MYELINATION OF NEURONS OF THE GENICULATE GANGLION IN GUINEA PIGS: AN ULTRASTRUCTURAL STUDY IN THE PERINATAL PERIOD AND ADULT LIFE.

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INTRODUCTION

The acousticofacial ganglion is mainly derived from the neural crest. It consists of a vestibulocochlear part which at first fused with the ganglion of the facial nerve but later, the two separate. The vestibular and cochlear ganglia each becomes associated with the corresponding division of the eighth nerve. Cells of the vestibular and cochlear ganglia remain bipolar throughout life and their neurons are unusual in that many of their somata become enveloped in thin myelin sheaths (Williams et al. 1995). Most of the vestibular ganglion neurons are surrounded by thin myelin sheath in humans (Ona and Kanzaki, 1991), in adult squirrel monkey (Fermin and Igrashi, 1982), in cat (Spasova, 1982), in rabbits and in bats (Ona, 1993).

Ultrastructural features of the human spiral (cochlear) ganglion revealed that large myelinated neurons were few. However, the majority of cells were myelinated in guinea pigs (Thomsen, 1966; Abdel Rahman et al., 1998) and in cats (Spoendlin, 1972).

Detailed studies on the geniculate ganglion have not been included in textbook descriptions. We postulated that neurons of the geniculate ganglion (GG) may be enveloped by thin myelin sheath like the vestibular and cochlear derivatives of the acousticofacial ganglion because all have the same embryological origin. Although **Kitamura et al. (1982)** stated that all neurons of GG in guinea pigs and monkeys are unmyelinated, yet we conducted this research to reinvestigate this issue with a trial to determine the age at which neuronal myelination starts. Moreover, a study of the capillary blood vessels of the GG will be performed to throw some light on the blood ganglion barrier.

MATERIALS AND METHODS

Five adult coloured male guinea pigs (Cavia Cobaya) weighing 950-1200 gm were caged with 10 females weighing 750-900 gm for 24 hours (Every male with two females). The presence of a vaginal plug in addition to detection of sperms in the vaginal smear were indicative of fertilization.

This was recorded as the first gestational or first pre-natal day in assessment of the age of the developing embryos. The gestation period varied between 66-72 days. Five pregnant females at 60 days of gestation were anaesthetized with intraperitoneal injection of sodium thiopental (35 mg/kg body wt.). Laparotomy was followed by extraction of ten embryos from the uteri of the five females. This was considered the prenatal age group (group I). Offsprings of the remaining five pregnant females (10) were sacrificed at the first post-natal day (group II). The adult age (group III) comprised five adult guinea pigs of both sexes (100-150 days). For all age groups (prenatal, postnatal and adults) the animals were sacrificed by decapitation after sodium thiopental anaesthesia (35 mg/kg body wt.). After decapitation of the animal, the skin of the scalp was incised in the midline down to the foramen magnum, the cartilaginous auditory canal was cut during dissection then the mandible was disarticulated at the joint and separated using the scissor. One of the scissor blades was introduced in the foramen magnum and the cranial cavity was opened in the midline. The temporal bone was gently separated and the surrounding muscles, tendons and the squamous part of the temporal bone were removed. Using the surgical microscope at 10 fold magnification, the tympanic bulla was then opened using a small mosquito artery forceps, one blade was inserted in the bony external canal and the other over the most bulging part (ventral wall) of the bulla. After opening the bulla, the structures of the middle ear (auditory ossicles and muscles) and the otic capsule (the cochlea, labyrinth and semicircular canals) were clearly exposed. The facial nerve canal could be demonstrated. The facial nerve enters its canal in the middle ear at the rostral portion of the pars labyrinthicus of the tympanic bulla from the most lateral extension of the internal auditory canal. The geniculate ganglion lies at this site. The tympanic segment of the facial canal runs in a horizontal course caudally, dorsal to the stapes then bends ventrally and laterally to exit from the stylomastoid foramen. The stylomastoid foramen lies caudal and dorsal to the external auditory canal. Then the nerve runs caudal to the cartilaginous external auditory canal to enter the parotid gland. Dissection of the canal is easier from the stylomastoid foramen. The bony roof was gently removed using a fine hook used in stapedectomy operations (stapes hook of Fisch), to expose the nerve. Dissection was continued along the whole canal till its proximal end at the internal auditory meatus. The ganglion lies near this proximal end. The nerve was gently held using a fine alligator forceps from its distal end and withdrawn from the opened canal.

The specimens were immediately fixed in cold buffered glutaraldhyde 3.5 % then processed to reach epon blocks. The blocks were trimmed and cut into semithin and ultrathin sections using LKB ultra microtome. The semithin sections were stained with toluidine blue for light microscopic examination and measurement of the cell diameters. This was performed by using an ocular scale grid calibrated with a micrometer stage slide at 400

fold magnification. The longest diameter was measured only in cells having the nucleolus. Then the widest diameter perpendicular to the longest diameter was measured (Nolte, 1999). The results were recorded and tabulated to get the averages. The ultra thin sections were doubly stained with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963) and examined by transmission electron microscope (TEM) Philips 201. Photographs were taken at TEM unit in Ain-Shams University, Military Academy and in Dr. Fakeeh Hospital in Jeddah.

RESULTS

Pre-natal age Group I (60 days old):

The neuronal perikaryon of the geniculate ganglion (GG) had an euchromatic nucleus with a prominent nucleolus showing a characteristic inner region of dense filaments (pars filamentosa) and an outer granular zone (pars granulosa) (Fig. 1). The neuronal cytoplasm was rich in free ribosomes that were present in aggregates. Few short stalks of granular endoplasmic reticulum were observed. Golgi complex was inconspicuous. Neurofilaments and neurotubules were sparse. Mitochondria were numerous and some of them were seen dividing. Schwann cells adhered to the neuronal somata. In semithin sections a cytoplasmic loop of Schwann cell extended around part of the circumference of the perikaryon (Fig. 2). In ultrathin sections the inner aspects of the plasma membrane of Schwann cell were seen parallel and very closely opposed to each other overlying the perikaryon (Fig. 3). The adjacent segment of the facial nerve showed variable degrees of axonal myelination. Myelinated axons had 1:1 relationship with their ensheathing myelin forming Schwann cells. The axons contained mitochondria, neurofilaments and neurotubules. The amount of Schwann cell cytoplasm was relatively large containing prominent parallel stalks of rough endoplasmic reticulum (Fig. 4). Non myelinated axons were seen either singly in between Schwann cells or in groups (Figs. 4, 5). These groups invaginated the cytoplasm of non myelin forming Schwann cells (Fig. 5). The nuclei of Schwann cells were heterochromatic but some of them showed multiple chromatin clumps. The basal lamina of Schwann cells was clearly seen.

Capillaries observed in between the neurons of geniculate ganglion were all of the continuous type. Endothelial cells had a smooth luminal surface with occasional filopodia and caveolae. The cytoplasm contained conspicuous vesicles (Fig. 6). Intercellular tight junctions were observed between adjacent endothelial cells. Collagen fibres were seen between the basal lamina of the capillaries and the basal lamina of Schwann cells. Unmyelinated axons were either embedded in the walls of capillaries or close to them (Figs. 7, 8).

First Post-natal Day: Group II

In toluidine blue stained semithin sections, the majority of the neurons of the GG whether large (28.1 μ m X 22 μ m) or small (20.3 μ m X 16.1 μ m) were enveloped by a thin myelin sheath which was either complete or partially surrounding the neuronal somata. The myelin envelope appeared as a dark blue line. However, unmyelinated neurons were observed (Figs. 9-11 inclusive). Nissl substance appeared clearly as clumps in the cytoplasm. Nuclei were characterized by having multiple nucleoli (up to 4) (Fig. 11) Binucleation was frequently observed in large and small neurons (Fig. 12).

In ultrathin sections, the neuronal myelin envelope was formed of 3-4 lamellae. It was less electron dense when compared to the myelin sheath of adjacent axons (Fig. 13). Rough endoplasmic reticulum became copious. Its stalks became larger, longer, sometimes parallel and more dilated compared to the prenatal age group (Fig. 14). Similarly neurofilaments and neurotubules increased. The neurofilaments were seen converging at the site of exit of the cell process (Fig. 15).

The ganglionic capillaries increased and were very close to the neurons and their process (Figs. 10-12).

Similar to the prenatal age groups, the capillary endothelial cells were connected by tight junctions. However, cytoplasmic intermediate filaments were more prominent and cytoplasmic vesicles increased compared to the prenatal age group. Collagen fibres outside the capillary basal lamina also increased (Fig. 16).

Adult Geniculate Ganglion (GG) 100-150 days:

The majority of neurons of the adult GG were large myelinated with spherical or ovoid somata aggregated in groups interspersed with fasciculi of myelinated and unmyelinated nerves. Their average diameter was 31.1 µm X 24.3 µm. Intermediate forms having the same basic morphological structure were observed in the adult GG. Their average diameter was 25 µm X 19 µm. Small neurons were few usually having eccentric nuclei. Their average diameter was 20 µm X 15 µm. Neurons appeared dark or light (Fig. 17). The majority of the cell somata were unipolar (Fig.18-a,b). A few neurons were large bipolar (32 µm X 25 µm) (Fig. 19). Myelination was very clear in toluidine blue stained semithin sections for the majority of the three types of neurons. Myelin appeared as a blue more dense line as compared to that of the first post-natal day. However, some of the large neurons were still unmyelinated (Fig.20-a,b). Only one myelinated heterotopic neuron (25 µm X 18 µm) was observed in this study in the adjacent segment of the facial nerve (Fig. 21). The neuronal myelin envelope was continuous with the myelin sheath of the cell process in semithin sections (Figs.18-a,b). Ultrastructural features confirmed this continuity (Fig.

22). As regards neuronal myelination at the ultrastructural level, the mature myelin envelope was formed of 11-14 dense regular period lines alternating with less dense intraperiod lines (Fig. 23). The adjacent mature axonal myelin sheaths were laminated with 18-20 regular dense period lines. The less dense intraperiod lines were more regular and narrower as compared to those of the myelin neuronal envelope. The latter had wider period to period line thickness (Compare Fig. 23 to Fig. 24 having the same magnification; Scale bar 200 nanometer).

Nonmyelinated neurons were surrounded only by Schwann cell cytoplasm containing the usual organelles. This type of Schwann cells was the mature non myelin forming cells. There was no observable ultrastructural differences between the mature myelin forming and the mature nonmyelin forming Schwann cells (Figs. 25, 26).

Concerning the ganglionic capillaries, they became more numerous and very close to the neurons (Figs. 25, 26). Similar to the capillaries of the previous ages, endothelial cells were connected by tight junctions (Figs. 27, 28). Unmyelinated autonomic nerves were observed very close to the capillaries (Fig. 25) or sometimes a group of nonmyelinated nerves surrounded the capillary (Fig. 26). Collagen of the extracellular space increased markedly (Figs. 27, 28).



Fig. (1): Electron micrograph of a neuron of the GG of a guinea pig 60 days old prenatal showing the nucleus (n) and a prominent nucleolus (ni). Note the pars filamentosa. (f) and the pars granulosa (g). Note the numerous mitochondria (m), aggregates of free ribosomes (r), short stalks of rough endoplasmic reticulum (\uparrow) and few neurofilaments and neuro-tubules (Head arrows). (Scale bar 2 µm)(X 4,900)



Fig. (2): Photomicrograph of a semithin section of GG of a guinea pig 60 days old prenatal showing a cytoplasmic loop of Schwann cell extending around part of the circumference of a neuron (\uparrow). Note the nucleus of Schwann cell (n), euchromatic nucleus of a neuron (N), myelinated axon (a) and capillary (c) **Toluidine blue; Scale bar 30 µm (X 1,000**)



Fig. (3): Electron micrograph of GG of a guinea pig 60 days old prenatal showing a Schwann cell (S) adherent to a neuron. Note that the inner leaflets of the plasma membrane of Schwann cell are closely opposed to each other (--). Note a Y shaped dividing mitochondrion in the adjacent neuron (m). The inset shows chromatin clumps (clu) in a Schwann cell nucleus. **Scale bar 2 µm (X 4,900)**



Fig. (4): Electron micrograph of the adjacent segment of the facial nerve of a 60 days old prenatal guinea pig showing axons at various stages of myelination. Note that myelinated axons have a 1:1 relationship with their ensheathing myelin forming Schwann cells and that the cytoplasm of Schwann cell contains prominent parallel stalks of rough endoplasmic reticulum (rer). The black arrow points to a single non myelinated axon. The white arrows point to thinly myelinated axons. Note the mitochondria in a myelinated axon (m), the transversely cut neurotubule in another axon (t) and the neurofilaments (f). Scale bar 2 μ m (X 4,900)



Fig. (5): Electron micrograph of the adjacent segment of the facial nerve of a 60 days old prenatal guinea pig showing many unmyelinated axons (multiple black arrows \uparrow) housed within infoldings of the cytoplasm of a non myelin forming Schwann cell (S). Note the mitochondria (m), neuro-tubule (t), neurofilaments (f), thick myelin (M), and single unmyelinated axon (U). The white arrow points to a thinly myelinated axon. Scale bar 2 µm (X 4,900)



Fig. (6): Electron micrograph of a capillary in the GG of a 60 days old prenatal guinea pig showing a smooth luminal surface, a filopodium (f), caveola (\rightarrow), many cytoplasmic vesicles (v). Note the basal lamina (bl) of Schwann cell (S) and that of the capillary. Note also the capillary lumen (L), collagen fibres (Cl), and unmyelinated axons (U), one of them adherent to the capillary wall. (X 10,000)



Figs. (7): Electron micrograph of capillaries in the GG of a 60 days old prenatal guinea pig showing tight junctions (arrow) between adjacent endothelial cells. Note the capillary lumen (L), and filopodia (f). **(X 20,000)**



Figs. (8): Electron micrograph of capillaries in the GG of a 60 days old prenatal guinea pig showing tight junctions (arrow) between adjacent endothelial cells. Note the capillary lumen (L), filopodia (f), and collagen (Cl). **(X 20,000)**



Figs. (9): Photomicrograph of a semithin section of GG at the first postnatal day showing myelin sheath appearing as a dark blue line surrounding the neurons (arrows). Note that some neurons are unmyelinated (U) and that Nissl clumps appear clearly in the cytoplasm (NI). Note also the myelinated axons (a), and Schwann cell nuclei (S). **Scale bar 30 µm (X 1,000)**



Figs. (10): Photomicrograph of a semithin section of GG at the first postnatal day showing myelin sheath appearing as a dark blue line surrounding the neurons (arrows). Note that some neurons are unmyelinated (U) and that Nissi clumps appear clearly in the cytoplasm (NI). Note also the myelinated axons (a), and capillary (C). Scale bar 30 μ m (X 1,000)



Figs. (11): Photomicrograph of a semithin section of GG at the first postnatal day showing multiple nucleoli (1-4) of variable sizes. Note that the neurons are non myelinated (U) or partially myelinated (\uparrow). Note also Schwann cell nuclei (S), capillary (C), and myelinated axons (a). The two insets show multiple nucleoli (2 and 3) of variable sizes Scale bar 30 µm (X 1,000)



Figs. (12): Photomicrograph of a semithin section of GG at the first postnatal day showing a binucleate small unmyelinated neuron (b). Note the unmyelinated neuron (U), myelinated neuron (\uparrow), myelinated axons (a), and capillary (C). The inset shows a binucleate large unmyelinated neuron (b). Scale bar 30 µm (X 1,000)



Fig. (13): Electron micrograph of the GG at the first postnatal day showing four myelin lamellae (\uparrow). Compare the myelin density to that of adjacent axon (a). Note the increased rough endoplasmic reticulum (rer). Its stalks appear dilated and almost parallel. *Scale bar 5 µm (X 2,750)*



Fig. (14): Electron micrograph of the GG at the first postnatal day showing part of a neuron. Note the nucleus (N), nucleolus (n), and the rough endoplasmic reticulum(rer). (X 6,000)



Fig. (15): Electron micrograph of the GG at the first postnatal day showing neurofilaments in two adjacent neurons (arrows). The neurofilaments in the upper neuron converge at the site of exit of the cell process (curved arrow). Scale bar 5 μ m (X 2,750)



Fig. (16): Electron micrograph of the GG at the first postnatal day showing a capillary with tight junctions (\uparrow). Note the filopodia (f), caveola (C), cytoplasmic vesicles (V), intermediate filaments (i), basal lamina (bl), and collagen (Cl). (X 15,000)



Fig. (17): Photomicrograph of a semithin section of an adult GG showing spherical or ovoid cell somata interspersed with fasciculi of myelinated and nonmyelinated nerves (\uparrow). Note the presence of large (L), intermediate (i) and small neurons (S) having eccentric nuclei. Note also the dark neuron (D), and the light neuron (Li). Toluidine blue; Scale bar 20 μ m (X 1,000)



Fig. (13-a): Photomicrograph of a semithin section of an adult GG showing myelinated unipolar neurons (1, 2, 3). Note that the myelin of the cell body is continuous with myelin sheath of its process (arrows). The capiliaries (c) are very close to the perikaryon or its process. The inset (low magnification) shows the single process of neurons (2&3) and a dark neuron (D). Toluidine blue; Scale bar 30 µm (X 1,000) Scale bar for the inset 20 µm (X 400)



Fig. (18-b): Photomicrograph of a semithin section of an adult GG showing myelinated unipolar neuron (4). Note that the myelin of the cell body is continuous with myelin sheath of its process (arrows). Note also the capillaries (c) are very close to the perikaryon. *Toluidine blue; Scale bar 30 µm (X 1,000)*



Fig. (19): Photomicrograph of a semithin section of an adult GG showing a large myelinated bipolar neuron (B). The arrows point to the continuity of neuronal myelin with myelin of the 2 processes ($\uparrow\uparrow$). Note the capillaries (C). Toluidine blue; Scale bar 30 µm (X 1,000)



Fig. (20-a): Photomicrograph of a semithin section of an adult GG showing a large unmyelinated neuron (U) lying beside a large myelinated one (M). Toluidine blue; Scale bar 30 µm (X 1,000)



Fig. (20-b): Photomicrograph of a semithin section showing an unmyelinated neuron (U), two small myelinated neurons (S). The inset shows a large unmyelinated neuron (U). The arrows point to Schwann cells capping the neuron but are non myelin forming. Toluidine blue; Scale bar 30 µm (X 1,000)



Fig. (21): Photomicrograph of a semithin section of the adjacent segment of the facial nerve of an adult guinea pig showing a single myelinated heterotopic neuron (H). The inset is a higher magnification to show the myelin sheath of the neuron. Toluidine blue; Scale bar 20 μ m (X 400) Scale bar for the inset 30 μ m (X 1,000)



Fig. (22): Electron micrograph of the GG of an adult guinea pig showing a myelinated neuron (mn) capped by a mature myelin forming Schwann cell (mS). The arrows point to the site of continuity of the myelin sheath of the neuron and its process. Note the myelinated axon (a), red blood cell (R), and capillary lumen (L). (X 1,200)



Fig. (23): Electron micrograph of the GG of an adult guinea pig showing myelin sheath of a neuron formed of about 14 dense regular period lines alternating with less dense intraperiod lines (\uparrow). Note the mitochondrion (m), and the rough endoplasmic reticulum of the neuron(rer). *Scale bar* **200 nm (X 35,000)**



Fig. (24): Electron micrograph of an axon in the GG of an adult guinea pig showing part of the myelin sheath formed of about 20 lamellae. Note that the less dense intraperiod lines are more regular and narrower (\uparrow) compared to those of the neuronal envelope. Note the mitochondrion (m), neurotubule (t), neurofilaments of the axon (f), and extracellular collagen (Cl). Scale bar 200 nm (X 46,000)



Fig. (25): Electron micrograph of two adjacent neurons (A + B) of the GG of an adult guinea pig. Neuron A is capped by a mature myelin forming Schwann cell (mS) and neuron B is capped by a mature non myelin forming Schwann cell (nmS). Neuron A is surrounded by myelin lamellae (↑) while neuron B is surrounded by Schwann cell cytoplasm (curved arrow). Note the nucleus (N) of the nonmyelin forming Schwann cell and its cytoplasm contains the usual organelles. Note that both neurons contain ribosomes (r), mitochondria (m), many transversely cut neurotubules (t), rough endoplasmic reticulum (rer), Golgi complex (G) in neuron B, and lysosomes (ly). The capillary above neuron B shows a tight junction (J), filopodium (f) and an unmyelinated axon (u) embedded in its wall. (X 4,000)



Fig. (26): Electron micrograph of the GG of an adult guinea pig showing a non myelinated neuron surrounded by the cytoplasm of a non myelin forming Schwann cell containing small sized mitochondria (arrows). Note the presence of lysosomes (ly) in the cytoplasm of the neuron and the capillary surrounded by unmyelinated axons (u). Note also the tight junction (J), nucleus of the endothelial cell (n), capillary lumen (L), long myelinated axons (a), and collagen in the extracellular space (Ci). (X 2,500)





Figs. (27) : Electron micrograph of the capiliaries of an adult GG showing tight junctions ($\uparrow\uparrow$), filopodia (f), increased collagen (Cl) and the basal lamina (Bl). Scale bar 500 nm (X 27,500)

Figs. (28) : Electron micrograph of the capillaries of an adult GG showing tight junctions $(\uparrow\uparrow)$, filopodia (f), increased collagen (Ci) and the basal lamina (Bi). (X 30,000)

DISCUSSION

The present investigation is the first study, as far as we know, to demonstrate the presence of a myelin envelope around the perikarya of neurons of the geniculate ganglion (GG) of the facial nerve. Myelination was observed clearly at the first post-natal day. The myelin sheath was formed of four layers and an associated state of neuronal activity in protein synthesis was observed as judged by the multiplicity of nucleoli and by better development of the channel system of rough endoplasmic reticulum and Nissi clumps. At the age of 60 days pre-natal, myelination was not observed in the perikarya. Only a cytoplasmic loop of Schwann cell in semithin sections or an opposition of the inner leaflets of cytoplasmic membrane at the ultrastructural level was seen. This could possibly represent the start of neuronal myelination process where fusion of the inner cytoplasmic aspects of Schwann cell membrane produces the major dense or period lines (Fawcett, 1994). This result is similar to our previous study on pre and post-natal development of the spiral ganglion in guinea pigs where the myelination started at late pre-natal life and became clear at the first postnatal day (Abdel Rahman et al. 1998).

In the present study, the fully developed neuronal myelin sheath was formed of 11-14 layers of compact and loose myelin. Although the majority of neurons were myelinated, yet nonmyelinated neurons were observed. Schwann cells capping all the neurons were either mature myelin forming or mature nonmvelin forming respectively. Kitamura et al. (1982) reported that neurons of GG in guinea pigs and monkeys were unmyelinated as seen by the electron microscope which contradicts the results of the present investigation. However, they added that most of the neurons were unipolar, a few were bipolar and some neurons were dark while others were light; this partly coincides with our results. Since unipolar neurons develop from bipolar ones (Gartner and Hiatt, 2001), the bipolarity observed in this work may be the stage before the final unipolar differentiation. The dark stain of some neurons may reflect a higher state of neuronal activity at the time of fixation of the specimen. Concerning the neuronal myelin envelope. Wakisaka et al. (2001) said that it has a protective role. They compared herpes simplex virus type I infection in neurons of the vestibular ganglion with that in neurons of the geniculate ganglion (nonmyelinated as they assumed). They observed Golgi vesicles in neurons of both ganglia. However, they reported viral infection in Schwann cells of the GG but not in the vestibular. They suggested that loose myelin of the vestibular neurons is an important barrier to herpes simplex virus type I infection and that it must play a role in prevention of viral spread from infected neurons to other cells. Their suggestion was based on the assumption that neurons of GG are nonmyelinated. Therefore, the spread of infection observed by Wakisaka et al. (2001) in the GG may be a spread from the nonmyelinated neurons observed in our work or their suggestion about the protective role of myelin is disputed.

In the present work, myelinated axons in the adjacent segment of the facial nerve had 1:1 relationship to their ensheathing Schwann cells. Unmyelinated axons were either sporadic or in groups invaginating the cytoplasm of a single supportive nonmyelin forming Schwann cell. Sometimes group of unmyelinated axons were seen surrounding the capillaries. Williams et al. (1995) stated that "nonmyelinated fibres include autonomic postganglionic axons which are exclusively nonmyelinated and other fine sensory fibres such as those of nociceptors". The latter may be processes of the small neurons observed in the GG in the present work. The unmyelinated nerves surrounding the capillaries or embedded in their walls in this study may raise the question of a nervous control on the capillaries which needs further investigation. Mvelin sheath around the axons increases the velocity of action potential conduction. Conduction proceeds from node to node in sequence along the axon. This mode of conduction is termed saltatory and is much faster than the mode of conduction in unmyelinated axons in which action potential sweeps continuously over the axolemma. Therefore, lack of myelin together with the small fibre diameter leads to relatively slow conduction of electric signals (Nolte, 1999). Moreover, when axons become demyelinated they transmit impulses 10 times slower than normal myelinated ones (Demyelination html Online, 2004).

In the present work, the single process of the cell somata of the facial ganglion was myelinated and its myelin was continuous with that of the perikaryon. This was observed in both semithin sections (as a continuous dark blue line) and ultrathin sections. This is contradictory to what is written in Gray's Anatomy about the somata of the facial ganglion which is described among other craniospinal sensory ganglia as having a single non-myelinated neurite termed the dendroaxonal process (Williams et al. 1995). The continuity of the myelin envelop of the perikaryon with that of its process may facilitate conduction of trains of impulses between its peripheral and central processes at a higher speed than in the nonmyelinated neurons.

Schwann cells in this investigation were seen capping both myelinated and nonmyelinated neurons.

Junqueira et al. (1998) mentioned that Schwann cells are the peripheral neuroglia which furnish a microenvironment suitable for neuronal activity.

Lieberman (1976) stated that "Schwann cells and their basal laminae help to demarcate the peri-neuronal compartment from the extracellular space in the ganglia". Thus, the perikarya of neurons do not come in direct contact with the connective tissue in the extracellular space. The myelin sheath surrounding the majority of the perikarya in this work will provide more insulation from the extracellular space of the ganglion. Concerning the Schwann cell lineage, neuronal signals appear to control the production of basal laminae by Schwann cells, the induction and maintenance of myelination and Schwann cell survival (Williams et al. 1995). This means that one neuronal signal leads to the development of a myelin forming Schwann cell while another neuronal signal leads to the development of a nonmyelin forming one as seen in the present work.

The two types of Schwann cells differ in their molecular phenotype. The adult myelin forming Schwann cells are characterized by the presence of several myelin forming proteins. The adult nonmyelin forming Schwann cells express nerve growth factor receptor, neural cell adhesion molecule and glial fibrillary acidic proteins (Williams et al., 1995). Immunohistochemical staining techniques can help to differentiate the two types of Schwann cells which should be conducted as a future approach. Both types, however, help to establish and maintain a controlled microenvironment around neuronal bodies providing electrical insulation as well as a pathway for metabolic exchanges (Ross et al. 1995).

In the present study, one myelinated heterotopic neuron was observed. Sensory ganglionic neurons are not entirely confined to craniospinal ganglia. They often occupy heterotopic positions distal or proximal to their ganglia. **Rupa et al. (1992)**, in an anatomic study with surgical implications, reported that a single ganglion cell in the region of the genu in one case lead them to postulate that geniculate ganglionectomy may be ineffective as the sole treatment for certain cases of geniculate neuralgia and that nervus intermedius section may also be required to achieve a more complete deafferentation.

The blood ganglion barrier of the geniculate ganglion (GG) has not been investigated before. The present study proved for the first time that capillaries in the grey matter of GG (area of neuronal density) are all of the continuous type. In the pre-and early postnatal period as well as in adult life, intercellular tight junctions were observed between endothelial cells of all detected capillaries. Tight junctions contribute to the restricted permeability of the blood ganglion barrier which is a functional barrier that protects neurons from harmful substances such as bacterial toxins and drugs. It also shields the neuron from contents of the blood that may act as neurotransmitters (Kessel, 1998). The continuous capillary endothelium with intercellular tight junctions observed by Saito et al. (1997) in the endoneurium of $7^{\underline{w}}$ and $8^{\underline{w}}$ nerves of guinea pigs was considered to form a blood nerve barrier. By immunohistochemical staining they reported the presence of P-glycoprotein in the capillary endothelium of these nerves. They concluded that immunoreactivity in these nerves was similar to that in the brain. Detection of P-glycoprotein in the capillary endothelium of GG should be performed to prove that a blood ganglion barrier similar to the blood-brain barrier and blood-nerve barrier exists at the capillary level.

SUMMARY

This study proved for the first time the issue of myelination of neurons of the geniculate ganglion (G.G) of the facial nerve, the myelination of the dendroaxonal processes of the neurons, the types of Schwann cells and the capillary blood supply as well as the blood ganglion barrier.

G.G. of 10 guinea pig embryos (day 60 prenatal), 10 offspring (day one postnatal) and 5 adults of both sexes (100 – 150 days old) were extracted with the aid of a surgical microscope. Specimens were processed to semithin and ultrathin sections, stained, examined and photographed.

At 60 days prenatal neurons of the G.G were nonmyelinated. However, axonal myelination in the adjacent facial nerve was clear. Myelinated axons had 1 : 1 relationship to their myelin forming Schwann cells. Nonmyelinated axons were single or in groups, which invaginated the cytoplasm of nonmyelin forming Schwann cells. Capillarles of G.G were of the continuous type. Intercellular tight junctions were observed between endothelial cells. Unmyelinated axons were embedded in the capillary walls. Basal jamina of capillaries and Schwann cells was clear.

At day-one postnatal the majority of neurons were myelinated. The myelin envelope was formed of 3 – 4 lamellae. The ganglionic capillaries as well as the collagen outside their basal laminae increased.

in adults the majority of neurons were myelinated unipolar cells. A few were bipolar. Myelin appeared as a dense dark blue line in toluidine blue stained sections. Neuronal myelin envelope was continuous with the myelin of the dendroaxonal process. The lamellae of the neuronal myelin envelope were 11 – 14 major dense or period lines as compared to 18 – 20 period lines enveloping the axons. The less dense intraperiod lines were more regular and narrower in the axons as compared to those of the neurons. Schwann cells surrounding the nonmyelinated neurons were of the mature nonmyelin forming type (MNMFSC) MMFSC could be differentiated from MNMFSC by immunchistochemical techniques only. Moreover, capillaries in the ganglion were seen surrounded by autonomic nonmyelinated nerves.

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الملخص العربي

تكونً الغمد النخاعى حول خلايا العقدة العصبية الركبية في الخنزير الغينى :دراسة بالمجهر الإلكترونى في الفترة حول الولادة وفي البالغين سوسن عبد الرحمن *، غادة وصيف *، عزة سليمان *

> ، محمد البجيرمى ** من قسمى التشريح * و الأنف والأذن والحنجرة ** كلية الطب ، جامعة عين شمس

أثبت هذا البحث لأول مرة وجود غمد نخاعى (ميلين) للخلايا العصبية المكونة للعقدة العصبية الركبية للعصب الوجهى (السابع المخى) كما أثبت وجود غمد نخاعى حول زوائد الخلايا ومعظمها من النوع الوحيد القطب. وأنواع خلايا شوان والـ شعيرات الدموية المغذية وكذلك الحاجز الموجود بين الشعيرات الدموية وخلايا العقد العصبية.

وقد استخدم فى هذا البحث عشر من العقد العصبية الركبية لأجنة الخنزير الغينــى فى اليوم الستين قبل الولادة وعشر فى اليوم الأول بعد الولادة ، بالإضافة إلى خمس من العقد العصبية الركبية من البالغين من الجنسين تتراوح أعمارهم ما بين مائــة ومائــة وخمسين يوماً. وقد تم إستخراج هذه العينات باستخدام الميكروسكوب الجراحى حيث تم تمرير العينات للحصول على مقاطع شبه رقيقة وفائقة الرقة وقد تم صبغها وفحـصها وتصويرها.

وقد ثبت أن الخلايا العصبية للعقد العصبية الركبية في عمر ستين يوماً قبل الولادة كانت غير مغطاه بالغمد النخاعي، مع أن الغمد النخاعي كان واضحاً بالنسبة للمحاور العصبية من الجزء المجاور للعقدة في العصب الوجهي. كما لوحظ وجود خلية واحدة من خلايا شوان المكونة للغمد النخاعي حول كل محور مغطي بالغمد النخاعي ، وكان هناك البعض من المحاور العصبية الغير مغطاه بالغمد النخاعي في صورة منفردة أو مجموعات والتي كانت منغمدة في السيتوبلازم في خلايا شوان غير المكونة للغمد النخاعي. وكانت الشعير ات الدموية للعقدة العصبية من النوع غير المنفذ. وأرتبط ت خلاياها المبطنة بإتصالات محكمة وظهرت بعض المحاور الصغيرة التي ليس لها غمد نخاعى مندغمه في جدر الشعيرات الرقيقة. هذا بالإضافة إلى وضوح الطبقة القاعدية للشعيرات الدموية ولخلايا شوان.

فى اليوم الأول بعد الولادة ظهر أن غالبية الخلايا العصبية الركبية كانت مغطاه بالغمد النخاعى ، الذى يتكون من ٣ إلى ٤ طبقات وقد لوحظ زيادة عدد المشعيرات الدموية المغذية للعقدة العصبية والكولاجين خارج الطبقة القاعدية.

أما فى البالغين فقد ظهر أن الغالبية العظمى من الخلايا العصبية كانت مغطاء بالغمد النخاعى ومعظمها خلايا وحيدة القطب وقليل جداً منها كان ذا قطبين. وقد ظهر الغمد النخاعى كخطوط زرقاء داكنة فى المقاطع المصبوغة بصبغة التلويدين الزرقاء. كما أن غطاء الغمد النخاعى للخلايا العصبية كان مستمراً مع الغمد النخاعى المغطى للزائدة القطبية والمحاور العصبية. وقد تراوح عدد طبقات الغمد النخاعى المعطى للزائدة القطبية من ١١ إلى ١٤ طبقة من الخطوط الداكنة مقارنة بـ ١٨ إلى ٢٠ طبقة حول المحاور . وكانت الخطوط الأقل كثافة ما بين اللفات الدورية أكثر ضيقاً وإنتظاماً فى المحاور مقارنة بالخطوط المقابلة لها فى الخلايا العصبية.

وقد لوحظ أن خلايا شوان التي تحيط بالخلايا العصبية غير المغطاه بالغمد النخاعي كانت من النوع الناضج غير المكون للغمد النخاعي ، ويمكن التفرقه بين النوع المكون للغمد النخاعي والنوع غير المكون للغمد النخاعي عن طريق إستخدام الطرق الهيستوكيميائية المناعية. أما الشعيرات الدموية في العقد العصبية فكانست محاطسة بالأعصاب اللإرادية وغير المغطاه بالغمد النخاعي.

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