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Light and scanning electron microscopic studies on subcommissural organ of the pig

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ABSTRACT

The subcommissural organ (SCO) of pig located on the ventral aspect of posterior commissure was constituted by an ependyma and a hypendyma. The pseudostratified columnar ciliated ependyma of SCO comprised 8–11 rows of round, oval to elongated type-I and type-II nuclei. Less basophilic type-I and darkly stained type-II nuclei were categorised on the basis of distribution of nuclear chromatin and staining affinity. The hypendyma contained hypendymal cells, micro- and macro-glial cells, blood capillaries and nerve fibres. The neurosecretory substance in the form of small, pink structures was mainly localised towards the supranuclear portion of the ependyma, free ventricular surface and lumen of blood capillaries. Scanning electron microscopy revealed ciliated and non-ciliated apical spherical protrusions along with tufts of cilia. A few apical spherical protrusions possessed small microvilli like structure.

Key words: Ependyma, Hypendyma, Pig, Subcommissural organ

The subcommissural organ (SCO) is an ependymal differentiation located in the diencephalon and under the posterior commissure (PC) forming partly the roof of III ventricle and cerebral aqueduct. A functional interrelation between anti-angiotensin II and the secretory activity of the SCO was noticed in goats by Castaneyra-Martin *et al.* (2005). The ependymal cells of the subcommissural organ and SCO-spondin secretion were suspected to play a crucial role in CSF flow and/or homeostasis (Meiniel 2007). The molecular structure and functional significance of these proteins remains to be elucidated. Hoyo-Becerra *et al.* (2010) in chicks found that at early stages of development, the SCO releases some attractive or permissive molecule(s) for the growing of the posterior commissure, whereas at later stages, the SCO has a repulsive effect over neurites arising from magnocellular nucleus of the PC region. The functional significance and lack of literature on the structure of the SCO in pig led to pursue the present study.

MATERIALS AND METHODS

Fresh brain tissues were collected from 10 adult apparently healthy pigs of either sex of non-descript local breed after decapitation at atlanto-occipital joint. The tissues from mammillary body to the level of rostral colliculi of mid brain having SCO were fixed in 10 % neutral buffered formalin for 48–72 h and processed for paraffin technique of light microscopy. sections of 6–15 µ were stained by routine Harris' haematoxylin and eosin, Gomori's for reticulum, Bielschowsky's for axis cylinder and dendrites, Holmes for nerve cells and fibres, Sevier-Munger for neural tissue, Kluver-Barrera for myelin and nerve cells, Lapham's for myelinated glial fibres (Luna 1968), chrome-haematoxylin Bargmann's modification for neurosecretory substance (Humason 1979).

For SEM, the fresh brain tissues containing SCO were washed with chilled 0.1M phosphate buffer (pH 7.4) and fixed in chilled 2 % gluteraldehyde solution prepared in 0.1M phosphate buffer (pH 7.4) at 4°C for 6–8 h. The tissues were rewashed thrice with 0.1M phosphate buffer, dehydrated; critical point dried and were sputter coated with gold before viewing under SEM at EM Laboratory, AIIMS, New Delhi.

RESULTS AND DISCUSSION

The SCO of the pig possessed 2 different zones i.e. an ependyma towards free ventricular surface and a hypendyma adjacent to neural tissue of the posterior commissure (PC) as reported in sheep, goat, elk, llama (Talanti and Kivalo 1960), camel and water buffalo (Dellmann and Fahmy 1966 a, b). An additional subependymal glial zone consisting of glial tissue and blood capillaries sandwiched in between the 2 was reported in buffalo (Ramkrishna and Saigal 1986), goat, camel (Kumar *et al.* 1997, 1999) and sheep (Saggar *et al.* 2002 a). In addition, a sub-hypendymal fibrous zone was also observed only in the horse (Kumar and Timoney 2008).

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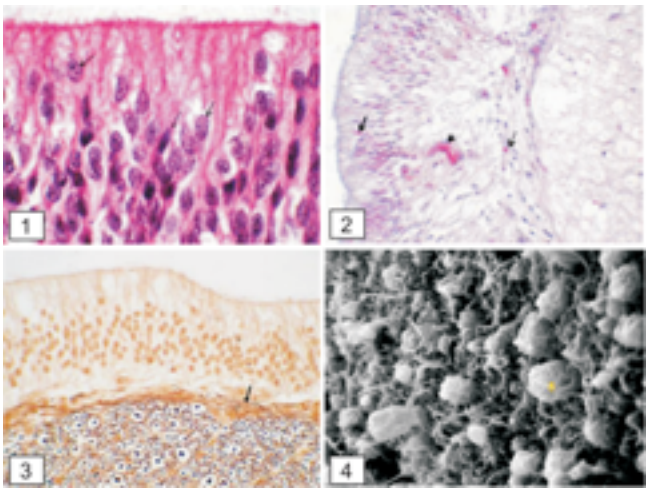

Figs 1–4. **1.** Photomicrograph showing type-I (arrow) and type-II (dotted arrow) nuclei of ependymal cells. Note vacuolated areas in the ependyma. H. & E.×1000. **2.** Neurosecretory substance (pink colour) in supranuclear portion of the ependyma (large arrow) and hypendyma (dotted arrow). Note an intense positive reaction in the lumen of blood capillaries (small arrow). Bargmann's chrome-haematoxylin method×400. **3.** Nerve fibres (arrow) placed horizontally in the hypendyma close to posterior commissure. Sevier-Munger method×400. **4.** Scanning electron micrograph at higher magnification showing apical spherical protrusions (S) having small microvilli like structure and the cilia of ependymal cells.×4840.

The ependyma of SCO in pig was pseudostratified columnar ciliated as described in camel, water buffalo (Dellmann and Fahmy 1966 a, b), sheep (Barlow *et al.* 1967), buffalo (Ramkrishna and Saigal 1986), goat and camel (Kumar *et al.* 1997, 1999), sheep (Saggar *et al.* 2002 a) and horse (Kumar and Timoney 2008). An ependyma having tall ciliated columnar cells were reported in rat, mouse, guinea pig, hamster, ox, horse, pig, dog, sheep, goat, elk, llama and cow calf (Talanti and Kivalo 1960, Isomaki *et al.* 1965).

The pseudostratified columnar ciliated ependyma of pig contained 8–11 rows of round, oval to elongated nuclei throughout the height of ependyma particularly towards mid and base of the ependyma (Fig. 1) as reported in domestic animals except water buffalo where only a few nuclei were present (Dellmann and Fahmy 1966 b). Two types of ependymal cells were identified on the basis of nuclear chromatin and staining affinity in pig as in goat (Kumar *et al.* 1997) and horse (Kumar and Timoney 2008) in contrast to 3 types in sheep (Saggar *et al.* 2002 a). Two types included a less basophilic round to oval shaped type-I and darkly stained type-II cells of irregular shapes (Fig. 1). The different types of nuclei represented various functional stages of the cell types.

The cytoplasm of ependymal cells in pig was eosinophilic and finely granular with increased eosinophilia towards the intercellular junctions, basal processes of the ependymal cells and free ventricular surface. The supranuclear cytoplasmic vacuolations in between the ependymal cells was a regular feature during the present study (Fig. 1). Ramkrishna and Saigal (1986) suggested the steroid secretory activity of the SCO by attributing these sites to contain lipids. The fine basal cytoplasmic processes of ependymal cells intermixed with the glial processes or terminated at the periphery of capillaries. A similar type of arrangement was described in the bovine calf (Isomaki *et al.* 1965), buffalo (Ramkrishna and Saigal 1986), goat (Kumar *et al.* 1997), sheep (Saggar *et al.* 2002 a) and horse (Kumar and Timoney 2008). The neurosecretory substance in SCO of pig demonstrated by Bargmann's modified chrome alum haematoxylin method was observed towards the supranuclear portion and free ventricular surface (Fig. 2) as demonstrated in domestic animals (Talanti and Kivalo 1960, Kumar *et al.* 1997, Saggar *et al.* 2002 a).

Subependymal glial zone could not be localized in the present study whereas, intermediate zone having mixed distribution of basal cytoplasmic processes, fine blood capillaries and small number of glial cells was reported in domestic animals (Ramkrishna and Saigal 1986, Kumar *et al.* 1997, Saggar *et al.* 2002 a, Kumar and Timoney 2008). The subependymal glial zone was considered as part of hypendyma in some domestic animals (Talanti 1958).

The hypendymal layer in pig was cellular zone adjoining the posterior commissure and was constituted by hypendymal cells, glial cells, fine blood capillaries and nerve fibres. The concentration of hypendymal cells was very less towards the basal portion of hypendyma. These cells were scattered singly throughout the extent of the organ however, at places these were arranged in small groups. Hypendymal cells had been called to all those secretory cells of SCO which were not located within ependyma. Single type of hypendymal cells was observed in pig whereas, 2 types of hypendymal cells were reported in goat (Kumar *et al.* 1999), sheep (Saggar *et al.* 2002 a) and horse (Kumar and Timoney 2008). The nuclei of hypendymal cells were round to oval in shape having fine distribution of moderately basophilic chromatin material. The cytoplasm of the cells was distributed in the form of fine eosinophilic granules. Similar characteristics of cytoplasm were also reported in goat (Kumar *et al.* 1997) and sheep (Saggar *et al.* 2002 a). The glial cells distributed among the hypendymal cells were categorized into two types. The darkly stained small sized microglial cells were mainly associated with fine blood capillaries whereas, the large sized macroglial cells distributed throughout the SCO were lesser in number. The glial cells, considered as fibrous astrocytes (Leonieni 1968), acted as a "relay or end station" because of their significant role in the transport of substances from the cerebrospinal fluid and blood vessels. Hypendymal cavities and vacuolations of different dimensions were present in between the hypendymal cells as observed in calf (Isomaki *et al.* 1965), buffalo (Ramkrishna and Saigal 1986), goat (Kumar *et al.* 1997), sheep (Saggar *et al.* 2002 a) and horse

(Kumar and Timoney 2008).

The blood capillaries of different dimensions were mainly longitudinally oriented. A close relation between blood capillaries, hypendymal and glial cells indicated an active transport mechanism of secretory substances as reported in ox, horse, pig, dog (Talanti 1958) and in some other ruminants (Talanti and Kivalo 1960, Isomaki *et al.* 1965, Barlow *et al.* 1967, Ramkrishna and Saigal 1986, Kumar *et al.* 1997, Saggar *et al.* 2002 a, Kumar and Timoney 2008). The neurosecretory substance was also observed in the lumen of blood capillaries (Fig. 2) of pig as reported in sheep (Saggar *et al.* 2002 a) and goat (Kumar *et al*.1997). However, Ramkrishna and Saigal (1986) in buffalo hypothesized endocrine nature of SCO due to transportation of its secretions through blood capillaries under neuro-hormonal control.

The special silver stains for nervous tissue revealed fine nerve fibres in hypendyma oriented horizontally parallel to the length of ependyma. The concentration of nerve fibres was more around the blood capillaries and in deeper portion of hypendyma close to PC (Fig. 3). Similar findings were reported in domestic animals (Talanti 1958, Dellmann and Fahmy 1966 a and Kumar *et al.* 1999), sheep (Barlow *et al.* 1967, Saggar *et al*.2002 a), buffalo (Ramkrishna and Saigal 1986), goat (Kumar *et al*.1997) and horse (Kumar and Timoney 2008). The origin of these nerve fibres could not be established owing to their tortuous course in hypendyma of bovine calf (Isomaki *et al.* 1965).

SEM revealed that the SCO (Fig. 4) present beneath the PC presented tufts of cilia, which originated from round to oval shaped structures called apical spherical protrusions (ASP) as reported in cow (Lindberg and Talanti 1975), goat (Kumar and Kumar 2000), sheep (Saggar *et al.* 2002 b) and buffalo (Kumar *et al.* 2006). The majority of ASP possessed depressions towards their centre in the SCO possibly represented absorption as an additional functional capacity of ependymal cells in the goat (Kumar and Kumar 2000). The tufts of cilia originated from ASP (Fig. 4) formed intricate meshwork with those of adjacent ones as reported in cow (Lindberg and Talanti 1975), goat (Kumar and Kumar 2000), sheep (Saggar *et al.* 2002 b) and buffalo (Kumar *et al*.2006). Some of the ASP presented small microvilli like structure (Fig. 4). Some of the smooth ASP devoid of cilia represented the non-ciliated ependymal cells of SCO as reported in goat (Kumar and Kumar 2000), sheep (Saggar *et al.* 2002 b) and buffalo (Kumar *et al.* 2006). The presence of amorphous, heterogeneous material between the protrusions and cilia in pig supported the findings as reported in cow, buffalo, goat and sheep (Lindberg and Talanti 1975, Kumar *et al.* 2006, Kumar and Kumar 2000, Saggar *et al.* 2002 b).

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