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Original article

Cytoarchitectural organization of the parabrachial/Kölliker-Fuse complex in man

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Abstract

While the parabrachial/Kölliker-Fuse complex has been described in a variety of animal species it has not been characterized in human brainstem. In the present study we investigated fetal and infant brainstems, focusing particularly on the dorsolateral part of the pontine tegmentum, with the aim of defining the precise cytoarchitecture of the medial parabrachial, lateral parabrachial, and Kölliker-Fuse nuclei in man, and analyzing the developmental stages of this complex. In serial sections of 28 human brainstems of subjects aged between 32 gestational weeks and 1 year we made a morphologic and morphometric analysis of the shape and size of the parabrachial/Kölliker-Fuse complex. We observed a homogeneous morphology in all cases, which enabled us to define the structure of the three nuclei. The features of the parabrachial nuclei are largely consistent with those reported in experimental studies. However, the Kölliker-Fuse nucleus appears to be more developed in human beings than in other animal species, showing a greater extension and a more complex structure. The neuronal maturation of these nuclei was seen to occur between the 35th and the 36th gestational weeks.

Keywords: Brainstem; Parabrachial nuclei; Kölliker-Fuse

1. Introduction

The parabrachial and Kölliker-Fuse nuclei have been described in several studies on a variety of animal species as a pontine complex which plays an important role in modulating the respiratory function [1–8]. Morphologically, the parabrachial/Kölliker-Fuse complex (PB/KF) has been described as a group of neurons that surrounds the superior cerebellar peduncle (scp), subdivided into three well defined regions: 1) the medial parabrachial nucleus (mPB), localized ventromedially to the scp; 2) the lateral parabrachial nucleus (lPB), located dorsally to the scp; and 3) the Kölliker-Fuse nucleus (KF), located ventrally to the lPB [9–13].

In humans, the impossibility of using experimental approaches has made it difficult to characterize the architecture and physiology of these structures. Only a few studies have reported morphologic data on the human PB complex, but these observations are imprecise and controversial [14–16].

We needed to define these structures when we began work on a research project on sudden infant death syndrome (SIDS) and sudden intrauterine unexplained death (SIUD) conducted by our Institute.

The SIDS is defined as the sudden death of an infant under 1 year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history [17]. SIUD is defined as late, unexplained fetal death before the complete expulsion of the fetus from the mother [18]. Despite a variety of proposed theories, the etiology of both SIDS and SIUD is still largely unknown. Recent observations have revealed morphologic alterations (hypoplasia and agenesis) of the arcuate nucleus, an important center of the ventral medullary surface which mainly modulates pulmonary ventilation, in over 30% of SIDS and SIUD cases [19–22].

The aim of the present study was to examine serial sections of fetal and infant brainstems in order to define the precise morphology, extension and cytoarchitecture of the human medial parabrachial, lateral parabrachial and

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Kölliker-Fuse nuclei and to evaluate the morphologic development of the PB/KF and the relative neurons from the 32nd gestational week to 12 months of age. Preliminary results have been reported in abstract form [23].

2. Material and methods

We examined 28 brainstems obtained at autopsy from ten stillborns (32–40 gestational weeks) and 18 infants (aged 1–12 months). Five of the stillborns had died of known causes (Potter's syndrome, dilated cardiomyopathy, septicemia, severe chorioamnionitis and umbilical cord torsion, respectively), whereas in five cases a precise cause of death had not been determined. Seven of the infants were SIDS victims and 11 had died of other causes, not related to disease of the central nervous system (Table 1).

Adult brainstems were excluded from the study because of the presence of myelinized fibers, which often obscure the parabrachial structures. Fetuses of only a few weeks, in which the pontine nuclei are not clearly defined, were also excluded.

The brainstems were fixed in 10% phosphate-buffered formalin for 3–4 days, and then were dehydrated with ethanol at increasing concentrations and embedded in paraffin. Transverse serial 5 μm sections were made proceeding in a rostrocaudal direction through the entire pons and mesencephalon (approximately 100–200 sections per case). Of each group of 12 sections, three were stained using alternately hematoxylin–eosin, Bielschowsky and Klüver-Barrera stains, and nine were kept and stained as deemed necessary.

Table 1
Stillborn and infant case profiles

Case no.	Age	Sex	Diagnosis of death
1	32 gw	M	SIUD
2	32 gw	M	Septicemia
3	34 gw	F	SIUD
4	34 gw	M	Dilated cardiomyopathy
5	35 gw	M	Potter's syndrome
6	36 gw	F	Severe chorioamnionitis
7	38 gw	F	SIUD
8	38 gw	M	SIUD
9	39 gw	M	SIUD
10	40 gw	F	Umbilical cord torsion
11	1 month	M	SIDS
12	1 month	F	Necrotizing enterocolitis, peritonitis
13	1 month	M	Hypertrophic cardiomyopathy
14	2 months	F	Pericarditis
15	2 months	M	SIDS
16	4 months	M	SIDS
17	5 months	M	Acute glomerulonephritis
18	6 months	M	Congenital toxoplasmosis
19	6 months	F	Cardiac fibroma
20	7 months	M	SIDS
21	8 months	F	Acute glomerulonephritis
22	8 months	M	SIDS
23	9 months	M	Micronodular cirrhosis
24	10 months	M	SIDS
25	10 months	F	SIDS
26	10 months	M	Hypertrophic cardiomyopathy
27	11 months	M	Bronchopneumonia
28	12 months	F	Extrahepatic biliary atresia

gw, gestational weeks.

2.1. Morphometric analysis

The morphometric study was performed with an image analyzer (Image-Pro Plus, Media Cybernetics, Silver Spring, MD). The profile section areas of all the neurons of the PB/KF with clearly recognizable edges, nucleus and nucleolus, and of the parabrachial surfaces were evaluated in all the serial sections stained with the Klüver-Barrera method.

The mean section areas of the neurons were expressed in μm^2 and the mean areas of the parabrachial structures were expressed in mm^2 (mean ± SD). These mean values have been calculated on the whole cases series and on five groups subdivided according to age.

The statistical significance of direct comparison of the means between groups was determined by one-way analysis of variance. The significant threshold level was set at $P < 0.05$.

3. Results

Homogeneous results were obtained from comparative analysis of the observations of SIUD and SIDS cases and fetal and infant deaths due to other causes. In fact, the PB/KF nuclei and neuron section areas in SIDS and SIUD did not differ from those of age-matched controls that died from other causes. Furthermore, these cases did not show decreased neuron numbers, glial reactions or other signs of hypoxic suffering. Thus, it was possible to define the general morphologic features of the PB/KF complex in man and of the three principal nuclei composing it (the lateral parabrachial, medial parabrachial and Kölliker-Fuse nuclei). The PB/KF structure extends from the rostral dorsolateral region of the pons to the caudal portion of the mesencephalon.

3.1. lPB

In transverse sections this nucleus is located between the lateral surface of the scp and the lateral lemniscus (Fig. 1). It extends rostrocaudally from the level of the pons–mesencephalon junction (cranial pole) to the level where the lateral lemniscus nucleus is clearly visible (caudal pole). The section marking the passage from the pons to the mesencephalon is recognizable because the scp forms a continuous line with their decussation.

The size of the lPB decreases from the cranial (mean area: 20.08 ± 0.35 mm^2) to the caudal pole (mean area:

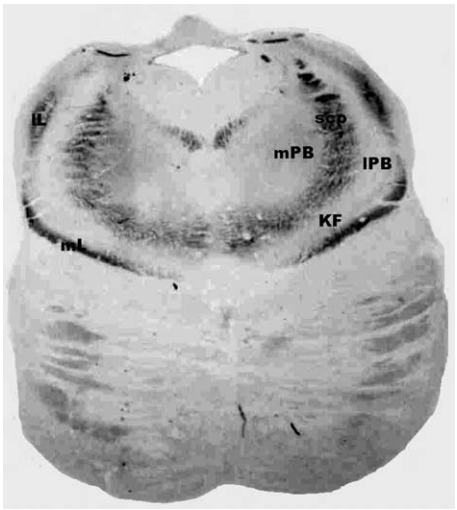

Fig. 1. Case no. 15 (male infant, dead suddenly and unexpectedly at 2 months of life). Photomicrograph of a transverse rostral section of pons. Klüver-Barrera stain; magnification, 5 ×; lL, lateral lemniscus; mL, medial lemniscus; mPB, medial parabrachial nucleus; lPB, lateral parabrachial nucleus.

6.05 ± 0.44 mm^2). The neurons are round or tapering, with a light, often central nucleus, prominent nucleolus, and scarce cytoplasm. Many neurons are dorsoventrally oriented, parallel to the scp axis. The mean area of the lPB neurons was 275 ± 0.36 μm^2.

3.2. mPB

In transverse sections this nucleus lies medially to the scp, running between the motor nucleus nervi trigemini and the locus coeruleus up to the ventral termination of the scp (Fig. 1). Longitudinally, it is constant in size from the rostral pole (pons–mesencephalon junction) to the caudal pole (where the lateral lemniscus nucleus is clearly visible). In fact, the mean areas of the two extremities (rostral and caudal) were 18.01 ± 0.40 and 17.8 ± 0.35 mm^2, respectively. The mPB nucleus contains oval and polygonal neurons, which are usually larger than the lPB neurons and have a darker and more evident cytoplasm. The mean section area of these neurons was 420.29 ± 0.20 μm^2.

3.3. KF

This extends from the caudal pole of the parabrachial nuclei in the rostral pons to the lower portion of the mesencephalon, up to the level where the caudal pole of the red nucleus is visible.

In transverse pontine sections, it appears as a group of large neurons, ventrally located to the lPB, between the medial limit of the scp and the medial lemniscus (Fig. 1). The mean section area of the KF nucleus is 2.55 ± 0.23 mm^2.

The neurons, which are noticeably larger than those of the PB nuclei (mean area: 957 ± 0.40 μm^2), have a large, distinct, eccentric nucleus with a very evident nucleolus,

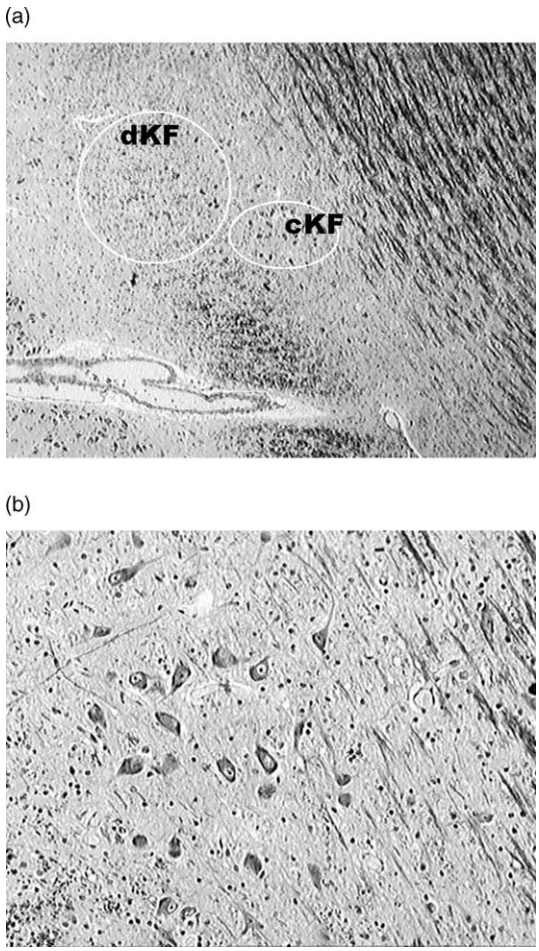

Fig. 2. Case no. 9 (male fetus, dead suddenly and unexpectedly at the 39th week of gestation). (A) Configuration of the KF subdivided into two subnuclei. The encircled area cKF corresponds to subnucleus compactus. The encircled area dKF corresponds to subnucleus dissipatus. Klüver-Barrera stain; magnification, 25 ×. (B) Neurons of the Kölliker-Fuse, subnucleus compactus. Klüver-Barrera stain; magnification, 500 ×.

and abundant cytoplasm with Nissl substance located at the cell periphery.

On the basis of the neuronal arrangement, it is possible at all levels to define two KF subnuclei: the subnucleus compactus, consisting of a cluster of a few neurons, whose outline is sometimes indistinct, and the subnucleus dissipatus, adjacent to the compactus (Fig. 2).

In the more rostral sections obtained at the level of the caudal mesencephalon, the KF, located between the lateral limit of the scp decussation and the medial lemniscus, shows similar cytological features and neuronal distribution.

3.4. Developmental analysis

Table 2 shows the mean section areas of the three nuclei of the PB/KF and of the relative neurons at the different developmental ages.

The mean values did not differ significantly among the groups, except for those related to neuronal cell body areas of the three PB/KF nuclei between the first and second

Table 2
Morphometric developmental analysis of the PB/KF complex

	lPB		mPB		KF	
	Mean area (± SD)	Mean neuronal area (± SD)	Mean area (± SD)	Mean neuronal area (± SD)	Mean area (± SD)	Mean neuronal area (± SD)
Group 1 (cases 1–5), age: 32–35 gw	16.8 ± 0.41	108 ± 0.18*	14.8 ± 0.42	265 ± 0.30*	2.71 ± 0.35	515 ± 0.13*
Group 2 (cases 6–10), age: 32–35 gw	18.9 ± 0.20	280 ± 0.15*	17.01 ± 0.61	418 ± 0.26*	2.99 ± 0.13	899 ± 0.41*
Group 3 (cases 11–16), age: 1–4 months	20.1 ± 0.43	268 ± 0.40	17.9 ± 0.28	425 ± 0.18	2.48 ± 0.42	970 ± 0.28
Group 4 (cases 17–22), age: 5–8 months	21.05 ± 0.25	299 ± 0.32	16.9 ± 0.13	418 ± 0.6	2.13 ± 0.37	1025 ± 0.5
Group 5 (cases 23–28), age: 9–12 months	28.1 ± 0.19	325 ± 0.32	20.3 ± 0.47	516 ± 0.25	2.85 ± 0.15	1079 ± 0.36

gw, gestational weeks. Mean nuclei areas are expressed in mm^2; mean neuronal areas are expressed in μm^2; level of significance, $^*P < 0.05$.

group of fetuses. Marked morphologic differences were also observed between these. In fact, in subjects who died within the 35th gestational week, the neurons have a rounded, apolar aspect with compact chromatin, a not clearly identifiable nucleolus and very scarce cytoplasm. Starting from the 36th week, the neurons generally appear larger, bipolar or polygonal with a large vesicular nucleus, loose chromatin and an evident nucleolus, and they have assumed the specific patterns described above.

4. Discussion

It has been observed in experimental studies that the PB and KF nuclei play an essential role in respiratory modulation [1–8]. Moreover, the KF also has an important function during intrauterine life, inhibiting the response of central and peripheral chemoreceptors (which are already fully formed and potentially functional), and therefore any respiratory reflex. From birth, the KF abruptly reduces its inhibitory effects and becomes active as a respiratory center able to coordinate the pulmonary motor responses to the blood oscillations of pO_2, pCO_2 and pH [24–26].

Experimental studies have clearly defined not only the physiological roles but also the cytoarchitectural organization of the PB/KF complex [9–13]. However, there is still a lack of precise morphologic data regarding this structure in man [14–16].

In the present study, based on examination of fetal and infant brainstems, we define the structure and extension of the three nuclei of the PB/KF complex. We chose subjects whose age ranged from the 32nd week of gestation to 1 year of age. In such subjects the process of myelinization of the nerve fibers is still incomplete [27], so it is easier to examine the PB structures. On the contrary, in adults the relative density of myelinized fibers of the scp and lateral lemniscus often covers the cells, making it difficult to recognize these structures.

The sample includes cases of both SIDS and SIUD. However, the morphology of the PB/KF complex was found to be homogeneous in all cases, even if the morphologic techniques we employed cannot rule out functional abnormalities of these nuclei in fetal and infant sudden unexplained deaths. For this reason, our next step will be to employ specific immunohistochemical techniques to ascertain whether the PB/KF complex harbors homogeneous or heterogeneous populations of nerve cells.

The developmental analysis of the PB/KF complex according to age seems to be of particular interest. In fact, we demonstrate that neuronal maturation, characterized by a significant increase of the neuronal body size and a morphologic differentiation, occurs between the 35th and the 36th gestational weeks.

Our concluding observations on the cytoarchitecture of the PB/KF complex are largely consistent with those of previous experimental studies. By means of electrical stimulation and somatosensory projection studies, different authors [9–13] have described the cytoarchitectural organization of the PB/KF complex in rat, cat and sheep as a collection of neurons, observable in the more rostral coronal sections of the pons, around the scp. From the cytological viewpoint, two major subdivisions, a medial division and a lateral division, have been made. The KF nucleus, situated ventrally to PB nuclei, is characterized by round boundaries of large neurons. While the morphology and extension of the two PB nuclei (lPB and mPB) we define correspond to the findings in experimental studies, we found some morphologic differences in the KF between man and other species. The KF in man consists of an area of cluster neurons (the compactus nucleus) and an adjacent area with scattered neurons (the dissipatus nucleus). Moreover, the human KF complex is also detectable in the caudal mesencephalon.

On the basis of these observations, it can be concluded that the KF appears to be more developed in human beings than in other animal species, and that it shows a more extensive and complex structure, with connections to the mesencephalon.

Further investigations should address an even larger number of cases of both explained and unexplained perinatal deaths. Moreover, we suggest that attention should be paid to another nervous center, the gigantocellular nucleus, extending from the rostral tract of the medulla oblongata to the caudal pons. This nucleus seems to be closely associated with the PB/KF complex in the control of respiratory activity [28].

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