RESEARCH ARTICLE

Histochemical Studies on the Different Nuclei of Hypothalamus of Indian Buffalo during Different Reproductive Stages

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Abstract

The study was aimed to elucidate the histochemical moieties in different nuclei in the hypothalamus of Indian buffaloes under different reproductive stages. Tissue samples were collected from three levels, the supraoptic region (anterior region), the tuberal region (middle region), and the mamillary region (posterior region) of the hypothalamus, and processed for routine histochemical studies and cryosectioning. The paraffin sections were stained for carbohydrate and protein localization, while the cryosections were used for lipid demonstration. The neurosecretory substance of the neurons in these nuclei was PAS-positive and mostly clustered along the periphery of the cell. An intense reaction of acid mucopolysaccharides was also present in the peripheral part of the perikaryon and weak nucleus. The neurosecretory materials were noticed in the perikaryon and axon fibers of the paraventricular nucleus. A strongly positive reaction for PAS -Alcian blue was observed in the neurons of all the reproductive phases. The neurons in the arcuate nucleus demonstrated intense PAS reaction in the peripheral part of the neuron during the follicular phase and pregnant buffaloes. The PAS reaction was moderate during the luteal phase and weak in prepubertal buffaloes. The reaction product was granular. Acid mucopolysaccharides (AMPS) were also strongly present in these neurons during the follicular phase and in pregnant animals. PAS reactive neurosecretory granules were observed in the perikaryon of neurons located in the ventromedial nucleus. AMPS were also located in the cytoplasm of these neurons. The reaction was moderate to strong in pubertal buffaloes while moderate in prepubertal buffaloes. Sudanophilic lipid droplets and Oil Red O positive lipids were observed in the neuronal substance of preoptic, supraoptic, and paraventricular nuclei of the buffalo hypothalamus during the pubertal period.

Keywords: Histochemical Studies, Hypothalamus, Indian Buffalo, Nuclei.

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Introduction

One of the most important components of the hypothalamo-hypophyseal-ovarian axis is the hypothalamus, which is a portion of the diencephalon. It is positioned ventral to the thalamus, with the optic chiasma as its anterior border, and the mammillary body is its posterior limit in buffaloes (Pathak and Bansal, 2015). In all reproductive phases of buffalo, well-defined cell clusters or nuclei have been recognized in the coronal and sagittal sections of the hypothalamus (Pathak and Bansal, 2021). Several distinct roles have been attributed to these nuclei. These nuclei have been involved in the control of biological rhythms, appetite, water balance, body temperature, cardiovascular performance, sexual behavior and activity, sleep, muscle tension, and emotion (Singh, 2018). It also acts as a neuro-endocrine organ and secretes hormones that control the functioning of the hypophysis. It is the master endocrine gland because it regulates the activity of the pituitary. Histomorphological studies on the hypothalamus have been performed by Pathak and Bhardwaj (2005) in Gaddi goats and Paramasivan *et al.* (2011) in Sheep; however, meager information is available about the distribution of histochemical moieties in the hypothalamus of buffalo. Keeping in view, the current study was conducted to determine the histochemical moieties in different nuclei in the hypothalamus of Indian buffaloes.

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Materials and Methods

The hypothalamus of buffaloes (n = 52) was collected from and Teaching Veterinary Clinical Complex, GADVASU, Ludhiana, and was utilized for the histochemical studies. The tissue samples were classified into four groups; prepubertal (based on the history and age of the animal), follicular phase (presence of a dominant follicle on the surface of the ovary and absence of corpus luteum), Luteal phase (presence of a fully developed corpus luteum), pregnant (presence of fetus in the uterus). Based on the known history and the

ovarian surface structures, the animals were classified into prepubertal, in the follicular phase, in the luteal phase, and pregnant. The tissue samples were collected from three levels i.e., the supraoptic region (anterior region), the tuberal region (middle region), and the mamillary region (posterior region) of the hypothalamus of buffaloes and were fixed in 10% Neutral Buffered Formalin, Zenker's fluid, and in Bouin's fluid and processed for paraffin sectioning by dehydrating in ascending grades of alcohols and acetone, cleared in benzene, and infiltrated and embedded in paraffin (Pathak and Bansal, 2012).

Histochemical demonstration of carbohydrates (neutral and acid mucopolysaccharides) and basic proteins in different components of the hypothalamus was carried out on the paraffin sections, while total lipids and phospholipids were demonstrated on cryostat sections of unfixed fresh tissue.

The stains used are Periodic Acid Schiff (PAS) for neutral mucopolysaccharides, alcian blue at pH 1 and 2.5 for acid mucopolysaccharides, PAS-alcian blue for neutral and acid mucopolysaccharides, bromophenol blue for basic proteins, Sudan black B for lipids and Oil Red O for lipids following the standard methods in practice.

Results and Discussion

The distribution of carbohydrates (acid and neutral mucopolysaccharides), basic protein and lipids, and phospholipids were observed in different nuclei of the buffalo hypothalamus.

Neutral and Acid Mucopolysaccharides

Supraoptic Region

The neurons in the preoptic nucleus were strongly PAS reactive, and neurosecretory PAS-positive material (magenta colored) in the peripheral part of the neuron was also observed (Fig. 1A). A similar pattern of blue-colored acid mucopolysaccharides was also present in these neurons. The PAS-Alcian blue reaction was weak during buffaloes' prepubertal phase, whereas it was strong during the follicular phase and pregnancy. The PAS reactive granules suggested higher physiological activity in these cells. Stanikova *et al.* (1985) observed increased secretory activity of neurons in the preoptic nucleus with the administration of Pregnant Mare's Serum Gonadotropin (PMSG). The mucopolysaccharides possibly act as ion exchangers, indicating that these substances might play an important role in the supply of Na+ and K+ ions in neuronal tissue.

The neurosecretory substance of the neurons in this supraoptic nucleus was PAS-positive and mostly clustered along the periphery of the cell. An intense reaction of acid mucopolysaccharides was also present in the peripheral part of the perikaryon and weak nucleus (Fig. 1B). Martinez-Rodriguez *et al.* (1976) detected intense PAS-positive reaction in the large-sized neurons while less intense in the smaller neurons of the supraoptic nucleus. Stanikova *et al.* (1985) observed an increased amount of PAS-positive substance in the supraoptic nucleus of gonadotropins treated sheep hypothalamus. Cotea *et al.* (2007) reported ranges of PAS-positive granules across the axons of the supraoptic nucleus.

The neurosecretory materials were noticed in the perikaryon and axon fibers (Fig. 1C) of paraventricular nucleus (PVN). A strongly positive reaction for PAS and Alcian blue was observed in the neurons of all the stages of reproduction. Cotea *et al.* (2007) reported ranges of PAS-positive granules across the axons of the paraventricular nucleus (PVN) in the cow hypothalamus. Soliman *et al.* (2015) recorded the presence of alcian blue positive cytoplasmic granules in the neurons of PVN of rabbits.

Tuberal region

The neurons in the arcuate nucleus demonstrated intense PAS reaction in the peripheral part of the neuron during the follicular phase and pregnant buffaloes (Fig.1D). The PAS reaction was moderate during the luteal phase and weak in prepubertal buffaloes. The reaction product was granular. Acid mucopolysaccharides were also strongly present in these neurons during the follicular and pregnant animals. Similar observations were also recorded by Bhattacharya and Saigal (1987) in the arcuate nucleus of the goat hypothalamus. Stanikova *et al.* (1985) observed increased secretory activity of neurons in the arcuate nucleus with the administration of PMSG.

PAS reactive neurosecretory granules were observed in the perikaryon of neurons located in the ventromedial nucleus. AMPS were also located in the cytoplasm of these

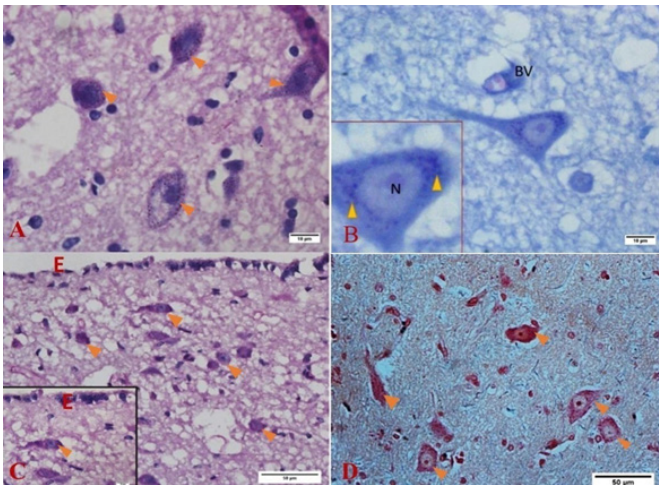

Fig. 1: Section of buffalo hypothalamus showing the presence of **A***. Neutral mucopolysaccharide in the peripheral part of neuron of the preoptic nucleus. **B***. Acid and neutral mucopolysaccharide in the peripheral part of neuron of the supraoptic nucleus. **C*.**PAS-positive reaction in bipolar neurons of PVN (arrow) and ependyma (E), **D*.** Granular PAS-positive substance in the peripheral part of neuron of the arcuate nucleus.

***A,C,D:** PAS, original magnification x400

***B:** PAS-Alcian blue, original magnification x1000

neurons (Fig. 2A). The reaction was moderate to strong in pubertal buffaloes while moderate in prepubertal buffaloes. Stanikova *et al.* (1989) observed an increased amount of PAS-positive substance in the ventromedial nucleus of hormone-treated sheep hypothalamus.

Mammillary Region

Accumulation of NMPS was observed in the cytoplasm of neurons in the lateral mammillary nucleus. NMPS was also observed in great amounts in the wall of blood vessels located within this nucleus (Fig. 2B).

AMPS and NMPS were accumulated in the cytoplasm of neurons (N) and blood vessels in the medial mammillary nucleus (Fig. 2C). The amount of accumulation was more in pubertal animals as compared to the prepubertal buffaloes.

Neutral and acid mucopolysaccharides were present in the cytoplasm and axons of the neuron in the posterior hypothalamic nucleus (Fig. 2D). The reaction was moderate to strong in the pubertal buffaloes, while it was weak in the prepubertal buffaloes. Similar findings were also reported by Arendarcik *et al.* (1983), who recorded the stimulation of neurosecretion and PAS staining by irradiation of the caudal hypothalamic nucleus.

Basic Proteins

A diffuse reaction for basic proteins was observed in the neuronal components of the supraoptic, preoptic, and paraventricular nucleus of buffalo and did not vary in different phases of reproduction buffaloes. The RBCs present in the blood vessels in the respective nuclei demonstrated a strong reaction for basic proteins (Fig. 3A).

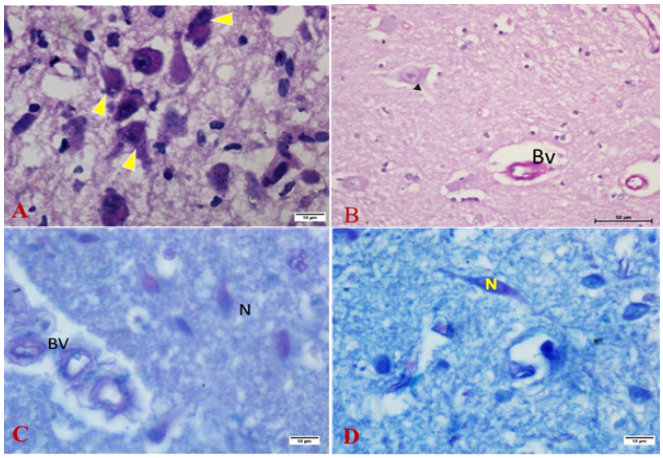

Fig. 2: Section of buffalo hypothalamus showing the presence of **A***. Granular PAS-positive substance in the peripheral part of neuron of the ventromedial nucleus (arrowheads) of pubertal buffalo. **B***. Accumulation of neutral mucopolysaccharide (arrowhead) in the peripheral part of neuron and blood vessels (Bv) of the lateral mammary nucleus. **C*.** Accumulation of AMPS and NMPS in the cytoplasm of neuron (N) and blood vessels in the medial mammillary nucleus. **D*.**Accumulation of AMPS and - A PAS method; original x1000
* A, PAS method; original x 1000
* B, PAS method; original x 400,
*C and D; PAS-alcian blue original x 1000

Neuronal elements of the arcuate and ventromedial nucleus of the tuberal region showed diffuse and weak reactions for basic proteins, but the RBCs present in the blood vessels in the respective nuclei demonstrated strong reactions for basic proteins (Fig. 3B).

Like the supraoptic and tuberal region, the neuronal elements of the posterior hypothalamic nucleus, medial mammillary nucleus, and lateral mammillary nucleus, the reaction for basic proteins was not distinct. The red blood cells present in the blood vessels showed a strong reaction to the basic proteins (Fig. 3C).

Lipids

Sudanophilic lipid droplets, as well as Oil Red O positive lipids, were observed in the neuronal substance of preoptic, supraoptic, and paraventricular nuclei of the buffalo hypothalamus during the pubertal period. The accumulation of lipids was in the form of droplets. The droplets were of varied sizes (Fig. 4). Sudanophilic lipid droplets and Oil Red O positive lipids were observed in the neuronal substance of arcuate and ventromedial nucleus of buffalo hypothalamus during the pubertal period (Fig. 5). Sudanophilic lipid droplets

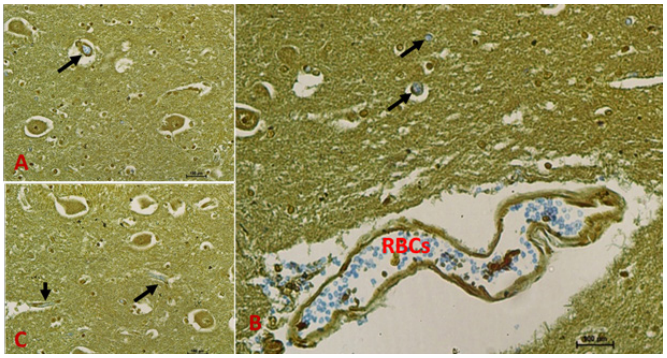

Fig. 3: Section of the hypothalamus of buffalo showing localization of basic proteins; **(A)** in blood vessels (arrow) present in the supraoptic region; (B) in blood vessels (arrows) present in the tuberal region; (C) in blood vessels (arrows) present in the posterior hypothalamic region; Bromophenol blue method x100

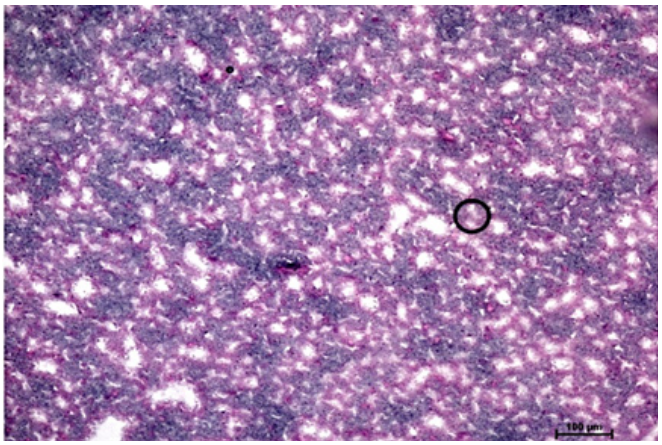

Fig. 4: Section of the hypothalamus of buffalo shows the localization of sudanophilic lipids in the preoptic nucleus—Sudan Black B. x 100.

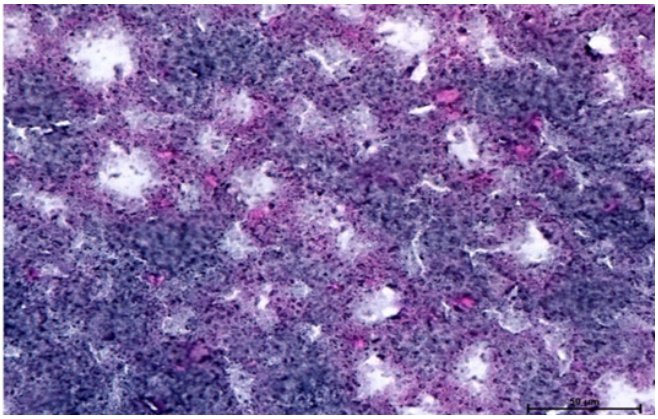

Fig. 5: Section of the hypothalamus of buffalo shows the localization of sudanophilic lipids in granular form in the supraoptic nucleus. Sudan Black Bx 400.

and Oil Red O positive lipids were observed in the neuronal substance of hypothalamic nucleus, medial mammillary nucleus, and lateral mammillary nucleus, the reaction for basic proteins was not distinct.

Conclusion

The neurosecretory substance of the neurons of different nuclei of the hypothalamus was neutral and acid mucopolysaccharides and mostly clustered along the periphery of the cell. A strongly positive reaction for PAS and Alcian blue was observed in the neurons of all the stages of reproduction. The neurons demonstrated intense PAS reaction in the peripheral part of the neuron during the follicular phase and pregnant buffaloes, while the PAS reaction was moderate during the luteal phase and weak prepubertal buffaloes. Sudanophilic lipid droplets and Oil Red O positive lipids were observed in the neuronal substance of preoptic, supraoptic, and paraventricular nuclei of the buffalo hypothalamus during the pubertal period.

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