 1st, 2nd and 3rd nn. thoracalis spinalis, *: rami communicantes

Şekil 2. Saanen keçisinde CTG ve MCG’nin sol taraf görünümü.
a) CTG, b) MCG, c) ansa sublavia’nın cranial kolu, c’) ansa sublavia’nın caudal kolu, d) CTG’dan ayrılan ve a. subclavia sinistra’nın kollan ile seyreden kol, e) n. vertebralis, f) CTG’dan T1 spinal sinire katılan kol, g) CTG’un caudoventral’inden ayrılan ve plexus aorticus’a katılan kol, h) CTG’un ventral’inden ayrılan ve auricula sinistra düzyeyinde kalbe dağılan kol, i) CTG’un ventral’inden ayrılan ve kalbe dağılan kol, j) CTG’dan ayrılan ve a. subclavia sinistra’ya boyunca uzanan kol, k) m. longus colli üzerinde seyreden sinir kolu, acc) a. carotis communis sinistra, ass) a. subclavia sinistra, np) n. phrenicus, nv) n. vagus, ts) tr. sympatheticus’un boyun bölümü C6, C7, C8: 6. 7. ve 8. cervical spinal sinirlerin ventral kolları T1, T2, T3: 1. 2. ve 3. thoracal spinal sinirlerin ventral kolları *: rami communicantes.
Figure 3. The expression of dopamine-β-hydroxylase (A), Neuropeptide Y (B), Tyrosine hydroxylase (C) and Substance P (D) in GCC (1), GCM (2), GCT (3), bar), 25µm.

Şekil 3. GCG (1), MCG (2), CGT (3)’da Dopamin - β-hidroksilaz (A), Neuropeptid Y (B), Tirozin hidroksilaz (C) ve Substans P (D)’nin ekspresyonu, bar), 25µm.

**Ganglion cervicale medium:** The oval-shaped GCM (Figure 2-b) was at the entrance of the apertura thoracis cranialis and where join of the ansa cranialis (Figure 2-c) and ansa caudalis (Figure 2-c’). The length, width and thickness average measurements of GCM that observed as total 5 numbers (three left, two right) were in 4.78 ± 0.46 mm, 4.25 ± 0.22 mm and 2.72 ± 0.46 mm, respectively. Whereas some branches that separated from GCM added to a. subclavia dextra and a. subclavia sinistra (Figure 2-d), it was determined that some branches extended to the heart. In one sample, one branch that came from the GCM joined to n. vertebralis (Figure 2-e). In same sample, one branch which separated from ventral branch of 7th nervus cervicalis participated to GCM (Figure 2- *).

**Ganglion cervicothoracicum:** The GCT located in first intercostal space (Figure 2-a) and between the m longus colli and esophagus on the left, m longus colli and trachea on the right. GCT was triangular, round or spindle shape. It observed to be formed by the combination of the last cervical ganglion sympathica and first thoracic ganglion sympathetica (in a sample, the second thoracic
ganglion sympathetica also joined). Thin nerve branches involved from GCT to ventral branches of 8th cervical n. spinalis and first thoracal n. spinalis (Figure 2-f). The mean length, width and thickness measurements of GCT were in 12.84 ± 1.07 mm, 5.56 ± 0.93 mm and 3.26 ± 0.66 mm, respectively.

It was observed that thin nerve branches added from GCC to the vertebral nerve (Figure 2-e), to ansa cranialis (Figure 2-c) and ansa caudalis (Figure 2-c') forming ansa subclavia and in addition, to plexus aorticus (Figure 2-g) and heart (Figure 2-h-i). Apart from these branches, there were one (Figure 2-j) or two more branches separating from the ventral of the ganglion. Some branches (Figure 2-d) originating from GCM also added to these branches that accompany with a. subclavia sinistra and dextra and forming perivascular plexus on them. It was seen to participation of one branch (Figure 2-e) from GCM to n. vertebralis in only one sample.

Neurons in the cervical sympathetic ganglion stained immunopositive with DβH, NPY, SP and TH antibodies (Figure 3). In all three ganglia, the cytoplasm of some neurons with DβH antibody stained medium intensity and homogeneous character. It was observed that the staining with NPY antibody in the perinuclear region of some neurons determined to be granular. Although the cytoplasm of neurons with SP antibody was homogeneous staining, it was noted that the intensity of staining in all three ganglia was different. It was determined with TH antibody that almost all neurons in each of the three ganglia reacted immunopositive and the intensity of staining of most neurons was intense.

Discussion and Conclusion

It is important to know the location of the sympathetic ganglia and the distribution of the nerves separated from them, as the conduction disorders in postganglionic nerve fibers in the sympathetic nervous system may cause important clinical symptoms (18, 19).

The GCC was oval and spindle shaped in our study. This situation was similar to literature (6, 14, 15) The dimensions of the GCC have been reported in goat (27), Tibetan cattle (34) and roe deer (15). In this study, the width and thickness measurements of the GCC were similar to those reported as 3.67 mm and 3.07 mm in the roe deer (15). The length of the ganglion in the Saanen goat was lower than the value reported as 13.85 mm in the roe deer (15), as and higher than the value reported as 8 mm in the goat (27).

The separating of three main nerves as the nn. carotici interni, n. caroticus externus and n. jugularis from the GCC and the formations a plexus around the same named arteries of branches separated from nn. carotici interni and n. caroticus externus was compatible with the literature (6, 14, 31, 34). It has been reported in the literature (6, 7, 15, 27, 31) that there are differences in the numbers of the nn. carotici interni and n. caroticus externus. In this study, while the separating as usually to two branches of the nn. carotici interni was similar to literature (7, 27), as very rarely, the separating of the single branch was created a difference. In this study, the separation as a single branch of the n. caroticus externus from the ventral of GCC was consonant to literature. (6, 7, 27, 31). In four materials, the nerve which separated as a single branch from the ganglion immediately divided to two. This situation was similar to state of Kabak and Onuk (15).

In the literature (14, 15), it has been reported that the nerve branch separating from GCC is involved to the n. laryngeus cranialis. In this study, although one nerve that separating from the ganglion and joined directly to n. laryngeus cranialis was not presence, the participation to the mentioned nerve of the one branch separating from the n. caroticus externus and cervical part of the truncus sympathicus constituted an important difference.

The presence of connecting branches one (6, 15, 27, 31, 35) two or three (14) between the GCC and the nn. cervicales is mentioned. The absence of any linkage in this study was suggest that this was due to dissection errors.

Differences are reported about the presence of GCM at the junction of ansa cranialis and ansa caudalis in the literature (27). A total of 5 numbers GCM, 3 on the left and 2 on the right were observed in this study. In cervical part of truncus sympathicus of goat, the GCM and ganglia intermedia have reported by Getty (27). While it was only present GCM in our study, the ganglia intermedia were not found. This situation was consistent with the reported in the roe deer (16).

The localization of GCT within the first intercostal space in the Saanen goat was consistent with the literature (16, 27, 32, 33). Although the shaped of GCT is reported as oval, star, half moon, pear and inverted L letter (17, 27, 32, 33), the appearance like as triangular and spindle in this study was similar to roe deer (16). The length, width and thickness measurements of the GCTs reported in different species (16, 17, 27, 32, 33) determined to be 12.84 x 5.56 x 3.26 mm in the Saanen goat, respectively, and these values were similar to the goat (27).

It has been reported that GCT is caused by the combination of the last ganglion cervicale with the first (33), second (16, 27) or third (32) ganglia thoracica. In Saanen goats, the same ganglion usually formed by the coalescence of the last ganglion cervicale and first ganglia thoracica. In one sample, the second ganglia thoracica also participated in the formation of GCT. Kabak et al. (16) has reported that one branch of each extended to the ventral branches of the last n. cervicalis and second n.thoracalis from GCT, and two branches extended to the ventral branch of the first thoracal n. spinalis. In this study,
similarly to the literature (17, 27, 32, 33), it was observed that one branch participated in the last cervical n. spinalis and first thoracic n. spinalis. In one sample and on the left side, the extending of one branch to the ventral branch of the 7th cervical n. spinalis from GCM considered as a significant difference. The presence and distribution of the other branches leaving the ganglion were consistent with the literature (16, 17, 27, 32, 33).

In this study, immunohistochemical examination of GCC, GCM and GCT revealed that DβH, NYP, TH and SP antibodies were immunopositive. The immunopositivity of SP varies between species and gangliaions. It has been reported that SP does not show immunoreactivity in pig GCT (13), in sheep (2) and dog GCM (8) and in rat GCC (4). The immunopositivity observed in all ganglia was an important difference for SP antibody in our study. While DβH, NPY, and TH antibodies have showed intense immunopositivity in GCM of sheep (2), it has been expressed that only NPY antibody has showed intense immunopositivity in pig (13) and cat (24) GCT, in dog ganglia trunci sympathici (8) in rat GCC (20). In the Saanen goats, three antibodies were observed to be positive and intensely stained in all the ganglia. This situation can be interpreted as having intensively synapses of sympathetic nerves in all ganglia.

In conclusion, the shape, size and location of cervical sympathetic ganglia (GCC, GCM and GCT), the nerve branches separating from ganglion, and the relation between these branches extremite and peripheral organs and vessels determined in detail in Saanen goat. Although there were some minor differences in shape, size, location of GCC, GCM and GCT and major nerves and interconnected branches separated from ganglion in Saanen goats, our results were observed to be generally consistent with the literature. Besides these similarities, it was remarkable some different findings. One of these was that the nerve branches separated from caudodorsal of GCT did not see. The other one was a branch extending from GCM to both the ventral branch of the 7th n. cervicalis and the n. vertebralis in one sample. Also, DβH, NPY, TH and SP showed immunopositive reaction in all examined ganglia. We think that the findings obtained as a result of this study will contribute to the literature of the anatomy and will be a source for the studies to be made about the subject.

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