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Analysis of the human locus coeruleus in perinatal and infant sudden unexplained deaths. Possible role of the cigarette smoking in the development of this nucleus

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Abstract

We investigated the immunohistochemical expression of the tyrosine hydroxylase (TH) enzyme and the morphometric parameters of the human locus coeruleus (LC) in the brainstems of 32 subjects aged from 17 gestational weeks to 12 postnatal month, died of unknown (sudden unexplained perinatal and infant deaths) and known causes. The goals of this study were: (1) to obtain basic information about the structure and physiology of the LC during the first phases of human nervous system development; (2) to evaluate whether there is altered expression of TH and/or structural alterations of the LC could be related to maternal cigarette smoking. Morphometric analysis showed homogeneous data in cases of sudden perinatal and infant death and in age-matched controls who had died of known aetiology. However, immunohistochemistry demonstrated in a wide subset of sudden and unexplained deaths a negativity or low positivity of TH. High distribution of TH protein were instead detectable in the LC neurons of foetuses aged 17–18 gestational weeks who had died of known causes. Therefore, we postulate the functional importance of the LC in the early phases of central nervous system development. Besides, the observation of a significant correlation between sudden unexplained death, negativity of TH staining and maternal smoking, prompted us to suppose a close relation between smoking in utero and a decrease of the noradrenergic activity of the LC, leading to sudden death in the last part of pregnancy and in the first year of life. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

The locus coeruleus (LC), a complex of catecholaminergic neurons located in the rostral dorsolateral pons, has been extensively studied in numerous animal species, particularly in the rat [75,45,1,19,78,23]. Projections from this nucleus are responsible for more than half of the noradrenergic connections throughout the brain, including

the neocortex, thalamus, amygdala, hippocampus, hypothalamus, cerebellum, medulla oblongata, and spinal cord [80,72,16,82,40,41].

The LC, known to be the major producer of noradrenaline, subserves several important physiological functions including the sleep–waking cycle [29,14,28] and control of the cardiovascular and respiratory systems [15,55].

The biosynthesis of noradrenaline, like that of other catecholamines (adrenaline and dopamine), occurs by means of the enzyme tyrosine hydroxylase (TH) [4,68,5,12,87,20]. For this reason, TH is a specific marker of catecholaminergic neurons. In rats, using immunohistochemical methods, high

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concentrations of TH have been detected in the LC, present from the early stages of prenatal development [71,73].

To date, the studies on the LC in man that have been reported are prevalently focusing on cell loss and physiological alterations of the noradrenergic system in relation to aging, depression, suicide as well as other psychiatric disorders and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [8,77,3,44,64,39,63]. No specific investigation of the morphological and functional development of the human LC has yet been described in the literature.

In the present study, we investigated the immunohistochemical expression of the TH enzyme and the morphometric parameters of the LC in the brainstems of subjects, aging from 17 gestational weeks to 12 postnatal months, who had died of known and unknown causes. Our first aim was to obtain basic information about the structure and physiology of this nucleus during the first phases of human nervous system development. The second purpose was to evaluate whether there is altered expression of TH and/or structural alterations of the LC in cases of sudden perinatal and infant death, that we have previously shown in various works to be associated with morpho-functional alterations of the cardiorespiratory autonomic nervous system [6,33– 35,46–49].

Finally, the higher incidence of SIDS among children of smoking mothers [10,21,43] and our observation in a recent study of a significantly increased incidence of alterations of the arcuate nucleus in the brainstems of stillborns and of Sudden Infant Death Syndrome (SIDS) victims with smoker mothers compared with victims with nonsmoker mothers [36], prompted us to verify whether maternal cigarette smoking could also be related to morphological and/or physiological developmental abnormalities of the LC.

2. Material and methods

A total of 32 brainstems were studied, from 11 foetuses (17–40 gestational weeks), 4 newborns who had died within the first week of life and 17 infants aged 1–12 months.

The autopsies, performed at least 24 h after death as prescribed by the Italian law (range 24–30 h), included examination of the placental disk, umbilical cord, and membranes in fetuses, and, in all cases, an in-depth histological examination of the cardiorespiratory autonomic nervous system.

A case was classified as Sudden Infant Death Syndrome (SIDS) when death was sudden, completely unexpected, and unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and a review of the clinical history [85].

A case was classified as Sudden Intrauterine Unexplained Death (SIUD) when a fetus after the 25th gestational week died suddenly and unexplainedly before the complete expulsion or retraction of the fetus from the mother, and results in a stillbirth for which there is no explanation despite postmortem examinations [51,37,17].

Similarly, a case was classified as Sudden Neonatal Unexplained Death (SNUD) when a newborn from birth to the end of the 1st postnatal month of life died suddenly and unexplainedly [34].

In 18 cases, the death resulted totally unexplained. A diagnosis of SIUD was established for 4 foetuses, of SNUD for 3 newborns and of SIDS for 11 infants. In the remaining cases, a precise cause of death was established at autopsy.

While taking the medical history, the mother was asked for information about any smoking habit before and during pregnancy. Smoking habit was assigned to two categories (smokers vs. nonsmokers). Women were defined as smokers if they smoked one or more cigarettes a day, and smokers were subdivided in relation to the number of cigarettes smoked daily (1-5: +; 6 or more cigarettes: ++).

Table 1 summarizes the case profiles in this study, with their relative diagnosis and the mother's smoking habit.

After fixing in 10% phosphate-buffered formalin, all brainstems were processed and embedded in paraffin, according to the protocol routinely followed by the Institute of Pathology, University of Milan [51] and available on the web site: http://users.unimi.it/~pathol/sids_e.html. Briefly, transverse serial sections were made through the entire extension of the pons and medulla oblongata: the block was cut at intervals of 30 µm. For each level, twelve 5-µm sections were obtained, two of which were routinely stained for histological examination using alternately hematoxylin-eosin and Klüver-Barrera stains. Two additional sections at each level were subjected to immunohistochemistry for the study of the TH protein and two for glial fibrillary acidic protein (GFAP). The remaining sections were saved and stained as deemed necessary for further investigations.

At the histological examination, the principal nuclei of the brainstem were identified and analyzed, namely the parabrachial/Kölliker–Fuse complex and the locus coeruleus in the pons and the hypoglossus, the dorsal motor vagal, the tractus solitarius, the ambiguus, the inferior olivary, the arcuate nuclei, and the ventrolateral respiratory reticular formation in the medulla oblongata. Plates by Olszewski and Baxter were used for reference [62].

2.1. Morphologic and morphometric analysis of the LC

Serial transverse sections of the rostral pons including the LC were observed under a light microscope to define, in the first step, the morphology of the nucleus and related neurons and its caudal and rostral limits. In these sections, we also defined the main surrounding structures, namely the superior cerebellar peduncle, the mesencephalic trigeminal tractus and nucleus, the lateral lemniscus, the medial lemniscus, the medial longitudinal fascicle, and the parabrachial/Kölliker–Fuse complex

Table 1 Cases of perinatal and infant death

| Case | Age | Sex | Diagnosis | Maternal |
|------|-------|-----|-------------------|----------|
| no. | C | | of death | smoking |
| 1 | 17 gw | F | Abortion | _ |
| 2 | 18 gw | F | Abortion | _ |
| 3 | 25 gw | M | Necrotizing | _ |
| - | 8 | | Chorioamnionitis | |
| 4 | 32 gw | М | Septicaemia | _ |
| 5 | 34 gw | М | Dilated | _ |
| | 6 | | Cardiomyopathy | |
| 6 | 35 gw | М | Potter's syndrome | _ |
| 7 | 36 gw | М | SIUD | + |
| 8 | 38 gw | М | SIUD | ++ |
| 9 | 39 gw | М | SIUD | _ |
| 10 | 40 gw | F | Severe | _ |
| | - | | Chorioamnionitis | |
| 11 | 40 gw | М | SIUD | _ |
| 12 | 1 pd | М | SNUD | + |
| 13 | 2 pd | F | SNUD | _ |
| 14 | 2 pd | М | SNUD | _ |
| 15 | 3 pd | М | Hypertrophic | ++ |
| | - | | Cardiomyopathy | |
| 16 | 1 m | F | SIDS | ++ |
| 17 | 2 m | М | SIDS | ++ |
| 18 | 2 m | М | Hyperacute | + |
| | | | Infarction | |
| 19 | 2 m | М | SIDS | _ |
| 20 | 2 m | F | Pericarditis | _ |
| 21 | 2 m | М | SIDS | ++ |
| 22 | 3 m | F | SIDS | _ |
| 23 | 3 m | М | SIDS | ++ |
| 24 | 3 m | М | Extrahepatic | _ |
| | | | biliary atresia | |
| 25 | 4 m | М | SIDS | + |
| 26 | 4 m | М | SIDS | + |
| 27 | 6 m | М | Congenital | _ |
| | | | Toxoplasmosis | |
| 28 | 6 m | F | Cardiac fibroma | _ |
| 29 | 7 m | М | SIDS | + |
| 30 | 8 m | F | SIDS | _ |
| 31 | 10 m | М | SIDS | ++ |
| 32 | 12 m | F | Extrahepatic | _ |
| | | | biliary atresia | |

gw=gestational week; pd=postnatal day; m=month. SIUD=sudden intrauterine unexplained death; SNUD=sudden neonatal unexplained death; SIDS=sudden infant death syndrome.

+=number of cigarettes smoked per day ≤ 5 ; ++=number of cigarettes smoked per day ≥ 5 .

(lateral parabrachial nucleus, medial parabrachial nucleus, Kölliker–Fuse/nucleus) [33].

The *morphometric analysis* was performed with an Image-Pro Plus Analyzer (Media Cybernetics, Silver Springer, MD) using a simplified method, following the suggestion by Mountjoy and Bondareff [56]. These authors demonstrated comparable mean morphometric values among those obtained from serial sections along the rostrocaudal extent of the LC and those obtained from a single section at the middle level. Therefore, special care was taken to establish the central section of the LC, recognizable by the presence of superior cerebellar peduncles not joined in their decussation and of the

mesencephalic trigeminal tractus and nucleus well evident adjacent to the LC.

The measurements were calculated after plotting the outline of the entire nucleus and cell perimeters, and then digitised and stored in the computer.

The following parameters were evaluated on the right and left side of the LC and were indicated as mean values and standard deviation (S.D.): nucleus area (expressed in mm^2), neuronal density (expressed as number of neurons per mm^2), and neuronal size (cell body area, expressed in μm^2).

2.2. TH immunohistochemistry

For tyrosine hydroxylase (TH) immunostaining, the sections were rinsed three times in 0.1 m Trizma-buffered saline (TBS) followed by a 48-h incubation at 4 °C with a 1:500 dilution of primary rabbit antiserum to TH (Novocastra Laboratories, Newcastle, UK). The dilutions were prepared with a solution of 1% normal goat serum (NGS) and 0.25% Triton X-100 in 0.1 m Tris-saline. This was followed by a 2.5-h incubation with biotinylated goat anti-rabbit immunoglobulin G (IgG; Vector Laboratories, Burlingame, CA, USA) diluted 1:200 with 1% NGS in Tris-saline. The tissue was then incubated for 2 h with the avidin-biotin complex, diluted 1:100 with 1% NGS in Tris-saline (Vector). Between each incubation, the sections were rinsed three times with 1% NGS in Tris-saline. The sections were then treated for 6 min with a 0.05% solution of 3.3' diamino-benzidine and 0.01%hydrogen peroxide, rinsed in phosphate buffer, mounted on gel-coated slides, cleared in xylene, and coverslipped with Depex mounting medium.

For the phenotypic characterization of TH expression, we calculated for each case the mean number of TH-expressing cell bodies in all the immunostained sections along the caudorostral extent of the LC.

We applied a qualitative rating system based on examination of all the immunostained sections along the caudorostral extent of the LC. Briefly, the scoring system of the bilateral LC, was: 0, no TH-positive neuron; +1, a few dark TH-positive cells (<20%); +2, a number of positive cells ranging from 20% to 50%; and +3, a high number of TH-positive neurons (>50%). The scores were assigned by two independent and blinded observers and comparison among the data was performed to evaluate the interobserver reproducibility. In case of discordance among the investigators, the case was reviewed and discussed until the same result was obtained.

2.3. GFAP immunohistochemistry

For astrocytes identification, the sections were stained by the indirect immunoperoxidase method with antibody to glial fibrillar acidic protein (GFAP) (bovine GFAP, Novocastra, Newcastle, Tyne, UK). The GFAP dilution was 1:200; the time of incubation 1 h. Sections were then counterstained with Mayer's hematoxylin. Astrocytes were counted if they displayed a GFAP-positive cytoplasm and counterstained nucleus.

Following the method of Bruce and Becker [7], the target of cell counting in all cases was within the cross-sectional areas of the dorsal vagal nucleus and the tractus solitarius nucleus. The density of astrocytes (mean glial cell number per mm²) was assessed for each case in the areas of both these nuclei with the Image-Pro Plus Analyzer and the same program was employed in the morphometric analysis for the evaluation of the LC neuronal number.

2.4. Statistical analysis

To perform a comparative evaluation of the results, the cases of this study were subdivided in four groups: (I) sudden perinatal deaths (SIUD+SNUD); (II) control perinatal deaths; (III) sudden infant deaths (SIDS); and (IV) control infant deaths. The distribution of morphometric data in the four groups was evaluated by Student's t-test. The different expression of TH among groups and relation between maternal smoking habit and tyrosine hydroxylase expression in cases of perinatal and infant death were evaluated using Chi-square test and Fisher's exact test. The statistical value of the correlation was established at the level of *p*<0.05.

3. Results

The histological examination of the brainstems on serial sections did not disclose morphological alterations in all the nuclei and structures analyzed (parabrachial/Kölliker-Fuse complex, locus coeruleus, hypoglossus nucleus, dorsal motor vagal nucleus, tractus solitarius nucleus, ambiguous nucleus, inferior olivary nucleus, arcuate nucleus, and ventrolateral respiratory reticular formation).

The in-depth morphologic examination of the LC, the main target of this study, performed on serial sections of the rostral pons stained with Klüver-Barrera, showed that this nucleus, located in the dorsolateral pontine tegmentum, has a "column-like" shape in its rostrocaudal extent, and is bilaterally symmetrical. The soma of the neurons are

Table 3

| Distribution | of | tyrosine | hyc | lroxy | lase | in | the | locus | coerul | leus | in | cases | of |
|---------------|------|------------|-----|-------|------|----|-----|-------|--------|------|----|-------|----|
| perinatal and | l in | fant deatl | 1 | | | | | | | | | | |

TH-scoring Groups

| iii seoiiig | Groups | | | | | | | |
|-------------|---|---|-----------|--|--|--|--|--|
| | Ι | II | III | IV | | | | |
| | Sudden perinatal death (SIUD+ SNUD) <i>n</i> =7 | Control perinatal death <i>n</i> =8 | SIDS n=11 | Control infant death <i>n</i> =6 | | | | |
| 0 | 5 | 1 | 7 | _ | | | | |
| +1 | 1 | _ | 4 | _ | | | | |
| +2 | _ | 5 | _ | 1 | | | | |
| +3 | 1 | 2 | _ | 5 | | | | |

TH=tyrosine hydroxylase; SIUD=sudden intrauterine unexplained death; SNUD=sudden neonatal unexplained death; SIDS=sudden infant death syndrome.

TH-scoring: 0=no TH-positive neuron; +1=<20% TH-positive neurons; +2=from 20% to 50% TH-positive neurons; +3=>50% TH-positive neurons.

Level of significance groups I and III vs. II and IV: p<0.05 (Chi-square and Fisher's exact tests).

polygonal or fusiform in coronal sections with a light, eccentric nucleus, evident nucleolus, and abundant cytoplasm. The dendrites are often seen to emerge from the perikarya and to ramify, forming a local plexus.

We subdivided the selected cases of this study into four groups to evaluate if there are morphometric or functional alterations of the LC in the sudden unexplained perinatal and infant deaths compared with control deaths of known aetiology.

Table 2 shows the mean morphometric evaluations related to the LC areas, neuronal cell body areas and neuronal density in the four groups, respectively.

No statistically significant difference of the morphometric parameters was found between the perinatal sudden death and perinatal control groups, and between the infant sudden death and infant control groups (p>0.05). There is a progressive increase of the LC outlines and of the neuronal cell body areas during development, whereas the neuronal density remains steady.

TH-immunohistochemistry showed in the medulla oblongata of all the analyzed cases immunoreactive neurons in the dorsal motor vagal nucleus, in the tractus solitarius nucleus,

Table 2

Morphometric analysis of the locus coeruleus in cases of perinatal and infant death

| Morphometric parameter | Groups | | | | | | | |
|--|--|-------------------------------|--------------------|---|--|--|--|--|
| | Ι | II | III | $\frac{\text{IV}}{\text{Control infant}}$ death <i>n</i> =6 | | | | |
| | Sudden perinatal death (SIUD+SNUD) <i>n</i> =7 | Control perinatal death $n=8$ | SIDS n=11 | | | | | |
| LC area (mm^2) Mean \pm S.D. | 2.79±0.11 | 2.77 ± 0.18 | 4.19±0.34 | 4.23 ± 0.47 | | | | |
| Neuronal area (μm^2) Mean \pm S.D. | 389.28 ± 13.05 | 395.00 ± 16.72 | 583.45 ± 12.89 | 584.50±17.99 | | | | |
| Neuronal density (neuron number/mm ²) Mean±S.D. | 22.14±2.41 | 23.00 ± 3.93 | 23.18 ± 3.31 | 22.83±3.19 | | | | |

LC=locus coeruleus; SIUD=sudden intrauterine unexplained death; SNUD=sudden neonatal unexplained death; SIDS=sudden infant death syndrome. S.D.=standard deviation.



Fig. 1. (A) TH-immunonegative neurons in the locus coeruleus of a Sudden Intrauterine Unexplained Death (SIUD) case (Case no. 9: stillborn male aged 39 gestational weeks). Magnification: $10 \times$. (B) TH-immunopositive neurons in the locus coeruleus of a control foetus (Case no. 10: female dead at 40 gestational week of severe chorioamnionitis). Magnification: $10 \times$.

and in the ventrolateral reticular formation. The percentage of positive neurons ranged from 20% to 50% (score: +2), without any difference between the sudden death victims and controls.

In addition, the astrocyte density, evaluated in the selected areas for GFAP analysis, did not show significant differences, with only slightly greater values in the groups I and III (mean value \pm standard deviation in sudden perinatal deaths: 25 ± 11 ; in SIDS: 28 ± 14) compared with the control groups (group II: 22 ± 7 ; group IV: 24 ± 12).

In the pons, we observed a variable expression of the TH limited to LC. Table 3 represents the density of the TH-expressing cell bodies of the LC in the four groups of our study. We observed a statistically significant difference between the cases of sudden death (groups I and III) and the corresponding age-matched control cases (groups II and IV). In fact, in SIUD, SNUD, and SIDS victims, TH resulted prevalently unexpressed (score: 0) or present only

in a low percentage of LC neurons ($<20\% \rightarrow$ score: +1), whereas in cases of death of known causes, even in foetuses of 17–18 gestational weeks, the LC showed a prevalent TH-positive score corresponding to +2 or +3 (>20%) (Figs. 1–3).

Finally, the different distribution of TH was correlated to the mother's smoking habit, as represented in Table 4. Totally, 19 of the mothers (60%) were nonsmokers and 13 (40%) were smokers from the onset of the pregnancy. Among smokers, 6 smoked \leq 5 cigarettes and 7 smoked more than 5 cigarettes a day. We observed a significant correlation between the sudden death groups, negativity of TH staining and maternal smoking (p<0.05). In fact, 9 of the 13 victims with smoker mothers, even of less than 5 cigarettes a day, showed absence of TH expression (TH-score: 0) in the LC neurons. All these cases were sudden deaths (2 SIUD, 1 SNUD, and 6 SIDS). Conversely, only in 4 of the 19 victims with nonsmoker mothers (1 SIUD, 1 SNUD, 1 SIDS, and 1 foetus that died of



Fig. 2. (A) TH-immunonegative neurons in the locus coeruleus of a Sudden Neonatal Death (SNUD) case (Case no. 13: 2-day-old newborn female). Magnification: $10\times$. (B) TH-immunoreactive neurons in the locus coeruleus of a control case (Case no. 15: 3-day-old male dead of hypertrophic cardiomyopathy). Magnification: $10\times$.



Fig. 3. (A) TH-immunonegative neurons in the locus coeruleus of a SIDS case (Case no. 19: a 2-month-old male). Magnification: $10 \times$. (B) TH-immunoreactive neurons in the locus coeruleus of a control case (Case no. 20: female died at 2 months of pericarditis). Magnification: $10 \times$.

severe chorioamnionitis) we observed negative TH-immunostaining in the LC.

4. Discussion

In this study, we aimed to elicit basic data on the morphologic and functional development of the human LC, so far little investigated. In fact, only a few authors have focused attention on the LC in man, prevalently in the field of neurodegenerative diseases [8,77,3,44,64,39,63].

We firstly defined, by morphometric analysis, the anatomical parameters of this nucleus from the 17th gestational week to the end of the first year of life.

We observed a slight, progressive increase of the LC outlines and of the neuronal cell body areas during development, whereas the neuronal density remained steady. In addition, we obtained homogeneous morphometric data in cases of sudden perinatal and infant death and in age-matched controls who had died of known aetiology.

On the contrary, our previous studies performed on cases of unexplained sudden foetal death (SIUD), neonatal death (SNUD), and infant death (SIDS), thus defined when it was not possible to identify a precise cause of death after a complete autopsy, demonstrated, after our in-depth histological examination of the cardiorespiratory autonomic nervous system, frequent structural alterations of the arcuate nucleus, an important respiratory center of the ventral medullary surface [6,33–35,47–49], frequently associated to hypoplasia of the respiratory reticular formation and, in foetuses, to pulmonary hypoplasia [49]. More recently, while studying the somatostatin distribution in the brainstem, we have also observed functional alterations of the hypoglossus nucleus in more than 50% of cases of sudden perinatal and infant death [34,35].

However, the present study adds new evidence of possible neurochemical abnormalities in these pathologies related to tyrosine hydroxylase (TH), a rate-limiting enzyme in catecholamine biosynthesis. These alterations did not involve the catecholaminergic nuclei of the medulla, as frequently reported in the literature. Several authors in fact [65,59,66] have observed significantly lower TH-immunoreactivity in the tractus solitarius nucleus, dorsal vagal nucleus, and the reticular formation in SIDS victims than in those of the control subjects. We instead,

Table 4

Relation between maternal smoking habit and tyrosine hydroxylase expression in the locus coeruleus in cases of perinatal and infant death

| Death groups | TH-scoring | Maternal smoking habit (number of subjects) | | | |
|--------------------------|------------|--|--------------------|--|--|
| | | Smokers (13) | Nonsmokers (19) | | |
| I Sudden perinatal death | 0 | (3) | (2) | | |
| (SIUD+SNUD) $n=7$ | +1 | _ | (1) | | |
| | +2 | _ | _ | | |
| | +3 | _ | (1) | | |
| II Control perinatal | 0 | _ | (1) | | |
| death $n=8$ | +1 | _ | _ | | |
| | +2 | (1) | (5) | | |
| | +3 | _ | (1) | | |
| III SIDS $n=11$ | 0 | (6) | (1) | | |
| | +1 | (2) | (2) | | |
| | +2 | _ | _ | | |
| | +3 | _ | _ | | |
| IV Control infant | 0 | _ | _ | | |
| death $n=6$ | +1 | _ | _ | | |
| | +2 | (1) | _ | | |
| | +3 | _ | (5) | | |

TH=tyrosine hydroxylase; SIUD=sudden intrauterine unexplained death; SNUD=sudden neonatal unexplained death; SIDS=sudden infant death syndrome.

TH-scoring: 0=no TH-positive neuron; +1=<20% TH-positive neurons+2= from 20% to 50% TH-positive neurons; +3=>50% TH-positive neurons. Level of significance groups I and III vs. II and IV: p<0.05 (Chi-square and Fisher's exact tests).

according to Kopp et al. [32], found the same distribution of TH-positive neurons in these nuclei both in unexplained and in explained deaths. In addition, in relation to gliosis, we did not observe significant differences. Oehmichen et al. [60] showed similar results, whereas in other studies, a significant higher presence of astrocytes in brainstem of victims of SIDS was reported [86,30,58].

We detected physiological defects of the TH limited to the LC, the major producer of noradrenaline, in a wide subset of sudden and unexplained deaths. These cases presented a prevalent negativity or low number of immunopositive neurons for the TH. In contrast, high concentrations of TH protein were already detectable in the LC neurons in foetuses aged 17–18 gestational weeks who had died of known causes.

Based on these results, we postulate a functional importance of the LC in the early phases of central nervous system development. The noradrenaline released by neurons of the LC might be involved in critical periods for brain plasticity and modulate different brain developmental steps.

The causes of damage to the noradrenergic system in the LC remain unclear. The proposed possibilities in studies on experimental field and on several human neurodegenerative disorders, include drugs, pharmacological stimuli, and oxidative stress [81,54,9,25,2,11,27,13,42].

In particular, after induction of oxidative stress in the LC of rats by local infusion of iron, Chen et al. [9] demonstrated an increase of lipid peroxidation, a biological marker of free radicals, as well as a reduction in TH-positive neurons in this nucleus.

High concentrations of oxidants and free radicals are contained in nicotine, the major constituent of cigarette smoke [69,83,70]. The smoke oxidants, in particular, are considered to be absorbed into the systemic circulation, injuring firstly the arterial walls, thus playing an important role in the initiation and progression of atherosclerosis [57,50,52,53], and then causing damage to various organs.

Besides, in case of maternal smoking in pregnancy, carbon monoxide, a gaseous combustion product of nicotine, may readily cross, by passive diffusion, the placenta, where it binds to hemoglobin. Consequently, the carboxyhemoglobin inhibits the release of oxygen into fetal tissues causing hypoxia especially in the most susceptible organs, including the brain.

The effects of prenatal nicotine on brain catecholaminergic system has been demonstrated in experimental studies. In particular, Tolcos et al. [76] showed that even moderate prenatal exposure to carbon monoxide affects cholinergic and catecholaminergic pathways in the foetal brainstem of guinea pig. These results were consistent with those of Storm and Fechter [74] who reported a decrease in the concentration of noradrenaline in the pons and medulla of newborn rats following prenatal carbon monoxide absorption. Wickstrom et al. [84] examined the catecholaminergic system in the LC in newborn mouse prenatally exposed to nicotine. These authors observed a significant reduced levels of TH mRNA and speculated that nicotine absorption in fetuses may affect the ability of the newborn to autoresuscitate during severe hypoxia.

Based on these studies, several works [18,67,24] hypothesized that, in man, the low TH-levels induced by maternal smoking in pregnancy could influence and alter the ventilatory and/or the arousal response to hypoxia in infants, thus leading to SIDS. Moreover, in adult human, it has been reported [31] a statistically significant association between long-term cigarette smoking and low levels of TH in the LC.

The fall in the TH observed in this study in sudden death victims, could be therefore linked to brain hypoxia following prenatal smoke exposure.

We have recently demonstrated a significantly increased incidence of cytoarchitectural alterations of the arcuate nucleus in stillborns and in SIDS victims with smoker mothers compared with victims with nonsmoker mothers [36].

In the present study, we suppose that smoking exposure in pregnancy could also be responsible for noradrenergic abnormalities of the LC. Since the LC is topographically organized with numerous catecholaminergic projections throughout the brain, this implies widespread negative effects of cigarette smoking on many activities of the central nervous system, including cardiorespiratory activity, during development, which may reasonably lead to sudden death in the last part of pregnancy and in the first year of life.

Our observation of a low score of TH-immunoreactivity in the LC of sudden death victims of nonsmoking mothers leads to consider that even other risk factors may contribute to defects of the catecholaminergic system. Since most of the victim's mothers and of infants of this study lived in large industrialized cities, we can hypothesize that, also, atmospheric pollution may have contributed to a wrong physiology of the LC. Furthermore, it should be considered that retrospective assessment of maternal smoking habit, mainly if performed after the fatal event, is sometimes unavoidable [26,79].

In conclusion, we support the hypothesis of various authors [38,22,61] of a close relation between maternal cigarette smoking during pregnancy and abnormal human brain development. Smoking in utero may strongly interfere with brain biology, giving rise not only to the structural developmental abnormalities of the arcuate nucleus we have previously documented [36], but also to a decrease of noradrenergic activity of the LC.

We think that foetal nicotine exposure is one of the possible preventable factors with a strong potential influence on the incidence of stillbirths and infant deaths.

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