Decreased Kainate Receptor Binding in the Arcuate Nucleus of the Sudden Infant Death Syndrome

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Abstract. The human arcuate nucleus is postulated to be homologous to ventral medullary surface cells in animals that participate in ventilatory and blood pressure responses to hypercarbia and asphyxia. Recently, we reported a significant decrease in muscarinic cholinergic receptor binding in the arcuate nucleus in victims of the sudden infant death syndrome compared with control patients that died of acute causes. To test the specificity of the deficit in muscarinic cholinergic binding, we examined kainate binding in the arcuate nucleus in the same database. We assessed $^{3}H$-kainate binding to kainate receptors with tissue receptor autoradiography in 17 brainstem nuclei. Analysis of covariance was used to examine differences in binding by diagnosis, adjusted for postconceptional age (the covariate). Cases were classified as SIDS, 47; acute control, 15; and chronic group with oxygenation disorder, 17. (Acute controls are infants who died suddenly and unexpectedly and in whom a complete autopsy established a cause of death). The arcuate nucleus was the only region in which there was a significant difference in the age-adjusted mean kainate binding between the SIDS group (37±2 fmol/mg tissue) and both the acute controls (77±4 fmol/mg tissue) ($p < 0.0001$) and the chronic group (69±4 fmol/mg tissue) ($p < 0.0001$). There was a positive correlation between the density of muscarinic cholinergic and kainate binding in the SIDS cases only ($R = 0.460; p = 0.003$). The neurotransmitter deficit in the arcuate nucleus in SIDS victims involves more than one receptor type relevant to carbon dioxide and blood pressure responses at the ventral medullary surface.

Key Words: Autoradiography; Glutamate receptors; Hypercarbia; Retrotropezoide nucleus; Sudden infant death; Ventral medulla.

INTRODUCTION

The sudden infant death syndrome (SIDS) is the leading cause of postneonatal infant mortality in the United States; its incidence is about 1/1000 live births. The cause(s) of the syndrome is unknown. SIDS is defined as the sudden death of an infant under one year of age that remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history (1). The most significant advance in SIDS research is the discovery of the relationship between prone sleeping position and the increased risk for sudden death: in countries with national campaigns for supine sleeping position, the SIDS rate has fallen by as much as one-half (2, 3). The relationship of the prone position to increased risk for SIDS is unexplained, but raises questions about the role of airway obstruction or asphyxial rebreathing (4–6), and failure of protective reflexes (7–9) in the pathogenesis of sudden death.

We hypothesize that SIDS, or a subgroup of SIDS, is due to developmental abnormalities of the ventral medulla that interfere with protective cardiorespiratory responses to potentially life threatening, but frequent, events during sleep, such as hypoxia, hypercarbia, and apnea. In support of this hypothesis, we recently reported a significant decrease in muscarinic cholinergic receptor (mACHR) binding in the arcuate nucleus of the medulla in SIDS victims compared with autopsy controls that were acutely ill (10). The human arcuate nucleus is composed of ventral medullary surface neurons that are considered homologous to neurons located in similar areas in cats that are involved in protective responses to hypercarbia and asphyxia (11–21). We postulated that the mACHR binding deficit in the arcuate nucleus of SIDS infants contributes to a failure of arousal responses to hypercarbia or asphyxia during sleep, particularly since acetylcholine has been implicated experimentally in mediating the ventilatory response to carbon dioxide (CO$_2$) via mACHRs at the ventral medullary surface (12, 18, 20, 22).

The question arises: is the decrease in receptor binding in the arcuate nucleus in SIDS infants specific to the mACHR, or is the deficiency of mACHR binding part of a generalized neuronal defect in which other neurotransmitter receptors are affected as well? To test the specificity of the deficiency in mACHR binding, we examined in
the present study binding to the kainate receptor (a subtype of the glutamate receptor) in the arcuate nucleus of SIDS and control infants. We chose the kainate receptor because developmental studies indicated that its binding is heavily concentrated in the human infant arcuate nucleus (23), and that it is therefore an excellent neurotransmitter marker of this region. Other neurotransmitter receptors examined, i.e., N-methyl-D-aspartate, nicotinic cholinergic, and opioid, have no or negligible binding to the human infant arcuate nucleus (24, 25). Moreover, direct application of glutamate agonists to the ventral medullary surface in experimental animals stimulates ventilation, whereas application of antagonists depresses ventilation and results in apnea (22, 26–30). Application of kainate to the cat ventral medullary surface dramatically decreases the response to hypercapnia, suggesting that kainate receptors accessible to topical application are involved in mediating responses to CO2 (22, 26, 27, 29). The arcuate nucleus is the only neuronal population at the very surface of the human ventral medulla that possesses kainate receptors (25). The idea that there is a defect in CO2 responsiveness in SIDS victims is supported by physiologic data that indicate that infants at risk for SIDS who also have repetitive apnea and subsequently die of SIDS demonstrate alveolar hypoventilation and decreased CO2 responsiveness during sleep (7).

We used tissue receptor autoradiography to test the hypothesis that the binding of [3H]kainate to kainate receptors is decreased in the arcuate nucleus in SIDS victims. We used brainstem sections from essentially the same population of SIDS victims and controls as was used in the mAChR study in order to assess the correlation between the two neurotransmitter systems.

MATERIALS AND METHODS

Definition of Sudden Infant Death Syndrome (SIDS) and Control Groups

We classified cases into 3 categories: (a) SIDS cases; (b) acute controls; and (c) chronic cases. SIDS was defined as above (1). The acute controls were infants who died suddenly and unexpectedly and in whom a complete autopsy established a cause of death. The chronic group was composed of infants with a history of chronic or repetitive hypoxemia from cardiac, pulmonary, or central breathing disorders. The chronic group with oxygenation or breathing disorders was included in the study in order to address the possibility that the putative kainate receptor binding deficit in the arcuate nucleus in SIDS infants is not specific, but rather reflects an effect of hypoxia-ischemia. To insure uniform classification of deaths, a pediatrician (FM) and pediatric pathologists (MVD and HFK) who were blinded to all neurotransmitter binding data reviewed the clinicopathologic data recorded from the sudden deaths and classified each case as SIDS or acute control.

Tissue Receptor Autoradiography

The [3H]-KA binding procedure used in the present study for tissue receptor autoradiography was based upon methods developed in experimental animals (31) and applied in human postmortem pediatric brain tissue by us (23–25). Tritiated kainate is known to bind with high affinity to the glutamate receptor subtypes GluR5–GluR7, kainate (KA)1, and KA2 subtypes, in contrast to low affinity to GluR1–GluR4 subtypes (32). Unfixed, frozen, slide-mounted sections were preincubated in 50 mM Tris-HCl buffer, pH 7.4, at room temperature for 30 minutes (min) to remove endogenous ligand. To determine total binding, a sample of sections was incubated with 4 nM [3H]-kainate (58 Ci/mmol, New England Nuclear) in 50 mM Tris-HCl, pH 7.4, for 1 hour (h) at 4°C. To determine nonspecific binding, an adjacent subset of sections was incubated with 4 nM [3H]-kainate and 100 uM kainate and 50 mM Tris-HCl at 4°C. The sections were washed 4 times for 15 seconds each time in Tris-HCl buffer at 4°C, and then one time for 10 seconds in water at 4°C. They were then dried under a gentle stream of anhydrous air. Sections were exposed to [3H]-sensitive film (LKB Ultrasfilm–[3H] for 24 weeks. Each cassette included a set of [3H]-standards (Amersham) for conversion of optical densities to specific activities of ligand bound to tissue in femtomoles/milligram tissue (fmol/mg tissue).

Quantitative densitometry of autoradiographs was performed with an MCID imaging system (Imaging Research Inc., Ontario). Optical densities were converted to specific activities in fmol/mg tissue with [3H]standards. The autoradiograms of the tissue sections incubated for nonspecific binding were indistinguishable from regions of the film that did not overlie the sections; moreover, there were no regional differences in specific activities within the sections. Therefore, the average specific binding for sections incubated for nonspecific binding was subtracted from each pixel in the total binding sections. Receptor density was determined in specific nuclei directly upon the specific activity section displayed on the color monitor. Reference to the cell-stained tissue sections that generated the autoradiograms was made in the determination of the nuclear boundaries when necessary. A standard atlas (33) was used as reference. In a study of kainate receptor binding in the developing human brainstem (23), we found that the anatomic boundaries of the arcuate nucleus were well-defined by the binding, and that there was a high correlation between the anatomy and autoradiography (Fig. 1). Therefore, we used the pattern of receptor binding in the arcuate nucleus as the indicator of its anatomic boundaries.

Specific activity measurements were made in a blinded fashion, without knowledge of clinical diagnosis or postconceptual age. Two sections from 4 precisely defined levels were analyzed for each nucleus. Measurements were made in the arcuate nucleus at 6 levels of the medulla. The arcuate neurons are small, round, or multipolar, with eccentric nuclei, prominent nucleoli, and indistinct Nissl substance. They occur in clusters of 3 or more cells in a fine fibrillary neuropil close to the surface of the lateral and ventrolateral medulla, and in a larger collection surrounding the ventromedian sulcus. Commonly, arcuate neurons are found dorsal to the amiculum of the inferior olive, and adjacent to the nucleus reticularis lateralis ventralis.

or nucleus paragigantocellularis lateralis, but ventral to the anterior margin of the spinocerebellar tract, inferior cerebellar peduncle, and spinal tract of the fifth cranial nerve. These correspond closely to cells along the ventrolateral medulla in the cat, which have been associated with the respiratory chemosensitive fields (16). In humans there are abundant arcuate neurons ventral and ventromedial to the inferior olive, contiguous with the larger cells of the n. raphe pallidus. Both the ventral and more laterally placed arcuate neurons demonstrate 3H-kainate binding to the kainate receptor, and both locations were used to measure 3H-kainate binding. The 4 levels containing the 17 nuclei of interest are defined, with their atlas plate numbers (33) in parentheses: (a) mid-medulla, level of n. Roller (Plate XII), for measurements of hypoglossal nucleus, principal inferior olive, n. tractus solitarius, and n. centrales medullae oblongata; (b) rostral medulla, level of n. praepositus (Plate XIV), for measurements of n. gigantocellularis, n. paragigantocellularis lateralis, and n. raphe obscurus; (c) rostral pons, level of n. parabrachialis lateralis (Plate XXVIII), for measurements of n. parabrachialis lateralis, n. pontis oralis, basis pontis, and locus coeruleus; and (d) caudal midbrain, level of the decussation of the superior cerebellar peduncle (Plate XXXII), for measurements of n. cuneiformis, periambulad gyr, n. raphe dorsalis, inferior colliculus, and interpeduncular nucleus.

Statistical Analysis

For the clinical and autopsy database, group characteristics were compared using the Student’s t-test or the Wilcoxon test for continuous variables, and Fisher’s Exact Test for categorical variables. Analysis of covariance (ANCOVA) was used to examine differences in kainate binding by diagnosis, adjusted for postconceptional age (the covariate) (10). The adjustment uses the estimated slope for age to shift all observations to the mean age of the sample, and then examines the resulting SIDS vs control mean difference that no longer reflects the effects of varying ages in the dataset. Postmortem interval was not used as an additional covariate because it did not differ significantly by diagnosis, and it was not significantly correlated with 3H-kainate binding in this data set. (The mean postmortem intervals [±1 standard deviation] were: SIDS, 13.4±7.3 h; acute controls, 11.6±6.5 h, and chronic group, 11.4±7.4 h [p = 0.5135]). When the relationship between age and binding was similar for the 3 groups, age-adjusted mean binding levels were estimated for the 3 groups using the mean age of the entire sample (34). When the ANCOVA p-value for case diagnosis was less than 0.05, signifying differences in mean binding across the 3 groups, pairwise comparisons of the age-adjusted means were conducted. The correlation between the density of kainate and mACHr binding was assessed using simple linear regression.

RESULTS

Clinical and Autopsy Information

Brainstems from a total of 79 cases were analyzed: 47 SIDS, 15 acute controls, and 17 infants in the chronic group. Causes of death in the acute control group were: acute respiratory infection, 3; myocarditis, 2; enteritis, 1; drowning, 1; accidental asphyxia due to a plastic bag, 1; asymptomatic maple syrup urine disease and sudden death, 1; palliated congenital heart disease with acute intraoperative death, 1; asymptomatic congenital heart disease and sudden death, 2; mild psychomotor retardation, thalamic damage, and sudden death, 1; noncyanotic chronic lung disease with sudden death from pneumonia, 1; and arthrogryposis multiplex congenita and sudden death, 1. The causes of death in the chronic group were: congenital cardiac and/or pulmonary malformations, 10; primary breathing abnormality, 3; severe perinatal asphyxia, 2; apnea of infancy, 1; and Werdnig-Hoffmann disease with chronic respiratory compromise, 1.

Comparison of the SIDS and acute control groups showed no significant differences between mean gestational age, birth weight, and Apgar scores, or between rates of complications of pregnancy, labor, or postnatal course, oxygen use in the delivery room, history of maternal cigarette smoking during pregnancy, history of a life-threatening event, or cerebral edema. All of the SIDS deaths were temporally associated with sleep, compared with 73% (11/15) of the acute group (p = 0.003). Intra-thoracic petechiae were found in 96% (43/46) of the SIDS group, compared with 73% (11/15) of the acute group (p = 0.030). There was also a significant difference in sleeping position (p = 0.018), with 86% (24/28) of the SIDS infants sleeping prone compared with 33% (2/6) of the acute group; however, data regarding sleep position were not recorded in the charts for almost half of the SIDS group and for 60% of the acute group.

Tissue Receptor Autoradiography

We assessed 3H-kainate binding to kainate receptors with quantitative autoradiography (Fig. 2). We compared mean kainate binding of the 3 groups in each of 17 nuclei (Table 1). The arcuate nucleus was the only region in which there was a significant difference in age-adjusted mean kainate binding between the SIDS group (37±2 fmol/mg tissue) and both the acute controls (77±4 fmol/mg) (p < 0.0001) and the chronic group with oxygenation disorders (69±4 fmol/mg) (p < 0.0001) (Table 1) (Fig. 3). Mean binding of the acute controls versus chronic group was not significantly different.

The association between kainate and mACHr binding densities was examined in the cases where both measurements were available (Fig. 4). (The mACHr binding data have been published previously [10].) There was a positive correlation between the density of kainate and mACHr binding in the SIDS cases (R = 0.460; p = 0.003) (Fig. 3), but not in the acute controls (R = 0.368, p = 0.216) or chronic group (R = 0.217, p = 0.42). We also compared the clinicopathologic variables between the SIDS group with low (i.e. below average) kainate and mACHr binding (n = 16) and the SIDS group with high (i.e. above average) kainate and mACHr binding (n = 11) to determine if there were any features that distinguished the "low binding" group as a distinct subset of...
Fig. 1. (A) Autoradiogram of a representative level of the medulla. The arcuate nucleus (ARC) (arrows) is located along the ventral surface, superficial to the pyramid (PYR). Tritiated kainate binding is high in the ARC and principal inferior olive (PIO), and colocalizes to the anatomic boundaries of the nuclei. (B) Histological section of the medulla in (A) stained with hematoxylin and eosin; the section is the same one that generated the autoradiogram. The anatomic boundaries of the arcuate nucleus are outlined with arrows. (C) High-power view of arcuate nucleus in tissue section in (B), ×40; arrows point to illustrative neurons.

the SIDS group. The cut-off values that we selected were the mean binding values in the arcuate nucleus in the SIDS group for kainate binding (37 fmol/mg tissue) and for mAChR binding (110 fmol/mg tissue). The only significant feature that distinguished the "low" and "high" SIDS groups from each other was postnatal age at death, with a mean ± S.D. of 15.5 ± 7.4 weeks for the "low" group and 10.5 ± 2.7 weeks for the "high" group (p = 0.044). Comparison of the "low" and "high" group showed no significant differences between mean gestational age, birth weight, and Apgar scores, or between rates of complications of pregnancy, labor, or postnatal
course, oxygen use in the delivery room, maternal cigarette smoking during pregnancy, history of a life-threatening event, or cerebral edema.

We observed developmental changes in 3H-kainate binding in the 17 brainstem nuclei. In all but 4 nuclei, kainate receptor binding decreased significantly with increasing age over the first few postnatal months in the SIDS and control groups, ranging from a decrease of 0.2 fmol/mg tissue/week (n. centralis) to 1.5 fmol/mg tissue/week (basis pontis). The 4 nuclei without a significant age effect were the arcuate nucleus, n. tractus solitarii, n. raphe obscurus, and interpudendal nuclei.

**DISCUSSION**

In this study, we observed a significant decrease in 3H-kainate binding to kainate receptors in the arcuate nucleus

<p>| TABLE 1 | Age-adjusted Kainate Binding (fmol/mg Tissue), Mean ± S.E.M. (n) for 17 Nuclei |
|---------|----------------------------------|------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Site</th>
<th>SIDS</th>
<th>Acute</th>
<th>Chronic</th>
<th>3 group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcuate n.</td>
<td>37 ± 2 (45)</td>
<td>77 ± 4 (14)</td>
<td>69 ± 4 (17)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Basis pontis</td>
<td>95 ± 5 (40)</td>
<td>86 ± 8 (15)</td>
<td>84 ± 9 (13)</td>
<td>0.418</td>
</tr>
<tr>
<td>Inferior olive</td>
<td>40 ± 2 (45)</td>
<td>43 ± 3 (15)</td>
<td>46 ± 3 (17)</td>
<td>0.251</td>
</tr>
<tr>
<td>Hypoglossal n.</td>
<td>22 ± 1 (42)</td>
<td>22 ± 2 (13)</td>
<td>23 ± 2 (16)</td>
<td>0.873</td>
</tr>
<tr>
<td>n. Tractus solitarii</td>
<td>28 ± 1 (43)</td>
<td>27 ± 2 (15)</td>
<td>31 ± 2 (17)</td>
<td>0.296</td>
</tr>
<tr>
<td>n. Centrals</td>
<td>16 ± 1 (45)</td>
<td>18 ± 1 (15)</td>
<td>18 ± 1 (17)</td>
<td>0.281</td>
</tr>
<tr>
<td>n. Gigantocellularis*</td>
<td>16 ± 1 (44)</td>
<td>18 ± 1 (15)</td>
<td>—</td>
<td>0.134</td>
</tr>
<tr>
<td>n. Paragigantocellularis*</td>
<td>16 ± 1 (44)</td>
<td>17 ± 1 (14)</td>
<td>—</td>
<td>0.385</td>
</tr>
<tr>
<td>n. Raphe obscurus</td>
<td>15 ± 1 (39)</td>
<td>15 ± 1 (12)</td>
<td>16 ± 2 (9)</td>
<td>0.688</td>
</tr>
<tr>
<td>n. Parabrachialis lateralis</td>
<td>18 ± 1 (39)</td>
<td>19 ± 1 (13)</td>
<td>18 ± 1 (13)</td>
<td>0.773</td>
</tr>
<tr>
<td>n. Pontis oralis</td>
<td>22 ± 1 (39)</td>
<td>23 ± 2 (13)</td>
<td>20 ± 2 (13)</td>
<td>0.762</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>21 ± 1 (39)</td>
<td>23 ± 2 (12)</td>
<td>24 ± 2 (13)</td>
<td>0.239</td>
</tr>
<tr>
<td>Periaqueductal gray</td>
<td>26 ± 2 (22)</td>
<td>28 ± 2 (13)</td>
<td>24 ± 2 (10)</td>
<td>0.362</td>
</tr>
<tr>
<td>IPN</td>
<td>29 ± 2 (22)</td>
<td>30 ± 2 (14)</td>
<td>32 ± 3 (9)</td>
<td>0.676</td>
</tr>
<tr>
<td>n. Cuneiformis</td>
<td>19 ± 2 (24)</td>
<td>24 ± 2 (14)</td>
<td>21 ± 3 (10)</td>
<td>0.178</td>
</tr>
<tr>
<td>n. Raphe dorsalis</td>
<td>26 ± 2 (24)</td>
<td>29 ± 2 (14)</td>
<td>25 ± 2 (10)</td>
<td>0.374</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>22 ± 2 (21)</td>
<td>28 ± 2 (14)</td>
<td>22 ± 3 (9)</td>
<td>0.114</td>
</tr>
</tbody>
</table>

* In the arcuate nucleus, SIDS cases differ from acute controls (p < 0.0001) and the chronic group (p < 0.0001). Mean binding of the acute control and chronic group does not differ significantly (p = 0.178). The raw means ± standard error of the mean are: SIDS, 37 ± 2 fmol/mg tissue; acute, 76 ± 5 fmol/mg tissue; chronic, 72 ± 4 fmol/mg. * Age-adjusted means are based on a submodel that excludes the chronic group due to a significant interaction between group and age in the nucleus gigantocellularis and nucleus paragigantocellularis (kainate binding of the chronic group in these two nuclei decreased with age at a greater rate than in the SIDS and acute groups). The p-value shown is from the SIDS versus acute submodel. Abbreviations: S.E.M., standard error of mean; n., nucleus; IPN, interpudendal nucleus.
in a group of SIDS infants compared with infants dying acutely of known causes and to infants with chronic oxygenation or breathing abnormalities. Given that the arcuate nucleus is a region of the human ventral medullary surface that is putatively involved in chemosensitivity to CO₂ (16), and that the kainate receptor at the ventral medullary surface is involved in CO₂ responsiveness in experimental animals, we speculate that the kainate binding deficit in the arcuate nucleus of SIDS infants contributes to a failure of responses to cardiorespiratory challenges (e.g. hypercapnia from rebreathing) during sleep and sudden death.

There are several points that distinguish the results of the kainate binding study from previous work demonstrating a mAChR binding deficit in the arcuate nucleus in SIDS victims. The present study indicates that the neurotransmitter deficit in the arcuate nucleus of SIDS infants is not specific to the mAChR (10), but involves the kainate receptor as well. This study presents a significant correlation between kainate and mAChR binding. Since both kainate receptors and mAChRs at the ventral medullary surface are thought to be directly involved in CO₂ responsiveness, the dual defect is likely to be disastrous in the face of hypercapnia or asphyxia. The mean decrease in binding for SIDS relative to acute control infants is greater for the kainate receptor (52%) than the mAChR (27%), and there is less overlap in kainate binding than in mAChR binding between SIDS and acute control cases (10). Moreover, in the mAChR binding study, there was no statistically significant difference in the mean binding in the arcuate nucleus between the SIDS and chronic groups, and there were 2 other nuclei in addition to the arcuate nucleus, whereas mean mAChR binding was significantly lower in the chronic group compared with the acute group (10). This suggested that mAChR binding in the chronically ill infant was lowered nonspecifically by hypoxia-ischemia or related factors, whereas it was low only in the arcuate nucleus in the SIDS victim (10). In the current kainate receptor study, mean binding in the arcuate nucleus was significantly different between the SIDS and both the acute control and chronic groups. Thus, the kainate data support the interpretation of a specific underlying deficit in the arcuate nucleus of SIDS victims.

The finding of decreased kainate binding in the arcuate nucleus in SIDS victims needs to be considered in the broader context of neural systems regulating autonomic and respiratory control. Further clarification of the hypothesis that a dysfunctional arcuate nucleus is involved in defective cardioventilatory control and sudden infant
death requires a greater understanding of the functional role of the arcuate nucleus. Because the key physiological experiments are not directly feasible in the human brainstem, a comparative anatomic strategy is essential. Based upon cytoarchitectonic criteria, we postulate that the arcuate nucleus is part of the ventral medulla and is homologous to neurons located in similar areas in cats that are involved in protective responses to hypercapnia and asphyxia (16).

By “ventral medulla,” we mean neuronal populations along the ventral and ventrolateral rim of the medulla that function in a coordinated way to affect chemoreception to carbon dioxide, provide tonic influence to prevent apnea, and modulate respiratory and cardiovascular responses. Operationally, we limit the definition of the “ventral medulla” in experimental animals to the retrotapezoid nucleus, caudal raphe, parapyramidal neurons, juxtafacial parts of the nucleus paragigantocellularis lateralis, and chemoreceptor regions along the rostral and caudal surfaces. Historically, central chemoreceptors were considered to be located at or within a few hundred micrometers of the ventral medullary surface. The direct application of acidic or basic artificial cerebrospinal fluid to specific regions of the surface of the ventrolateral medulla using small cotton pledgets or a superfusion apparatus delineated a rostral chemoreceptive area, referred to as Mitchell’s (M) area, and a caudal chemosensitive region, referred to as Loescheke’s (L) area (35, 36). An intermediate area (IMA), lying between the M and L areas and referred to as Schlaefke’s (S) area, was also described (37), and was thought to be integrative and not chemosensitive. The surface application studies, however, were criticized for not accurately delineating the precise location of central chemoreceptors because substances applied to the ventrolateral medullary surface can be transported more deeply by the numerous blood vessels that penetrate the ventral surface (38). Recently, Nattie and coworkers developed a method to produce a focal region of acidosis in the brainstem that results in a respiratory system response (39, 40): in vivo microinjections of acetazolamide, a carbonic anhydrase inhibitor, into putative chemoreceptive sites brought about large increases in phrenic nerve activity. Nattie and others have identified the following chemosensitive sites in the brainstem: (a) sites within 800 um of the surface at sites corresponding to the L, M, and IMA areas (41); (b) caudal raphe (42, 43); (c) retrotapezoid nucleus (41); (d) sites near the nucleus of the solitary tract (41, 44); (e) sites in the vicinity of the locus coerules (41, 45); and (f) sites in the vicinity of the ventral respiratory group (40).

The functional anatomy of the ventral medulla is poorly characterized in humans. The arcuate nucleus along the ventral medullary surface in humans is postulated on the basis of cytoarchitectonic criteria to be homologous to widespread surface chemoreceptor regions in experimental animals (16), and is potentially part of the caudal raphe, retrotapezoid nucleus, and parapyramidal neurons. In experimental animals, neurons in the surface chemoreceptor regions have connections with catecholaminergic neurons of the lateral reticular formation of the medulla (46), premotor neurons of the pterygopalatine ganglia (47), nucleus of the solitary tract (48), ventral respiratory group (49), intermediolateral cell column, dorsal horn, phrenic nucleus, and parabrachial nuclei (50). The neurotransmitters of neurons in the surface chemoreceptor regions are not completely known, although acetylcholine and glutamate have been suggested as key neurotransmitters (18). The experimental application or microinjection of many neuroactive agents (cholinergic agonists, glutamate, kainate, thyrotropin-releasing hormone, and substance P) to the ventral medullary surface stimulate ventilation, whereas muscarinic and glutamate antagonists, opiates, and GABA inhibit it (18).

The chemical anatomy of the human arcuate nucleus is largely unexplored, particularly the neurotransmitter-specificity of the cell bodies. Muscarinic cholinergic (51), kainate (23), and serotonergic (52) receptors are heavily concentrated in this region in human infants. It is unknown, however, if the receptors are pre- or postsynaptic, or if they are restricted to neurons and not glia. Historically, the arcuate nucleus was thought to project to the cerebellum via the external arcuate fibers (53). In a recent study, however, DiI, a lipophilic dye which labels cells and cell processes by lateral diffusion along cell membranes, applied to 23 paraformaldehyde-fixed human fetal brainstems at 19 to 22 postconceptional weeks, demonstrated DiI diffusion from the arcuate nucleus to labeled fibers and cell bodies in the medullary raphe and the external arcuate fibers (54). Diffusion from the medullary raphe labeled the nucleus of the solitary tract, reticular formation, medullary raphe, and arcuate nucleus (54). DiI diffusion from the pyramid and basis pontis (negative control) labeled the corticospinal tract, with no labeling of the medullary raphe or arcuate nucleus. The results suggest the existence of cellular connections between the arcuate nucleus and the caudal raphe. It is noteworthy that the human midline arcuate nucleus is cytologically homologous to the small cells located at the ventral aspect of the cat raphe pallidus (16). A role for the arcuate nucleus in human ventilatory control is supported by its absence in an autopsied infant with congenital central hypoventilation syndrome (55), and in two SIDS victims (Filiano and Kinney, 1992). The idea that the ventral medullary surface populations are involved in the response to hypercapnia in humans is supported by functional magnetic resonance imaging in adults exposed to a hypercapnic challenge in which carbon dioxide responsiveness is localized to the region of the arcuate nucleus, as well as other regions (57).
We propose that the maturational regulation of the arcuate nucleus is abnormal in SIDS brains, such that there is a developmental deficiency of neurons and/or neuropil with an associated deficiency of neurotransmitter binding. The finding of severe hypoplasia in some SIDS cases (56, 58) indicates a failure in the proliferation and/or migration of neuroblasts destined to become arcuate neurons. Cell counts are needed to determine if there is a subtle deficiency of neuron number in a larger group of SIDS cases. On the other hand, in comparing baseline developmental studies of kainate (23) and mAChR binding (51) to the SIDS data, the SIDS values for mean binding in the arcuate nucleus for both kainate receptors and mAChRs are low and more comparable to values in the fetal and perinatal, rather than the infant, range. This observation suggests that there may be a lag in neurotransmitter maturation, regardless of a deficiency of neuron number; an underlying common signal of neurotransmitter maturation may be at fault.

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