Expression pattern of histaminergic neurons in the human fetal hypothalamus at second and third trimester

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ABSTRACT

Background: The hypothalamic nuclei constitute that part of the corticodiencephalic mechanism that activates, controls and integrates the peripheral, autonomic mechanisms, endocrine activity and many somatic functions. Their full integration into behavior requires the neocortex, in particular the limbic system. **Purpose:** An antiserum against histamine was used to reveal the location and time of appearance of the amine in developing fetal hypothalamus. Although the expression pattern of histamine is well studied in mammals, less is known about it in humans. The neurotransmitter histamine plays a crucial role in co-ordinating mutiple inputs from various brain centres. **Methods:** In the present investigations the hypothalamus was studied in the human fetus ranging from 19 weeks of gestation (GW) to term by using immunocytochemistry. Distinct neurons that stained with Pischinger's methylene blue were obsereved from 19 GW to term. Sections adjacent to those that contained neurons were stained for histamine labeling. Immunoreactive neurons in the hypothalamus at 19 GW of gestation show relatively meager population. **Results:** Histamine immunoreactive (His-ir) neurons of the hypothalamus were divisible in lateral and ventrolateral subgroups at 19 GW to 24 GW At 32 GW along with the neuronal cell bodies some beaded fibers were visible. There was progressive increase in the histamine expressing neurons as the fetus grows. In term fetal specimens, two to three small groups of his-ir neurons gradually merged in a single large ventrolateral group. **Conclusion:** The findings of the present study provide for a better understanding of the chemoneuroarchitecture of histamine containing neurons in hypothalamus during second and third trimester of human fetal development.

Introduction

The hypothalamic nuclei constitute that part of the corticodi-encephalic mechanism that activates, controls and integrates the peripheral, autonomic mechanisms, endocrine activity and many somatic functions, e.g. a general regulation of water balance, body temperature, sleep, food intake and the development of secondary sex characteristics. However, their full integration into behavior requires the neocortex, in particular the limbic system. In man, the massive development of neocortex and limbic structures means that these have a proportionately much greater influence on hypothalamic functions than in lower animals and hence the areas dealing with the above functions are of major significance. The posterior hypothalamic area receives inputs from diverse multiple neuronal populations and is of considerable importance as it is implicated in modulating hippocampal, autonomic and cortical functions.[1] Histamine is known to occur in significant amounts in the hypothalamus and plays various roles not only as a neurotransmitter but also acts as a local hormone on neurons, glial cells and blood vessels in a concerted manner.[2] The widespread distribution of histamine in the central nervous system is associated with many functions such as fluid balance, eating, thermoregulation and cardiovascular regulation.[3–6]

Histamine containing neurons have been demonstrated in the hypothalamus in adult human brain,[7,8] in Alzheimer's disease brains,[9] in the posterior hypothalamus,[10–14] median eminence, caudal magnocellular nucleus,[15,16] tuberomamillary nucleus of rats,[17,18] and in the posterior hypothalamic nucleus of cats.[19] However, there is very little information on the ontogeny of histaminergic neuronal system of the hypothalamic nuclei during human fetal development. In the present study, we have attempted to determine the appearance of histaminergic neurons in the hypothalamus of developing human brain and to follow the chemocytoarchitectonic changes between second and third trimester until term.

Methods

Sample collection

Fetal specimens for this study were obtained from the Government Medical College, Nagpur. The clearance from the local ethical committee and written consent of the parents were also obtained. Gestational age was determined by measuring the crown rump length.[20,21] Thirty nine fetal specimens were collected and after dissection, brains of only ten human fetuses that had minimal post-mortem changes as apparent with Pischinger's methylene blue staining for Nissl substance were used for the present study. Table I list the specimens, their age and post-mortem time interval, used for histological and immunocytochemical studies. Hypothalamic area was dissected and immersed in cold 4% paraformaldehyde for 48 hrs.

Histology and Immunocytochemistry

Tissues were subjected to several washes of cold phosphate buffered saline during the next 24 h, and transferred to 15% polyvinyl pyrrolidone (K 30) for 24 h (4oC). Ten microns cryosections were cut in coronal plane, fixed in chilled acetone, and stored at -20oC. Sections at regular intervals were stained with Pischinger's methylene blue (PMB) for histological examination. Those sections with neuronal cell bodies were demarcated and near adjacent sections were used for histamine immunolabelling. They were washed in cold PBS, subjected to endogenous peroxidase blocking for 20 minutes in 0.5% hydrogen peroxide

Table I: Age in weeks of gestation (GW), *post mortem* interval, sex and status at birth of human fetal specimens used for histological and immunocytochemical studies on posterior hypothalamic nucleus

S. no.	Age in GW	*Post mortem* time interval in hours and minutes	Sex	Status at birth and cause of death
1.	19	1	F	Abortion.
2.	21	3.20	F	Pre term new born.
3.	22	2	F	Pre term new born.
4.	22	2.10	F	Cardiorespiratory failure.
5.	24	1.15	F	Pre term still born, low birth Weight.
6.	28	6.35	M	Pre term new born.
7.	32	1.25	M	Still born.
8.	33	2.40	F	Cardiorespiratory failure.
9.	36	4	M	Cardiorespiratory failure.
10.	38	5	F	Still born.

in 20% methanol in Tris bufferred saline, pH 7.4. The sections were treated with histamine antibody, (Sigma, U.S.A) at 1:50 dilution for 2 hours at room temperature or overnight at 4°C. Different dilutions such as 1:500, 1:200, 1:100 and 1:50 were tried for antibody reactivity, but best results were observed at 1:50. Goat, anti rabbit Ig G conjugated to biotin was used as the secondary antibody. Sections were treated with streptavidin peroxidase (Sigma, U.S.A) for 45 minutes, followed by 3 - 3' diaminobenzidine tetrahydrochloride (Sigma, USA) in 0.05 M TRIS buffer with 0.01% hydrogen peroxide to obtain a brown immunoreaction product. Slides were dehydrated, coverslipped and photomicrographs obtained using a Zeiss microscope.

Results

Histological studies

Histological studies were carried out on developing hypothalamic nucleus in 10 human fetuses ranging from 19 GW through 38 GW. Neurons were stained with Pischinger's methylene blue. At 19 weeks of gestation hypothalamus is characterized by differentiating structures of the lateral hypothalamic zone, which give rise to the lateral hypothalamus (LH) and posterior hypothalamus. Nissl stained preparation revealed distinctly stained neurons along the lateral zones. At 20, 22 and 24 GW, neurons were anchored along the lateral and ventrolateral margins of the fetal hypothalamus. In 32-36 GW hypothalamus the lateral and ventrolateral subgroups became very conspicuous with several cells showing ovoid, rounded profiles (Fig. 1: A, B, C). At 38 GW hypothalamus, numerous neurons along the lateral and ventrolateral margins were seen. (Fig. 3)

Immunohistochemical studies

Fig. 2: D through I, illustrates histamine positive neurons in the lateral part of the hypothalamus in the second and third trimester human fetuses.

Figure 3 is a schematic representation of the stage wise appearance of histaminergic neurons in the lateral part of the hypothalamus. They were first observed in a small area along
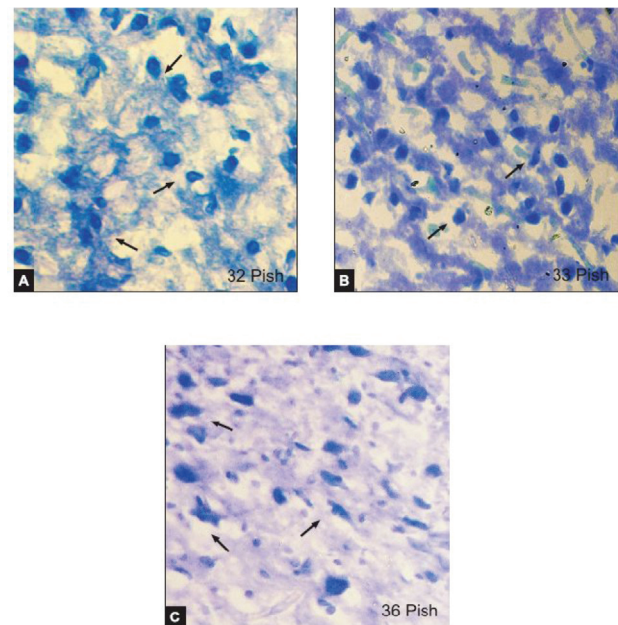

Fig 1: A through C. Histology of developing human fetal hypothalamus. Photomicrographs of coronal sections. A. Distinct aggregation of neurons stained with Pischinger's Methylene blue stain (arrow) 32 GW. B. 33 GW. Neurons (arrow) stained with Pischinger's Methylene blue C. 36 GW. Distinct oval shaped neurons (arrows) stained with Pischinger's Methylene blue can be seen. Bars = 50µm.

the ventrolateral margins of the 19 GW fetal hypothalamus. At 22 GW another group of cells is seen along the lateral margins. At 32 and 38 GW the lateral and ventrolateral groups appeared to merge into a single group. The rise in the population of Histamine-positive neurons was more conspicuous with the increase in gestational age of the fetuses.
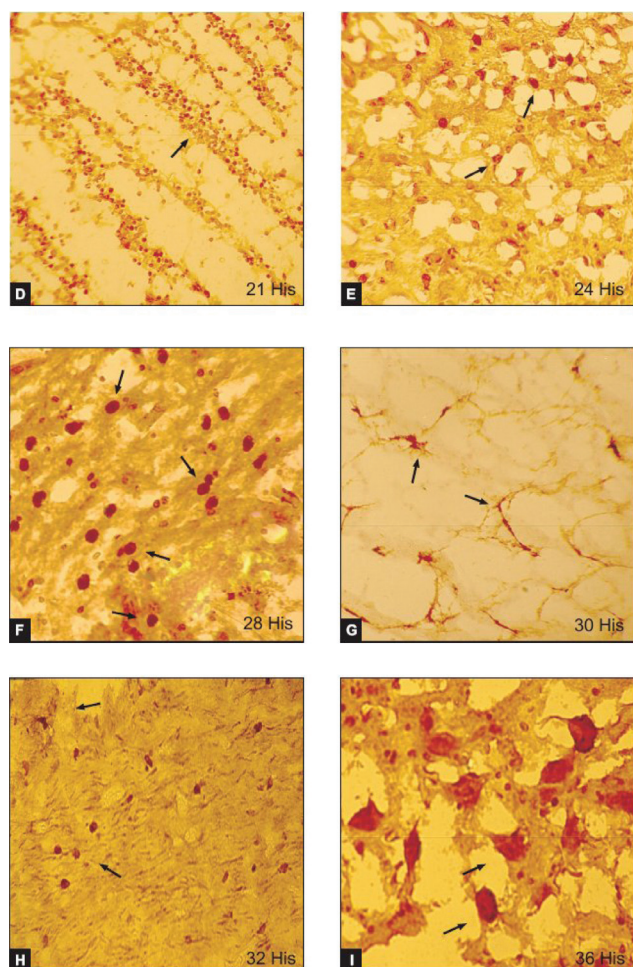
Fig. 2: Histamine immunocytochemistry of human fetal hypothalamus. D. 21 GW. Note the immunoreactive neurons (arrows). E, F, G, 24, 28 30 GW. Diistinctly positive His-ir neurons (thick arrow) are located in the ventrolateral and lateral part of the hypothalamus. H. Note the beaded axonal fibres (thin arrow) at 32 GW along with Rounded to oval neurons (arrow) with reaction product. I. 36 GW. Note the robust staining of neurons (arrow) located along lateral and ventrolateral part of hypothalamus. Scale bars= 50μm.

A few histamine positive cells were seen along the ventrolateral marginal area of the hypothalamus at 19 GW. At this stage histaminergic neurons were in their incipient stage of development at this stage. At 21 GW small rounded to oval shaped histamine labeled neurons were seen in the ventrolateral part of the hypothalamus (Fig. 2: D). In 24 and 28 GW hypothalamus, neuronal cells with reaction product were also seen along the lateral part of the hypothalamus. At 28 GW numerous neuronal cells expressing histamine were seen (Fig. 2: F). The cells were oval, to rounded with beaded axonal fibers. Some cells were spindle shaped with a short thin tail. Numerous thin fibers expressing histamine were seen distributed all along the ventrolateral margins of the hypothalamus. They were more numerous ventrolaterally than laterally (Fig. 2: G). At 32 GW rounded to oval shaped cells were seen dispersed along the lateral and ventrolateral margins (Fig. 2: H). At 36 GW there appears to be a significant increase in staining intensity of
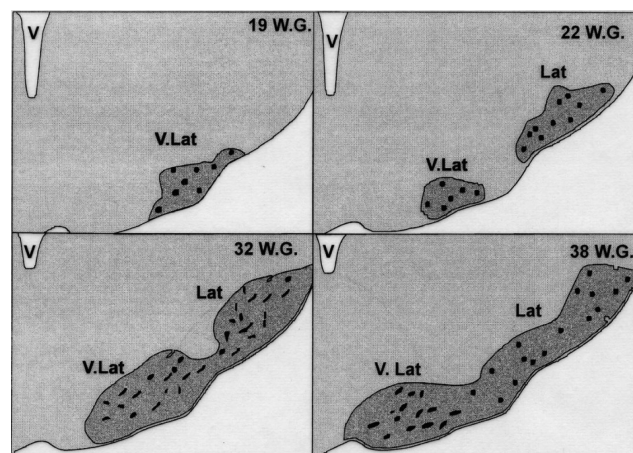

Fig. 3: Schematic representation of cells and fibers immunoreactive for histamine at 19 GW., 22 GW, 32 GW and 38 GW in the developing human fetal hypothalamus. A ventrolateral group appears first followed by a lateral group. The two groups merge into one during the late third timester. V = ventricle, Lat = lateral, V. Lat = ventrolateral group of neurons.

labeled cell bodies. Robust expression of histamine in the labeled cell population was detected all along the ventrolateral part of the hypothalamus (Fig. 2, 1). Seldom, if any, histamine immunoreactive neurons were seen in the midline area of the hyothalamus.

Discussion

Present study revealed the cyto and chemoarchitecture of histaminergic system of some hypothalamic areas during fetal development. The complex cyto and chemoarchitecture of the hypothalamus is the reflection of the diverse functions and connectivity of hypothalamic cell groups. Hypothalamic development is considered to be initiated with cell proliferation in ventricular marginal zone adjacent to the wall of the third ventricle, followed by formation of neuroblasts or postmitotic cell populations external to the germinal layer during the second half of gestation in rat,[23,23,24] in the middle of gestation in cat,[25] and in the first quarter of gestation in the rhesus monkey.[22,26] Few reports on human fetal chemoarchitecture[27,28,29,30] and cytoarchitecture[31,32] suggest early hypothalamic neurogenesis. During development aggregates of compact cell groups termed nuclei or more dispersed populations termed areas, but the mechanism of such aggregation is still largely unclear.[33] In an Australian marsupial, the tammar wallaby (*Macropus eugenii*), the developing hypothalamus mainly consisted of a ventricular marginal zone with a thin marginal layer at twelve days after birth, but by postnatal day twenty five, most hypothalamic nuclei were well differentiated.[34] In the present studies, neurons could be identified histologically at 19 GW in the hypothalamus, probably because humans are at an evolutionarily higher scale as compared to other mammals.

Although organization of histaminergic system has been well documented in adult human hypothalamus,[9] rat posterior hypothalamus and mamillary complex[7,35–37] cat and guinea pigs[15,38,39] relevant information on the developing system in humans is much restricted.

Airaksinen *et al*,[9] have demonstrated histamine neurons in adult human hypothalamus and they have been reported to

occupy a large proportion of the hypothalamus. There is scanty information on the chemoneuroarchitecture of developing human brains. Recent studies have contributed significantly to our understanding of the developmental dynamics of specific neuronal circuits in human fetal brains. Koutcherov et al,[40] reported that the human fetal hypothalamus is differentiated into lateral and posterior hypothalamus, by using imunohistochemical procedure, during the first trimester. They observed that both of these structures were marked by transient immunoreactivity for parvalbumin and strong immunoreactivity for GAP 43, the neuropeptide associated with development and restructuring of axonal connections.[41] They stated that the most significant event in the differentiation of lateral and posterior hypothalamic areas was the appearance of large calretinin and calbindin immunoreactive neurons at 13 and 16 GW respectively and observed that they are a permanent and prominent characteristics of lateral and posterior hypothalamus. Furthermore, it was observed that by 16 GW neurons already exhibited mature morphological appearance.

In the present studies, histamine immunoreactivity was observed in the developing lateral and ventrolateral part of the hypothalamus of human fetal brain. In early gestational weeks (19 GW) histaminergic cells were observed in incipient stage of development. There was an increase in histamine immunoreactive structures with increasing fetal age. The labeled cells exhibited rounded to oval shape. At 36 GW intensely stained histaminergic cells were observed. These cells were oval in shape. However, present studies reported some differences as compared to earlier reports (Smits et al, 1990).[39] They reported that the major groups of histaminergic cells were confined to the nucleus mammillaris medialis and the nucleus caudalis magnocellularis.

Panula et al,[7] found numerous histamine immunoreactive cell bodies in the posterior basal hypothalamus and around the tuberomammilary nucleus of rats.

Panula et al,[35] and Watanabe et al,[36] demonstrated that histaminergic neurons of adult rat are confined to a small region of the posterior hypothalamus and the tuberomammillary nucleus and these neurons have widespread diffuse projection patterns. Wouterlood et al,[37] observed histaminergic neurons by HDC (histamine synthesizing enzyme histidine decarboxylase and stated that HDC-immunoreactive perikarya occur in the posterior hypothalamic area, rostral and lateral to the mammillary complex.

Histaminergic neurons in the posterior hypothalamic region in rat are innervated by fibers arising from neurons in the medial preoptic region (MPO) was examined by using Phaseolus vulgaris leucoagglutinin (PHA-L) combined with immunohistochemistry of histidine decarboxylase as a marker.[11] The PHA-L labeled fibers from all parts of MPO were detected at ipsi and contralateral clusters of histaminergic nurons located in the posterior hypothalamus.

Histaminergic transmission and presence of vesicles in dendrites of histaminergic neurons was examined in mammalian brain.[5] Although some regions receive low density of histaminergic fibers, there was a considerable mismatch between histaminergic fiber density and density of histaminergic receptors in different regions.

Immunohistochemical studies in ground squirrel *Citellus lateralis* reported increase in fiber density during the hibernating

stage, further biochemical analysis revealed that there was an increase in turnover of histamine.

Present studies reported fibers expressing histamine immunoreactivity at 30 GW and 32 GW in the posterior part of the hypothalamus. Earlier to these gestational stages, histaminergic fibers were not observed which suggests increased activity of histamine with increased fiber density. These findings seems comparable with the report described by Sallmen et al, 1999.[42]

Some workers have reported co-localization of histamine with other peptides in various hypothalamic regions. Histidine decarboxylase immunoreactive neurons also contained glutamate decarboxylase in posterior hypothalamus, tuberal magnocellular, the caudal magnocellular nucleus and post mamillary caudal magnocellular nucleus in rat.[10] Histidine decarboxylase and Neuropeptide Y are reported in caudal magnocellular nucleus,[43] histidine decarboxylase and Substance P in posterior hypothalamus[13] and histidine decarboxylase, Substance P and Neuropeptide Y in posterior hypothalamus of rat[14] Histamine, GABA, thyrotropin releasing hormone (TRH), met-enkephalin-arg-phe, and Substance P are observed in the tuberomamillary nucleus of rat, mouse and guinea pig.[8]

Histamine has excitatory effect on the thermosensitive neurons in the anterior hypothalamic preoptic area and posterior hypothalamus.[44] Considering the pivotal role of histamine in neuroendocrine regulation, it may be conjectured that distribution of his-ir perikarya in the developing hypothalamus may hold an important clue to our knowledge of the neuronal circuitry of the hypothalamus. The present investigations have demonstrated sequential development of histaminergic neurons of human fetal hypothalamic area. The data gathered in the present study provides a good relationship of chemoarchitectural organization of this nuclear group to that of the adult and facilitates the establishment of nuclear homologies.

Conclusion

The present study reveals the chemoneuroarchitecture of the histaminergic system in developing hypothalamus in human fetal brain from 19 GW until term. Chemoarchitecture is advantageous in revealing the hypothalamic nuclei during development. His-ir neurons appeared first as a small group along the ventrolateral margins followed by another group along the lateral margin that gradually merged into a single group at term. The use of chemoarchitecture in human development permitted a more confident identification of nuclear organization compared with that afforded by cytoarchitecture. We conclude that the appearance of histaminergic system in the hypothalamus may be important to development during 19 GW.

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