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Histochemical studies on the subcommissural organ of the pig

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ABSTRACT

The present study, conducted on 10 healthy adult pigs of local mixed breed, revealed distribution of mucopolysaccharides, lipids and proteins in ependyma and hypendyma of the subcommissural organ. Periodic acid Schiff's reaction demonstrated by McManus' method was present in different components of ependymal cells, and hypendymal cells with more affinity in the blood capillaries of the organ and indicated presence of glycogen. Aldehyde thionin PAS activity was mainly localized towards supranuclear portion of the ependymal cells and the blood capillaries of the hypendyma. PAS Alcian blue demonstrated negligible concentration of acidic mucopolysaccharides whereas the distribution of neutral mucopolysaccharides was similar to that of PAS technique. Neutral and Sudanophilic lipids were localized in the form of very small sized droplets and fine dust particles towards base of the ependyma, respectively. The SCO secretions towards free ventricular surface were qualitatively proteinaceous in nature.

Key words: Subcommissural organ, Histochemistry, Ependyma, Hypendyma, Pig

The subcommissural organ (SCO) comprising an ependyma and a hypendyma was present on the ventral aspect of posterior commissure (PC) extending from its attachment with the pineal gland to the recessus mesocoelicus forming partly the roof of III ventricle and cerebral aqueduct (Kumar *et al*. 2010). Stutinsky (1950) was first to demonstrate Gomori positive secretory material in the ependymal cells of the SCO which was confirmed as polysaccharide-protein complex (Diederen 1970). Murphy and Woods (1966) observed an intense staining reaction of the ependymal secretory material in saline infused cats reflecting an important role of the subcommissural organ in homeostatic mechanism of diuresis and regulation of blood pressure. Lectin histochemistry suggested that the SCO secreted N-linked complex type glycoproteins with the possibility that an additional sialytated O-linked oligosaccharides branch on the protein (Meiniel *et al*. 1988). A functional interrelation between anti-angiotensin II (AAGII) and the secretory activity of the SCO has been reported in goat 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 neuronal development by modulating cell aggregative mechanisms (Gobron *et al.* 1996) and might be involved in cerebrospinal fluid (CSF) flow and/or homeostasis (Meiniel 2007). Molecular and functional features evidenced that SCO-spondin, a novel relative of thrombospondin family might be involved in neuronal development by modulating cell aggregative mechanisms (Gobron *et al.* 1996). At early stages of chick 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 (Hoyo-Becerra *et al.* 2010).

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MATERIALS AND METHODS

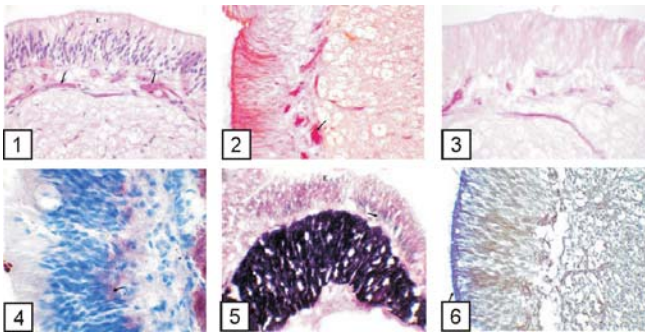
Adult apparently healthy pigs (10) of either sex of local mixed breed were used for the study. Fresh brain tissues were collected from mammillary body to the level of rostral colliculi of corpora quadrigemina immediately after decapitation at atlanto-occipital joint. Tissues from 5 animals were fixed in 10% neutral buffered formalin and processed for light microscopy. Paraffin sections of 6 µ cut serially in transverse and sagittal planes were stained by aldehyde thionin PAS for CNS, McManus' PAS method for glycogen, diastase digestion method for glycogen (Luna 1968), Best's Carmine method for glycogen (Drury and Wallington 1967), mercury bromphenol blue method for protein, and performic acid Alcian blue method (Pearse 1968). For demonstration of lipid content, the fresh tissues from 5 animals were frozen to –20° C and frozen sections of 10–12 µ cut by cryostat were stained by oil-red-o in propylene glycol method for fats, Sudan black B method for fats (Luna 1968), and Nile blue method for neutral and acidic lipids (Drury and Wallington 1967).

RESULTS AND DISCUSSION

The SCO of pig possessed two histologically different zones i.e. a pseudostratified columnar ciliated ependyma towards free ventricular surface and a deeper hypendyma adjacent to neural tissue of the posterior commissure.

Polysaccharides: The periodic acid Schiff (PAS) reaction was present in the form of uniformly distributed fine granules in the supra and infra nuclear portions of cytoplasm of the ependymal cells, hypendymal and glial cells. The reaction was more intense towards free ventricular surface, intercellular junctions, basal cytoplasmic processes and blood capillaries present in the hypendyma (Fig. 1). Similar observations had been reported in dog (Bargmann and Schiebler 1952, Talanti 1958, Leonieni 1968), ox, horse, pig (Talanti 1958), sheep (Barlow *et al.* 1967, Nesic and Babic 1979, Saggar *et al.* 2002), buffalo (Ramkrishna and Saigal 1987), goat (Kumar *et al.* 1996) and sheep (Saggar *et al.* 2002). Best's carmine method demonstrated the PAS activity of lesser intensity in pig as compared to McManus' method of glycogen, as reported in goat (Kumar *et al.* 1996) and sheep (Saggar *et al.* 2002). The diastase treatment did not affect much the PAS activity. In contrast, the reactivity was reduced after diastase treatment in buffalo (Ramkrishna and Saigal 1987 a), goat (Kumar *et al.* 1996) and sheep (Saggar *et al.* 2002) depicting the presence of mucopolysaccharides other than the glycogen. Glycogen had been reported to be absent in rodent (Wislocki and Leduc 1952), domestic animals (Talanti 1958) and sheep (Barlow *et al.* 1967). The presence of glycogen in neuroglia cells suggested its role in mediation of nutrition from blood vessels to nervous tissue proper (Shimizu and Kumamoto 1952). Glycogen present in glial cells had been attributed to the transport of nutrients from blood vessels to ependymal and hypendymal cells (Ramkrishna and Saigal, 1987). The authors also suggested that the glycogen acted as a transportation media for the secretory material synthesized in the ependymal and hypendymal cells. More concentration towards free and basal surfaces of ependyma led the SCO as an active metabolic organ in relation to absorption and detoxification of CSF (Sterba 1969).

The aldehyde thionin PAS activity in the SCO of pig was mainly localized towards supranuclear portion of the ependymal cells (Fig. 2) whereas, the activity towards basal cytoplasmic processes and free ventricular surface was less in contrast to sheep (Saggar *et al.* 2002) where middle and basal portions of ependyma showed significant activity. PAS activity was intense in the blood capillaries of hypendyma (Fig. 2). Alcian blue method (pH 2.5) depicted absence or a negligible amount of weakly acidic sulphated mucosubstances in ependyma, hypendyma and its junction with posterior commissure. Similar observations were reported in goat (Kumar *et al.* 1996) and sheep (Saggar *et al.* 2002). A very weak Alcianophilia revealed the presence of negligible amount of chondroitin-4 and/or 6 sulphates.



Figs 1–6. **1.** Photomicrograph showing PAS positive granules in ependyma (E) with their more concentration towards intercellular junctions. Note intense PAS activity towards the lumen of blood capillaries (arrow) present in hypendyma. McManus' PAS method × 400. **2.** PAS positive material in ependyma especially towards supranuclear portion and in the blood vessels of hypendyma (arrow). Aldehyde thionin PAS method. × 400. **3.** Presence of neutral mucopolysaccharides (pink colour) and a negligible distribution of acidic mucopolysaccharides (blue colour) only towards free ventricular surface. PAS Alcian blue method. × 400. **4.** Neutral lipid in the form of fine droplets (arrow) towards base of ependyma. Oil-red-o × 400. **5.** Sudanophilic lipids present in the form of fine dust particles (arrow) towards base of ependyma. Sudan black B method. × 100. **6.** Proteinaceous secretions (arrow) in blue colour towards free ventricular surface of ependyma. Mercury bromphenol blue method. × 100.

Alcianophilic sites in buffalo corresponded to the sites positive for neuro-secretory substance whereas, free ependymal surface presented glycoproteinaceous nature of the neurosecretory material (Ramkrishna and Saigal 1987). PAS Alcian blue method presented distribution of neutral mucopolysaccharides (Fig. 3) similar to that of McManus' PAS method, however, a very negligible amount of acidic mucopolysaccharides was observed towards free surface of ependyma.

Lipids: The neutral lipids demonstrated by oil-red-o method were localized in small amount in the form of fine small sized droplets towards the base of the ependyma in pig (Fig. 4). Only a few isolated droplets were observed in between the nuclei of ependymal and hypendymal cells. The neutral lipids had been reported absent in buffalo (Ramkrishna and Saigal 1987). However, Kumar *et al.* (1996) in goat found that lipid droplets of different dimensions were uniformly distributed throughout the height of ependyma. The large sized droplets had a tendency to occur at the base of ependyma. The lipid droplets were not observed towards the deeper portion of the hypendyma.

Sudanophilic lipids were localized in the form of fine dust particles (Fig. 5) corresponded to the sites as demonstrated by oil-red-o method during present study. In contrast, a somewhat stronger Sudanophilia was observed at the base of ependyma, moderate in the hypendyma and lesser activity in subependymal glial zone in goat (Kumar *et al.* 1996). Sudanophilic lipids had been reported in ependyma of

domestic animals (Talanti 1958). Ramkrishna and Saigal (1987) observed large lipid droplets along with smaller one in both the ependyma and hypendyma of the buffalo, corresponded to the sites of vacuolations. Sudanophilia might be due to bound lipids like phospholipids or lipoproteins. The lipid bodies containing esterified cholesterol being surrounded by membranous whorls were distributed throughout the SCO in sheep (Barlow *et al.* 1967). Nile blue sulphate method demonstrated distribution of lipids which corresponded as depicted by oil-red-o and Sudan black B methods.

Proteins: The proteinaceous nature of secretions of the SCO towards the ventricular surface was demonstrated by mercury bromphenol blue method (Fig. 6). The surface and contents in the lumen of blood capillaries showed mild activity however, the intercellular spaces of ependymal and the hypendymal cells presented very weak to negligible reaction as reported in goat (Kumar *et al.* 1996) and sheep (Saggar *et al.* 2002). A negligible reaction to performic acid Alcian blue method indicated less than 4% content of cysteine as observed in buffalo (Ramkrishna and Saigal 1987), goat and sheep (Kumar *et al.* 1996, Saggar *et al.* 2002). However, cysteine, tyrosine, tryptophan and arginine had been demonstrated in domestic animals (Talanti 1958, Leonieni 1968). The water stable mucoproteins demonstrated by Bismarck brown method confirmed glycoproteins nature of the secretion in SCO of buffalo (Ramkrishna and Saigal 1987).

REFERENCES

Bargmann W and Schiebler T H. 1952. Histologische und cytochemische untersuch ungen am Subkomissuralorgan Von Saugern. *Z. Zellforsch*. **37**: 583–96.

Barlow R M, D'Agostino A N and Cancilla P A. 1967. A morphological and histochemical study of the subcommissural organ of young and old sheep. *Zeitschrift fur Zellforschung and mikroskopische Anatomie* **77**: 299–315.

Castaneyra-Martin M, Carmona-Calero E M, Perez-Gonzalez H, Martinez-Pena Y, Valenzuela I, Ormazabal-Ramos C, Perez-Garcia C G, Marrero-Gordillo N, Trujillano-Dorado A, Gonzalez-Marrero I and Castaneyra-Perdomo A. 2005. Postnatal development of the secretory activity of the goat subcommissural organ. A Reissner's fibre and angiotensin-II immunohistochemical study. *Anatomia Histologia Embryologia* **34**: 247–51.

Diederen, J.H.B. (1970). The subcommissural organ of *Rana temporaria*. A cytological, cytochemical, cytoenzymological and electron-microscopical study. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **111**: 379–403.

Drury, R A B and Wallington, E A. 1967. *Carlton's Histological Technique*. 4th edn. Oxford University Press, New York.

Gobron S, Monnerie H, Meinel R, Creveaux I, Lehmann W, Lamalle D, Dastugue B and Meinel A. 1996. SCO-spondin: a new member of the thrombospondin family secreted by the subcommissural organ is a candidate in the modulation of neuronal aggregation. *Journal of Cell Science* **109**: 1053–61.

Hoyo-Becerra C, Lopez-Avalos M D, Cifuentes M, Visser R, Fernandez-Llebrez P and Grondona J M. 2010. The subcommissural organ and the development of the posterior commissure in chick embryos. *Cell Tissue Research* **339**: 383–395.

Kumar P, Kumar Pawan and Singh G. 2010. Topography of the subcommissural organ of the pig. *Haryana Veterinarian* **49**: 35–37.

Kumar Pawan, Kumar S and Singh Y. 1996. Histochemical studies on the subcommissural organ of goat. *Indian Journal of Animal Sciences* **66**: 877–80.

Leonieni J. 1968. A contribution to the morphochemistry of the subcommissural organ of young dogs. *Folia Morphologica* **27**: 13–21.

Luna L G. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd edn. McGraw Hill Book Co., New York.

Meiniel A, Molat JL, Meiniel R. 1988 Complex-type glycoproteins synthesized in the subcommissural organ of mammals. Light and electron-microscopic investigations by use of lectins. *Cell Tissue Research* **253**: 383–95.

Meiniel A. 2007. The secretory ependymal cells of the subcommissural organ: which role in hydrocephalus? *International Journal of Biochemistry and Cell Biology* **39**: 463–68.

Murphy G D and Wood J G. 1966. Functional and microscopic studies on the subcommissural organ in the cat. *Texas Report Biology Medicine* **24**: 729–35.

Nesic L and Babic L. 1979. Histophysiological characteristics of the subcommissural organ of ewes over the annual cycle. *Veterinaria Yugoslavia* **28**: 53–63.

Pearse A G E. 1968. *Histochemistry: Theoretical and Applied*. 3rd edn. Vol. I. Churchill Livingstone, London.

Ramkrishna V and Saigal R P. 1987. Carbohydrate, protein and lipid histochemistry of buffalo subcommissural organ. *Indian Journal of Animal Sciences* **57**: 391–97.

Saggar D, Kumar Pawan and Kumar S. 2002. Structural and histochemical study of the subcommissural organ in sheep. *Indian Journal of Animal Sciences* **72**: 1064–67.

Shimizu N. and Kumamoto, T. 1952. Histochemical studies on the glycogen of the mammalian brain. *Anatomical Rec*ord **114**: 479–97.

Sterba G. 1969. Morphologic and Funktion des-subkommissural organs. *Zirkum. ventrikulare organ und Liquor* **5**: 17–27.

Stutinsky F. 1950. Colloide, corps de Herring et substance Gomori-positive de la neurohypophyse. *CR Seances Society Biology Fil* (Paris) **144**: 1357–60.

Talanti S. 1958. Studies on the subcommissural organ in some domestic animals with reference to secretory phenomena. *Annales Medicinae Experimentalis Biological Fenniae* **36**: 1–97.

Wislocki G B and Leduc E H. 1952. The cytology and histochemistry of the subcommissural organ and Reissner's fibre in rodents. *Journal of Comparative Neurology* **97**: 515–44.