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Light and Electron Microscopic Studies on the Cranial Cervical Ganglion of the Arabian Camel (*Camelus dromedarius* L.)

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ABSTRACT. The cranial cervical ganglion (CCG) of the one humped camel was studied by light and electron microscopy. Light microscopy has shown that the CCG of the camel is completely invested with a thick connective tissue/perineurial cell capsule, and that the ganglion is lobulated. The nerve cell bodies were never seen in groups and they all belonged to the class of multipolar neurons. Electron microscopy has revealed that the perineurial cells of the capsule contain heterophagic vacuoles and this suggests that they are phagocytic. The nerve cell bodies showed the general ultrastructural features of sympathetic neuronal perikarya. Intranuclear inclusion bodies were frequently encountered and this suggests that neurons of the CCG of the camel are highly active. A small number of neuronal perikarya characterized by many large granules were seen in the CCG of the camel. They were considered to be somata of peptidergic neurons. The small granule – containing cells reported in the CCG of some other mammals were not seen in this study.

The present concept of the structure and function of sympathetic ganglia is derived mainly from studies on the superior cervical ganglion (SCG) (Dail and Barton 1983). Its large size and accessibility, in addition to the multiplicity of its target organs

(Flett and Bell 1991), make the SCG a favoured organ for histological and physiological studies. The ultrastructure of the SCG of man, bovidae, and experimental animals such as the rat, rabbit, mouse and cat has been studied (see review by Dail and Barton 1983). There is as yet no information on the general structure of the cranial (superior) cervical or any other sympathetic ganglion in the camel. The purpose of this study is to disclose the general histological and ultrastructural features of the cranial cervical ganglion of the camel (*Camelus dromedarius*).

Materials and Methods

The right and left cranial cervical ganglion of seven camels (age 8 months to 6 years) were obtained from the slaughter house. Immediately after slaughter the region of bifurcation of the common carotid artery was exposed. In each case the CCG was identified as a whitish fusiform organ about 3 cm long, located at the cranial end of the sympathetic trunk just craniomedial to the cranial end of the common carotid artery. In three animals the CCG was first excised, sliced and fixed by immersion in 10% neutral formalin or Bouin's solution. Tissues were then processed for paraffin sections and stained with haematoxylin and eosin (H&E), Masson's trichrome method for collagen fibres, Gordon and Sweet method for reticular fibres and Glees and Marslands method for nerve cell bodies (Culling *et al.* 1985).

In the other four animals, the CCG was fixed by perfusing, through the common carotid artery, 2.5% glutaraldehyde in sodium cacodylate buffer at pH 7.2. The CCG was then excised, sliced and further immersed in the fixative for two hours. Tissues blocks were post-fixed in 2% osmium tetroxide, dehydrated, cleared and embedded in epoxy resin. Semithin sections (1 um thick) stained with 0.15% toluidine blue were examined with the light microscope. Ultrathin sections (80 nm thick) stained with uranyl acetate and lead citrate were examined with an EM 10 C Zeiss electron microscope.

Results

Light microscopy has shown that a thick connective tissue capsule (Fig. 1) invested the CCG of the camel. It consisted of two layers; the outer one was made up of a collagenous connective tissue and the inner was composed of many (4 to 10) layers of perineurial cells. The perineurial cell was characterized by a dark flattened nucleus and very long slender cytoplasmic processes. Septa consisting of perineurial cell and collagen fibres originated from the capsule (Fig. 1) dividing the ganglion into incomplete lobules. Blood vessels and bundles of nerve fibres (Fig. 2) were often seen within these speta. The nerve bundles contained many unmyelinated

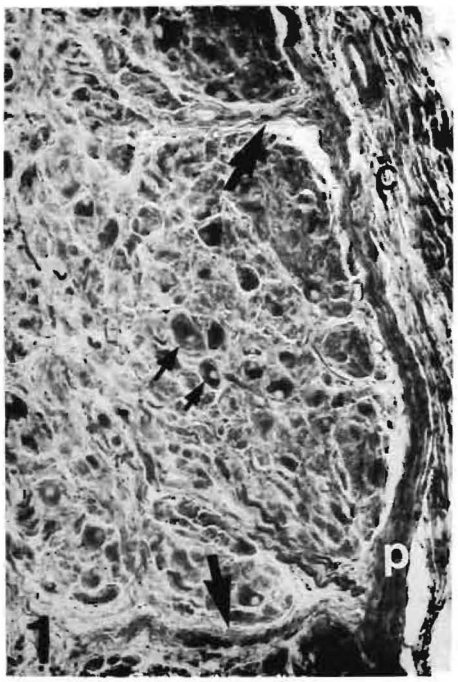

Fig. 1. Light micrograph of CCG of the camel showing septa (large arrows) originating from the capsule. The capsule has an outer collagenous layer (C) and an inner perineurial layer (P). Small arrows, nerve cell bodies. H & E. X100.

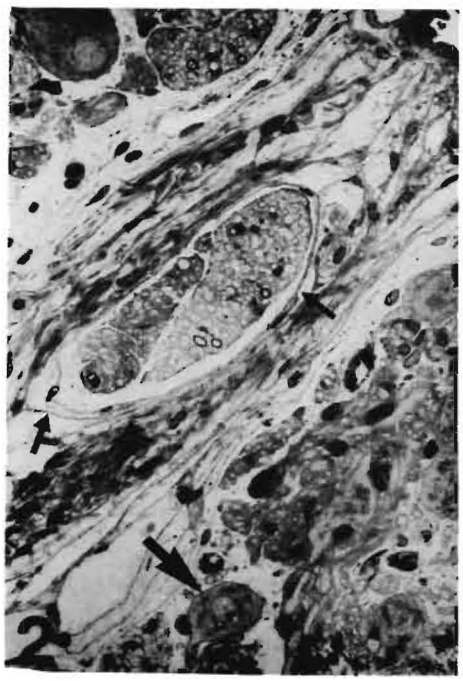

Fig. 2. Higher magnification of a CCG septum containing a bundle of nerve fibres delineated from the surrounding tissues by slender perineurial cell processes (small arrows). The nucleus of the nerve cell body (large arrow) at the bottom of the micrograph shows a prominent nucleolus. Toluidine blue, X320.

nerve fibres and a few myelinated ones. They were usually completely surrounded by 2 to 3 concentric perineurial cell lamellae. The substance of the ganglion comprised nerve cell bodies, many small blood vessels, nerve bundles and reticular fibres. The nerve cell bodies appeared ovoid, round or angular in H & E or toluidine blue stained sections (Figs. 1, 3). In silver stained sections it was apparent that the cell bodies belonged to multipolar neurons (Fig. 4). The nerve cell bodies often lay close to each other but they never formed contiguous cell groups. The neuronal nucleus was usually pale and showed one or more prominent nucleoli that were often peripherally located (Fig. 3). The small blood vessels located within the ganglionic substance were always invested with concentric perineurial cell lamellae. (Fig. 3).

Electron microscopy revealed that the collagenous layer of the capsule consisted of collagen fibrils (about 70 nm thick) and fibroblasts whereas the perineurial layer comprised perineurial cells made up of a cell body and slender processes. The perineurial cell body contained a nucleus rich in peripheral heterochromatin (Fig. 5) and a cytoplasm that showed a few polysomes, occasional flattened cisternae of rough endoplasmic reticulum (RER) and mitochondria. The perineurial cell surface was often covered with a basal lamina (Fig. 5). Adjacent cell processes were joined by cell junctions (maculae adherentes). Heterophagic vacuoles were freqently encountered within the cytoplasm of perineurial cell processes.

The nerve cell bodies were always solitary and were never seen in contiguous groups. They were about 15 to 30 um in diameter. The nerve cell body usually contained a smoothly round or oval nucleus, though on rare occasions the nuclear envelope had a wavy appearance. The nucleus contained abundant euchromatin in addition to few fine chromatin granules. A prominent nucleolus was often present. The nucleus frequently showed an intranuclear inclusion body (rodlet) characterized by cross striations (Fig. 6). The neuronal perikaryon usually contained many polysomes, cisternae of RER and mitochondria (Fig. 7), in addition to large electron dense granules, Golgi complexes, microtubules, neurofilaments and small dense-cored vesicles. A cilium was occasionlly encountered (Fig. 7). The cisternae of RER were usually flattened and present in stacks of 7 to 10. The mitochondria were elongate oval with transverse cristae. Some of the mitochondria were exceptionally long while others were swollen and devoid of cristae. The Golgi complexes were usually perinuclear but were occasionally seen in the perikaryal periphery, particularly at the regions of origin of dendrites. The large electron dense granules (large granules) were polymorphic. They were about 150-350 nm in diameter. At higher magnification it was possible to discern a limiting membrane around these granules. The number of large granules in the perikaryal cytoplasm was usually small but perikarya containing numerous large granules (Fig. 8) were occasionally encountered. The small dense-cored vesicles (granular vesicles) were sporadic and small being about 50 to 70 nm in diameter. They comprised an electron

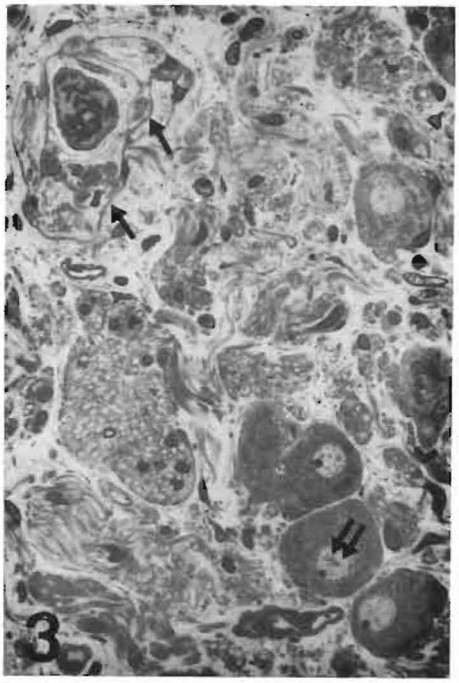

Fig. 3. The upper left hand corner of the micrograph shows a small blood vessel (venule) surrounded by slender perineurial cell processes (small arrows). The bottom right hand corner of the micrograph shows three nerve cell bodies. The nuclei of these cells are pale and each shows a prominent peripheral nucleolus. One of these cell bodies (the middle) shows an intranuclear rodlet (double arrows). Toluidine blue. X440.

Fig. 4. Light micrograph showing multipolar neurons of CCG stained by Glees and Marsland's method. X400.

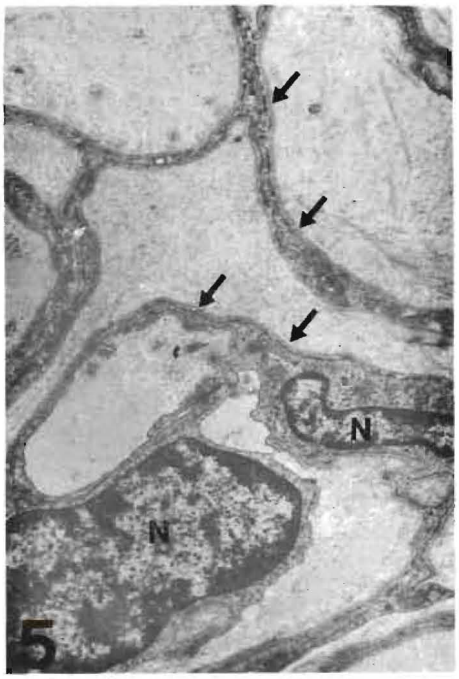

Fig. 5. Electron micrograph from the perineurial layer of the capsule. The perineurial cell nucleus (N) shows peripheral hetero-chromatin whereas the cell processes are slender and covered by a basal lamina (arrows). X5000.

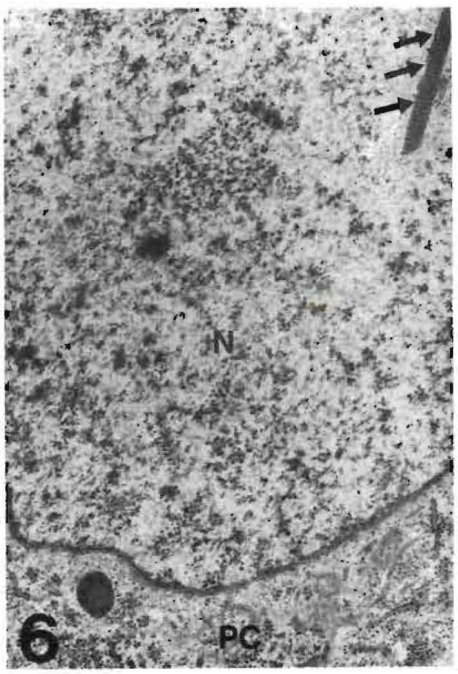

Fig. 6. Electron micrograph showing part of the nucleus (N) and perinuclear cytoplasm (PC) of a CCG neuron. The nucleus shows a intranuclear inclusion body (arrows) with cross striations. The cytoplasm contains a dense body, polysomes and cisternae of RER. X10000.

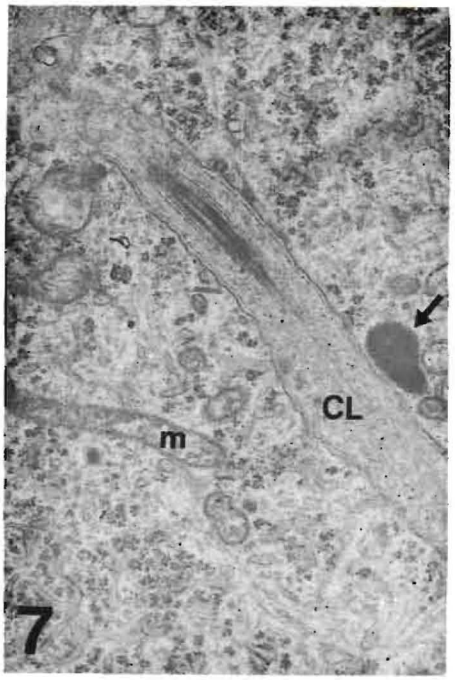

Fig. 7. The electron micrograph shows the perikaryal periphery of a CCG neuron traversed by a cilium (CL). The perikaryal cytoplasm contains many polysomes, a few cisternae of RER, an elongate slender mitochondrion (m) and a large pear shaped dense granule (arrow). X20000.

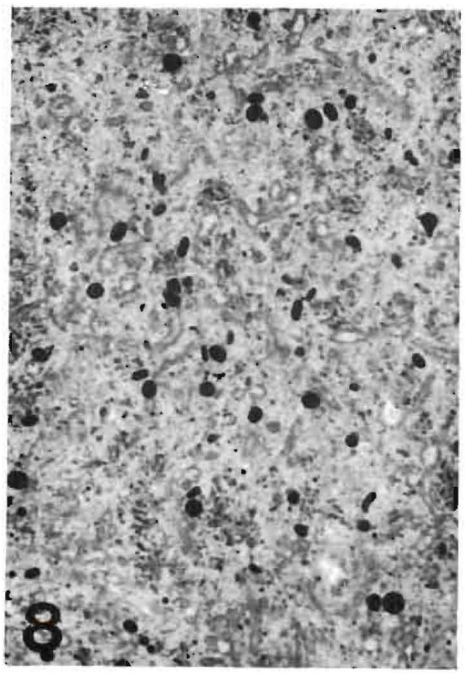

Fig. 8. Electron micrograph showing part of the perikaryal cytoplasm of a neuron containing numerous large dense granules. X8000.

dense core surrounded by a narrow electron lucent halo and were delineated by a limiting membrane. They were either round or oval; the oval ones were usually seen in the proximal parts of neuronal processes where they lay parallel to longitudinally oriented microtubles.

They perikarya of the ganglionic neurons were invested by satellite cells. The satellite cells were narrow, attaining a maximum width of about 4um in the nuclear region. The perinuclear cytoplasm contained a few cisternae of RER and mitochondria in addition to numerous microfilaments. The satellite cell processes were narrow and rich in microfilaments. Hemidesmosomes were frequently seen on the neuronal cell membrane facing the satellite cell. The free (convex) surface of satellite cells was covered with a distinct basal lamina and often showed hemidesmosomes. Adjacent satellite cell processes were highly interdigitated.

Sheath (Schwann) cells completely covered dendrites, axons, nerve fibres and nerve endings present within the ganglion. They had a smoothly oval or highly indented nucleus with dense peripheral heterochromatin and a cytoplasm that contained numerous microfilaments, in addition to a few polysomes, cisternae of RER and mitochondria. The sheath cell surface was invested by a distinct basal lamina. Desmosomes were present between adjacent sheath cell processes and as well between the apposing cell membranes of mesaxons.

The blood capillaries seen with the electron microscope were all of the continuous type. Their endothelial cells were invested with a basal lamina that was often stratified. Other types of blood vessels seen within the ganglion were invested by perineurial cell processes.

Discussion

The general histological features of the cranial cervical ganglion of the camel as seen in this study conform to those of other mammals (Dail and Barton 1983). In particular, it resembled SCG of man and rabbit (Pick 1970 and Dail and Barton 1983) in being surrounded by a thick connective tissue/perineurial capsule, As in man (Pick 1970), septation by connective tissue and perineurium imparts a lobulated appearance to the cranial (superior) cervical ganglion of the camel. The significance of lobulation is unknown but is thought to be an early step in the development of sympathetic ganglia (Canfield 1978).

The ultrastructure of neuronal perikarya of the CCG of the camel as seen in this study is in general similar to that of autonomic neurons (Gabella 1976). Of particular interest was the presence of intranuclear inclusion bodies, large cytoplasmic electron dense granules and cytoplasmic dense-cored vesicles in neuronal perikarya of the

CCG of the camel. Intranuclear inclusion bodies which are also known as nuclear rodlets, have occasionally been reported in sympathetic neurons of some mammalian (Seite *et al.* 1977) and avian species (Masurovsky *et al.* 1970, Abdel-Magied 1986). The frequent presence of intranuclear inclusion bodies is indicative of a high level of physiological activity of the neuron (Seite *et al.* 1977). Their frequent presence in neurons seen in this study reflects that neurons of the CCG of camels are highly active. The numerous blood vessels seen in this study in the CCG of the camel is in line with this suggestion. According to Dail and Barton (1983) numerous blood vessels in sympathetic ganglia are indicative of a high metabolic activity.

The small dense-cored vesicles seen in the CCG of the camel in this study are similar to those reported in the CCG of other mammals and are considered to be the storage site of noradrenalin (Hokfelt 1969). Unlike the small dense-cored vesicles which are found only in sympathetic neurons, large granules have been reported in the cell bodies of both sympathetic (Richards and Tranzer 1975) and parasympathetic neurons (Knight 1980) and are considered to be peptidergic. Thus, the nerve cell bodies which showed numerous large granules in this study in the CCG of the camel are considered to be belonging to peptidergic neurons. Consistent with this, is that immunocytochemistry revealed a few peptidergic neurons in the SCG of the rat and cat (Schlutzberg *et al.* 1983); The neuropeptides detected included somatostatin, vasoactive intestinal peptide (VIP), enkephalin, avian pancreatic polypeptide (APP) and substance P. However the exact projections of peptidergic neurons are yet to be determined. Nevertheless, it appears that enkephalin immunoreactive fibres are preganglionic (Schlutzberg *et al.* 1983). According to Lundberg *et al.* (1983) the APP immunoreactive catecholaminergic neurons of the SCG project to arteries of the nasal mucosa and salivary glands.

Small granule-containing cells (SCG), "also known as the small intensely fluorescent (SIF) cells" have been reported in SCG of many vertebrates (Taxi *et al.* 1983) but were not seen in this study. This does not eliminate the presence of SGC in CCG of the camel as no serial section studies were made. It is recalled here that the number of these cells is very small and that Kondo and Fujiwara (1979) could find only three such cells in a thousand serial sections of the SCG in man.

Finally it may be added that the strong connective tissue/perineurial cell capsule surrounding the ganglion, the presence of heterophagosomes in perineurial cell processes, and investment of the blood vessels of the SCG with perineurial cell processes and the stratified basal lamina, are all indicative of an efficient barrier separating neuronal elements in the CCG of the camel from the surrounding environment.

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دراسات مجهرية ضوئية والكترونية على العقدة العصبية العنقية القحفية في الابل العربية وحيدة السنام

التهامي محمد عبدالمجيد

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تم دراسة العقدة العصبية العنقية القحفية بواسطة المجهرين الضوئي والالكتروني . أظهرت المجهرية الضوية أن تلك العقدة محاطة تمامًا بمحفظة كثيفة من نسيج ضام وخلايا حول عصبية وأن العقدة فصيصية . أجسام الخلايا العصبية لم تكن موجودة في مجموعات وكانت اجسامًا لعصبونات متعددة الاقطاب . أوضحت الدراسة المجهرية الالكترونية أن الخلايا الحول عصبية في المحفظة تحتوي على فريغات بلعمية مغايرة ويدل هذا على أنها خلايا بلعمية أبدت أجسام الخلايا العصبية السمات التركيبة الفوقية لحول النواة العصبية الودية . كثيراً ما تم رؤية أجسام مشتملية داخل النواة ويشير ذلك إلى أن عصبونات هذه العقدة في الابل العربية نشطة جداً . أتسمت أعداد قليلة من أجسام الخلايا العصبية بالعديد من الحبيبات الكثيفة الكبيرة واعتبرت اجسامًا لعصبونات ببتيدية . لم تلاحظ في هذه الدراسة الخلايا المحتوية على حبيبات صغيرة التي سجلت من قبل في تلك العقدة في بعض من الثدييات الأخرى .