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Scanning electron microscopic observations on the third ventricular floor of the rat following cervical sympathectomy

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Various investigators have shown that unilateral ganglionectomy or transection of the internal and external carotid nerves leads to a regenerative response in the ipsilateral superior cervical ganglion and to uninjured mature sympathetic neurons sprouting into bilaterally innervated shared target organs. In this study changes in the supraependymal neuronal network following unilateral and bilateral cervical sympathectomy on the infundibular floor of the third ventricle were studied by scanning electron microscopy in comparison with normal and sham-operated control animals. After unilateral cervical sympathectomy there was a great increase in the number of varicose nerve fibres on the infundibular floor as compared to the normal and sham-operated control animals. Not only was there an increase in the number of nerve fibres, but also their varicosities were substantially larger than those normally present on the ependymal surface. This study indicates the possible sympathetic projections from the superior cervical ganglia to the ependymal surface of the third cerebral ventricle.

Key words: third cerebral ventricle, supraependymal nerve fibres, superior cervical ganglia, cervical sympathectomy, rat

INTRODUCTION

Mature mammalian neurons of the peripheral nervous system show successful axonal regeneration following injury [23] as compared to the neurons of the central nervous system (CNS). Following a crush injury to the peripheral nerve two phenomena occur, one being the formation of growth cones and sprouting and the other fibre elongation over long distances. In CNS, following injury to the axons, growth cone formation and sprouting occurs, although fibre elongation in the vertebrate CNS is often limited to less than 1 mm [28, 29]. This limited fibre elongation is due to the presence of specific CNS growth inhibitory factors [4, 10, 28, 30]. Furthermore, a glial scar represents an additional mechanical and biochemical obstacle to CNS regeneration [2, 11]. On the other hand, the Schwann cells that surround the peripheral nerve provide a suitable environment for neuronal growth [1, 9, 14, 15, 34]. Of the various elements required for successful nerve regeneration, the production of growth factors by the Schwann cells in the distal region of the injured nerve is considered crucial [9, 40]. Recent studies have indicated that Schwann cells express motor and sensory phenotypes that regulate axon regeneration [16]. Thus normal growth of mammalian neurons involves a complex interplay between extrinsic influences and intrinsic neuronal genetic mechanisms.

In a previous study from our laboratory [22] it was shown that the supraependymal neuronal plexus, which enjoys an environment different from that of the peripheral neurons and their central counterpart,

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shows considerable growth following axotomy. Furthermore, recent studies on the origin of the supraependymal nerve fibres indicate that at least some of these fibres could have originated from the superior cervical ganglia (Mathew, forwarded for publication). It is known that the sympathetic axons from the superior cervical ganglia normally innervate cerebral vasculature and reach the different regions of the brain. Ingrowths of sympathetic fibres into different regions of the vertebrate CNS are also not uncommon [6, 27].

The post-ganglionic sympathetic axons of the internal carotid nerve unilaterally and bilaterally innervate a number of target organs. The shared target organs of the superior cervical ganglia (SCG) include the cerebral vasculature and pineal gland [18, 23, 33]. Some of the principal neurons of SCG also unilaterally innervate the ipsilateral iris via the internal carotid nerve. The fibres arising from the two SCG mingle in some of these shared target organs. A considerable growth of sympathetic fibres is observed in the pineal gland following unilateral cervical sympathectomy [23]. These changes following unilateral cervical sympathectomy have been monitored using a specific molecular marker for neuronal growth, Tα1 α-tublin mRNA [23]. Previous studies from our laboratory [22, 23] and recent observations on the sympathetic projections to the ependymal surface of the third cervical ventricle (Mathew, forwarded for publication) prompts an investigation of the changes that occur to the ependymal lining of the cerebral ventricular floor in rats that had undergone unilateral and bilateral cervical sympathectomy as compared to normal and sham control animals.

MATERIAL AND METHODS

Surgical procedure

A total of 24 Wistar rats were used in the study. They included both sexes and ranged in weight from 150 to 200 g. The experimental procedures involved in the research were reviewed according to the guidelines of Kuwait University Research Administration and all the animal experiments performed in this study were approved by the Kuwait University Ethical Committee. The animals were divided into four equal groups (I–IV): Group I was used as a normal control, Groups II and III for unilateral and bilateral cervical sympathectomy respectively and Group IV as a sham control. For all the surgical procedures the animals were anaesthetised with sodium pentobarbital (35 mg/kg). In order to perform either unilateral or bilateral cervical sympathectomy the cervical region was opened so as to expose the location of the bifurcation of the common carotid artery into internal and external carotid arteries; either unilateral or bilateral cervical sympathectomy was then performed. For the sham control the sham operation involved exposing the superior cervical sympathetic ganglia but not removing them.

The processing of tissues for scanning electron microscopy

On days 7 and 15 following the unilateral and bilateral sympathectomy and the sham operation the animals were perfused transcardially, under deep anaesthesia, with a brief wash of 0.9% oxygenated Sorenson's buffer (0.1 M at pH 7.2) followed by 3% gluteraldehyde in Sorenson's buffer for scanning microscopy. The brains were dissected out and the tissues from the floor of the third ventricle were post-fixed in the same fixative. The tissues were further processed for scanning microscopy. For each time point three experimental and three sham control rats were used. To observe the entire third ventricular floor by scanning electron microscopy the horizontal method of dissection of the hypothalami [24] was followed. The procedure used for processing tissues for scanning electron microscopy is, briefly, as follows. After gluteraldehyde fixation, the tissues were further post-fixed in 1% osmic acid for 3 hours and dehydrated in graded acetone as per routine. Following dehydration, the tissues were critical-point dried using Balzer's drying system (Balzer's CPD 030). The specimens were then coated with gold using Balzer's sputter coater SCD 050 and examined with JSM-35 SEM.

RESULTS

With the use of scanning electron microscopy the entire infundibular floor of the third ventricle could be observed by following the horizontal dissection method described by Naik and Mathew in 1985 [24]. Ciliated and non-ciliated regions were observed at the ependymal surface of the third ventricle. A thin strip of transitional zone with ciliated and non-ciliated areas was present between the non-ciliated and richly ciliated surfaces of the ependymal floor (Fig. 1A). The ependymal lining of the infundibular floor, the tuber-infundibulum, the mamillary body and regions of the arcuate nucleus were lined with non-ciliated ependyma. In addition, a part of the *organum vasculosum* of the *lamina terminalis*, the preoptic and

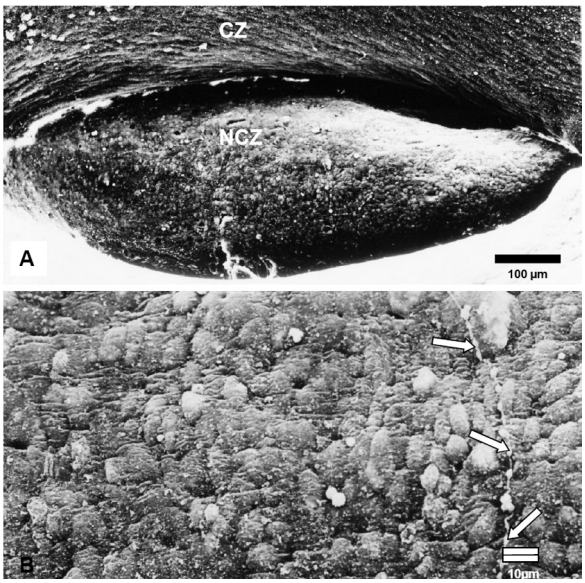

Figure 1. Scanning electron micrographs showing the general morphology of the ependymal surface of the infundibular floor of the third ventricle in adult rats. The ependymal surface of the third ventricle can be divided into non-ciliated (NCZ) and ciliated zones (CZ) on the basis of the absence or presence of cilia on the luminal surface of ependymal cells. The ependyma of the dorsolateral wall of the third ventricle was highly ciliated. A thin transitional zone with both non-ciliated and ciliated ependyma was present between these two zones (**A**). The ependymal surface of the infundibular floor is shown at high magnification (**B**). The luminal surface of the ependyma lining the infundibular floor possesses microvilli and blebs of various size and shape. Note the varicose nerve fibres indicated by white arrows that innervate the ventricular surface. Only very few nerve fibres were present on the luminal surface of the infundibular floor and adjacent areas of normal adult rats (**B**).

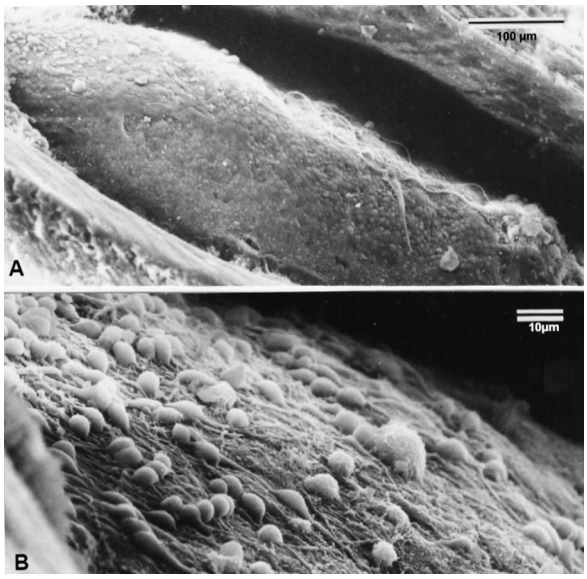

Figure 2. Scanning electron micrographs showing the morphology of the infundibular floor of the third ventricle in adult rats at 15 days following unilateral cervical sympathectomy (**A**). Note the increase in the number of supraependymal nerve fibres of the infundibular floor following unilateral cervical sympathectomy. The ependymal surface of the infundibular floor is shown at high magnification (**B**). Note that in addition to the increase in the number of supraependymal nerve fibres there is a great increase in the size of the axonal varicosities.

the paraventricular regions were also non-ciliated. The ependyma of the dorsolateral wall of the third ventricle was highly ciliated (Fig. 1A).

The luminal ependymal surface possesses microvilli and blebs of various size and shape (Fig. 1A, B). Supraependymal nerve fibres were observed on the surface of the non-ciliated infundibular floor and adjacent areas, although there were very few of these (Fig. 1A, B). Both fibres with varicosities (varicose fibres) and non-varicose fibres were present on the ependymal surface of the third ventricular floor. After unilateral cervical sympathectomy there was a profound increase in the number of nerve fibres found on the floor of the third ventricle (Fig. 2A, B). Their varicosities were substantially larger (Fig. 2B) than those usually observed in the varicose fibres of the ventricular surface (Fig. 1B). Generally the axonal varicosities of the supraependymal nerve fibres are within the range of 1–2 μm in diameter and 3–4 μm in length. On the other hand, after unilateral sympathectomy the varicosities were in the range of 4––5 μm in diameter and 4–10 μm in length along the axon.

The increase in the number of nerve fibres on the infundibular floor following unilateral cervical sympathectomy was much larger at 15 days than that observed after 7 days. Following bilateral cervical sympathectomy there was no significant change in the number of nerve fibres observed on the infundibular floor of the third ventricle either at 7 or at 15 days. Similarly, no change was observed in the number of nerve fibres in the sham-operated control animals.

DISCUSSION AND CONCLUSIONS

Supraependymal neuronal elements consist of *bona fide* neurons and an extensive network of nerve fibres [3, 5, 7, 13, 21, 24, 31]. In general the supraependymal nerve fibres of the ventricular system are characterised as catecholaminergic, cholinergic or peptidergic in nature [3, 5, 22, 24]. Depending on the size of these fibres, they may be further classified as being small or large in diameter.

The majority of the fibres of small diameter are varicose in nature [21]. Experimental studies from our laboratory using 5,7-dihydroxytryptamine injected into the cerebral ventricles have convincingly showed that at least some of these fibres are serotonergic in nature [24]. Serotonergic axons are characterised by numerous varicosities along their course [24, 35, 36]. Similar axonal varicosities are shown by adrenergic nerve terminals [17].

Axonal varicosities are considered to be specialised sites of uptake, storage, synthesis and release of the neurotransmitter substance [17]. Within the adrenergic nerve the neurotransmitter is located in special submicroscopic granules [17]. A high concentration of neurotransmitters is present in these varicosities [17]. Hence the increase in size of the varicosities indicates an increase in the storage of the neurotransmitter in these varicosities following unilateral cervical sympathectomy.

The efferent fibres arising from the postganglionic neurons of the superior cervical ganglion course primarily into two major nerve branches, namely the internal and external carotid nerves. The preganglionic input from the sympathetic preganglionic neurons in the spinal cord arrives via the cervical sympathetic trunk. The post-ganglionic sympathetic axons of the internal carotid nerve bilaterally innervate a number of shared target organs including the cerebral vasculature and the pineal gland. Fibres arising from the two SCG mingle at some of these target organs [18, 33].

It has been shown that unilateral transection of the internal and external carotid nerves leads to a regenerative response in the ipsilateral SCG and indirectly affects the physiology of the contralateral SCG. Several investigators [8, 18, 23] have demonstrated the sprouting of contralateral uninjured sympathetic neurons innervating the pineal gland following unilateral ganglionectomy or transection of the internal and external carotid nerves. In the present study a similar situation is observed. Following unilateral cervical sympathectomy, uninjured mature contralateral neurons sprout to the degenerated regions of the infundibular floor. Ingrowth of peripheral sympathetic nerve fibres is observed in the brain under certain experimental conditions [19].

A similar ingrowth of peripheral sympathetic nerve fibres is observed in the brain following the degeneration of septohippocampal cholinergic neurons [27]. An intact cholinergic mechanism is necessary for some of the hippocampal-dependent memory processing. As a consequence of the degeneration of septohippocampal cholinergic neurons, ingrowth of sympathetic fibres from superior cervical ganglia to the hippocampus is observed [27]. Normally these axons innervate the cerebral vasculature only. The role of nerve growth factors in triggering sympathetic ingrowth has been well established [6, 23]. Recent studies have investigated the functional impact of these sympathetic projections on hippocampal synaptic physiology and shown that the autonomic and central nervous system experience a structural rearrangement which replaces the lost cholinergic innervation and restores altered functions in the hippocampus [27]. The mechanism by which the adrenergic sympathetic innervation restores cholinergic function is not well understood. It is known that sympathetic neurons have the ability to switch adrenergic or cholinergic phenotypes and/or functionally alter their phenotype from adrenergic to cholinergic [12, 25–27, 37, 39]. However, unilateral cervical sympathectomy removes the above-mentioned re-established cholinergic innervation and function of the ipsilateral side [27].

Further studies are required to understand the molecular mechanism that leads to the profound growth of nerve fibres to the ventricular lumen following unilateral cervical sympathectomy. Interestingly, it has been found that these fibres regenerate well following injury as compared to other neurons confined within CNS [22]. This may be due to the environment in which these axons grow. In addition to various bioactive substances, CSF may also contain several neuronal growth factors belonging to the nerve growth factor family [20, 32, 38]. The presence of these and/or similar growth factors in CSF may be responsible for the enhanced growth of supraependymal nerve fibres. Biochemical characterisation of CSF may lead to the identification of new neural growth factors that induce growth in CNS neurons.

A significant decrease in the number of nerve fibres is not observed following bilateral cervical sympathectomy. One possible reason is the presence of very few neurons that project to the ventricular floor from SCG. Secondly, as the supraependymal floor of the third ventricle is innervated by a mixture of fibres of varying origin, scanning electron microscopy may not be sensitive enough to distinguish the exact change in the supraependymal neuronal network. The sympathetic origin of the supraependymal nerve fibres is indicative of novel pathways of neuroendocrine signalling mechanisms in the mammalian brain.

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