Alpha₂ Receptor Binding in the Medulla Oblongata in the Sudden Infant Death Syndrome

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Abstract. The sudden infant death syndrome (SIDS) is the leading cause of postnatal infant mortality in the United States. Its etiology remains unknown. We propose that SIDS, or a subset of SIDS, is due to a failure of autoretusatrication, a protective brainstem response to asphyxia or hypoxia, in a vulnerable infant during a critical developmental period. Gasping is an important component of autoretusatrication that is thought to be mediated by the "gasping center" in the lateral tegmentum of the medulla, a region homologous in its cytoarchitecture and chemical anatomy to the intermediate reticular zone (IRZ) in the human. Since we found that [³H]para-aminoclonidine ([³H]PAC) binding to alpha₂-adrenergic receptors localizes to this region in human infants and, thereby, provides a neurochemical marker for it, we tested the hypothesis that [³H]PAC binding to alpha₂-adrenergic receptors is decreased in the IRZ in SIDS victims. Using quantitative tissue autoradiography with [³H]PAC as the radioligand and phentolamine as the displacer, we analyzed alpha₂-receptor binding density in the IRZ, as well as in 7 additional sites for comparison, in 10 SIDS and 10 control medullae. There were no significant differences in alpha₂ receptor binding in the IRZ, vagal nuclei, or other medullary sites examined between SIDS and control cases. These results suggest that the putative gasping defect in the IRZ in SIDS victims is not related to [³H]PAC binding to alpha₂-adrenergic receptors.

Key Words: Autoretusatrication; Intermediate reticular zone; Receptor autoradiography; Respiration; Sudden infant death; Vagal nuclei.

INTRODUCTION

The sudden infant death syndrome (SIDS) remains the leading cause of postnatal infant mortality in the United States, with an overall incidence of 0.8/1,000 live births, despite the reduction in incidence since the 1994 national recommendation for the supine sleeping position. SIDS is defined as the sudden, unexpected death of an infant under 1 yr of age that remains unexplained by a complete autopsy and death scene investigation (1). Our over-riding hypothesis is that SIDS, or a subset of SIDS, is due to a failure of protective brainstem responses to asphyxia, hypoxia, and/or hypercapnia in a vulnerable infant during a critical developmental period. Gasping is a protective brainstem response to hypoxia, which emerges as an autoretusatricative mechanism to ensure survival (2–5). During an hypoxic episode, hyperpnea is initially observed in challenged mammals, followed by primary apnea. Within a variable period of time, gasping emerges as a final respiratory mechanism to restore eupnea. If oxygen does not become available, secondary or terminal apnea ensues, leading to death (2, 4, 5). The mechanism(s) underlying the transition from primary apnea to the initiation of gasping respiratory efforts is currently unknown.

Gasping in response to apnea has been observed as an autoretusatricative mechanism in many species, including human infants and other mammals, and its control has been localized to the intermediate reticular zone (IRZ) in the rat and cat (4, 5). Huang et al (6) demonstrated that the human intermediate reticular zone is heavily innervated by catecholaminergic neurons and contains the A1 and C1 catecholamine neurons in its ventrolateral portion. In the adult rat, Fung et al (7) observed axonal connections between the IRZ and nucleus ambiguus, hypoglossal and facial motor nuclei, as well as to the contralateral IRZ. Such connections suggest the involvement of a highly coordinated neural mechanism involving the upper airway in the regulation of gasping, as the nucleus ambiguous provides major efferent motor innervation to the laryngeal and pharyngeal muscles, the hypoglossal nucleus innervates the tongue, and facial neurons innervate muscles in the region of the alae nari. The neurons in the IRZ of experimental animals project to the ventral surface as well, suggesting that central chemosensitivity may be involved in its function (7).

A putative defect in gasping has been postulated to underlie the pathophysiology of SIDS (3). In a detailed analysis of death scene investigations involving SIDS cases, Thach et al demonstrated that certain factors, specifically soft infant bedding (e.g., beanbags), can lead to sudden death in infants when they rebreathe expired gases in the face-down position (prone), and become hypoxic and apneic (8). This apnea initiates gasping, and any dysfunction in this elicitation potentially could be fatal. More recently, recordings of infants with a history of apparent-life-threatening events (ALTE) who subsequently die while being monitored indicate gasping as part of...
In this study, we examined responses to life-threatening challenges (9, 10, 13, 15). Chemosensitivity is a neuronal cell population along the ventral and ventrolateral rim of the medulla that are involved in respiratory drive, chemoreception, and blood pressure responses. We include the following nuclei as components of the ventral medulla: intermediate reticular zone (IRZ), caudal raphe complex, parapyramidal neurons, n. paragigantocellularis lateralis, and rostral and caudal chemosensitive surface areas. We postulate that these rostral and caudal chemosensitive surface areas identified in various animal models are homologous, at least in part, to the human arcuate nucleus (14). In previous investigations, we reported isolated deficiencies of muscarinic and kainate receptor binding in the arcuate nucleus of SIDS victims compared with controls (9, 10). In a recent study by Panigrahy et al (13), deficient serotonergic receptor binding was observed in SIDS cases in the IRZ (“gasing center”), n. gigantocellularis, n. paragigantocellularis, n. raphe obscurus, principal inferior olive as well as the arcuate nucleus, using [3H]-lysergic acid diethylamide ([3H]-LSD) as the radioligand. All of these findings lend support to the idea that, at least in a subset of SIDS victims, there may be a primary defect in chemosensitivity, respiratory drive, and/or blood pressure responses to life-threatening challenges (9, 10, 13, 15).

In this study, we examined α2- adrenergic receptor binding with [3H]PAC in SIDS and control infant brainstems to test the specific hypothesis that α2-adrenergic binding is decreased in the IRZ of SIDS victims. We purposely chose [3H]PAC because it is a broad radioligand that binds to all three human α2-adrenergic receptor subtypes (α2A, α2B, and α2C).

**MATERIALS AND METHODS**

**Tissue Preparation and Storage**

The cases analyzed in this study are part of a database collected between 1985 and 1995 for comparing brainstem chemical anatomy between SIDS victims and controls (9–13, 16, 17). Alternate brainstem sections from the same SIDS and control cases have been analyzed for muscarinic (9), kainic (10), nicotinic (11), opioid (12), and serotonergic (13) binding. Acute controls included those infants who died suddenly and unexpectedly as a result of a brief illness and in whom a complete autopsy established the cause of death. Chronic controls included autopsyed infants who died after a lengthy illness, involving repetitive hypoxic episodes as a result of cardiopulmonary or neurological disorders. Brainstems were obtained from individuals with postmortem intervals ≤24 h in accordance with the guidelines of the Clinical Protection Committee. Our procedures for tissue preparation and sampling have been described in detail (16). Brainstems were stored frozen at –70°C and later cryostat-sectioned at 20 µm in thickness prior to radioligand binding experiments. The ages of all individuals in this study are expressed in postconceptional (gestational plus postnatal) weeks.

**Binding Studies and Generation of Brainstem Autoradiograms**

The [3H]PAC binding protocol used in this study was based upon methods developed in experimental animals (18, 19), and was used in the developing human brainstem (20). Unfixed, slide-mounted sections were preincubated in 50 mM NaH2PO4, (pH 7.4) and 10 mM (ethylenedinitrilo)-tetraacetic acid (EDTA) for 20 min at room temperature and then in 170 mM Tris-HCl (pH 7.6) and 10 mM MgCl2 for 10 min at room temperature. To determine total binding, sections were incubated with 1.0 nM [3H]PAC (58.2 Ci/mmol, New England Nuclear, Boston, MA) in 170 mM Tris-HCl (pH 7.6) and 10 mM MgCl2 for 1 h at room temperature. To determine nonspecific binding, a subset of sections was placed in the incubation buffer described above for 1 h at room temperature with the addition of 10 µM phentolamine as a displacer. Our procedures for washing and drying sections after incubation and generating autoradiograms have been described in detail (16, 17). Sections were exposed to [3H]sensitive film (LKB Ultrasprint-3 H, Sweden) for 12 wk. Each cassette included a set of [3H]standards (Amersham, Princeton, NJ) for calibration and conversion of optical densities to specific activities expressed in femtomoles of ligand bound per milligram of tissue.

**Quantitative Analysis of Brainstem Autoradiograms**

For the quantitative comparison of the 10 SIDS and 10 control cases, 2 sections from 2 medullary levels from each case were analyzed for 8 nuclei. In order to define the anatomical boundaries of brainstem nuclei, the sections were stained with cresyl violet or hematoxylin and eosin and compared to their corresponding autoradiograms. The 2 anatomic levels were defined using the atlas of Olszewski and Baxter (21), as a reference and are listed with their atlas plate numbers in parentheses: 1) mid-medulla, level of n. Roller (Plate XII), for measurements of the hypoglossal n., principal inferior olive, medial accessory olive, dorsal accessory olive, n. of the solitary tract (NTS), dorsal motor n. of X, and intermediate reticular zone; 2) rostral medulla, level of n. praepositus (Plate XVI), for measurements of the n. paragigantocellularis lateralis and intermediate reticular zone. The anatomical boundaries of the intermediate reticular zone were defined using the human brainstem atlas of Paxinos and Huang (22). Quantitative densitometry of autoradiograms was performed with a MCID imaging system (Imaging Research Inc., Ontario). Optical densities were converted to specific activities expressed in fmol/mg of tissue using [3H]standards.

**Statistical Analysis**

For each of the 8 medullary brainstem nuclei sampled, analysis of covariance (ANCOVA) was used to examine differences in α2 receptor binding between SIDS and control cases, adjusted for postconceptional age (Table 1). Statistical analysis of differences in selected clinicopathologic variables between SIDS and control groups was carried out using Fisher’s exact test for
results

Clinicopathologic Data

Twenty brainstems (10 SIDS, 10 controls) were analyzed: “acute” and “chronic” cases were combined as 1 control group since no statistical difference in α2 receptor binding was found between them (data not shown). The causes of death in the acute control group (n = 4) included pneumonia (2), acute laryngotracheobronchitis (1), and myocarditis (1). The causes of death in the chronic group (n = 6) included congenital heart disease (3), pulmonary hypertension (1), brain hypoxia (1), and arthrogryposis multiplex congenita (1). Postmortem interval (PMI) was not significantly different between the 2 groups (data not shown).

Alpha-2 Receptor Autoradiography

We examined α2-adrenergic receptor binding with quantitative autoradiography using [3H]PAC in 10 SIDS and 10 control infant brainstems. Alpha-2 receptor binding was highest overall in the DMX (Table 1, SIDS, age-adjusted mean ± standard error, 69.04 ± 7.50 fmol/mg tissue; controls, 65.29 ± 7.50 fmol/mg tissue; p = 0.730), and the relative pattern of binding across nuclei was similar, although not identical, between the SIDS and control groups. Mean [3H]PAC binding values between the SIDS and control groups were essentially the same for all nuclei examined (p > 0.30), including the IRZ (SIDS, 19.59 ± 4.03 fmol/mg tissue; controls, 25.10 ± 4.03 fmol/mg tissue; p = 0.350) (Fig. 1A–C). Binding in the arcuate nucleus was not detectable in either SIDS or control brainstems.

Discussion

This study using [3H]PAC does not demonstrate a significant alteration in α2 receptor binding in the IRZ in SIDS compared with control cases. Moreover, there were no differences in other medullary nuclei sampled, including the vagal nuclei (NTS, DMX). The pattern of [3H]PAC binding in the controls was essentially the same as that observed by us in a baseline developmental study (20). Of note, α2-receptor binding was not detected in the arcuate nucleus. A caveat of this analysis, and in all human postmortem studies, is that the autopsy controls do not necessarily represent the normal population and inherently do not comprise a homogeneous group. Most non-SIDS infants who die at this age suffer from illnesses with a protracted clinical course (e.g., congenital heart disease). There was no significant difference in binding levels between the acute and chronic controls in any of the nuclei analyzed in the present study. Furthermore, these controls are part of a larger database in which there were no differences in receptor binding between acute and chronic controls of any neurotransmitter we have studied thus far (9–13, 16, 17). For this reason, we combined the acute and chronic cases as 1 total control group. One caveat to keep in mind is that the lack of difference in [3H]PAC binding between SIDS and control cases does not rule out subtle differences in α2 subtype receptor binding; the use of more selective α2 receptor ligands is necessary for delineating the distribution of these receptor subtypes in regions such as the IRZ.

SIDS and Catecholaminergic Neurotransmission

Several studies of brainstem abnormalities in the catecholaminergic system in SIDS victims have been reported (23–27) (Table 2). Most of these abnormalities are reported in cardiorespiratory nuclei, notably the vagal nuclei and reticular formation of the ventrolateral medulla (Table 2). Takashima et al (25) described a delay in catecholaminergic dendritic development (i.e. increased spine density) in the ventrolateral medulla and vagal nuclei of SIDS victims compared with controls. They suggested a pattern of abnormal or slowed neuronal maturation in these infants, with a failure to “prune back” spine density with maturation. Kopp et al (26) reported an absence of phenylethanolamine-N-methyltransferase activity, the synthetic enzyme for epinephrine, in the nucleus gelatinosus of the NTS in SIDS victims compared with controls. They speculated that a deficiency of epinephrine in the NTS could underlie abnormal cardioventilatory reflex integration in the NTS in SIDS victims. More recently, Obonai et al (27) found decreased levels...
of immunoreactivity to tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, in the DMX and area reticularis superficialis in the brainstems of SIDS victims compared with controls. Since the vagal nuclei exert an important role in cardiorespiratory function and have been shown to have relatively high levels of $\alpha_2$ receptors during early human development (20), we evaluated the possibility of any differences in $\alpha_2$ receptor binding between SIDS and controls in cardiorespiratory nuclei, including the IRZ, NTS, and DMX, and found the current negative results. While other investigators have measured levels of catecholaminergic enzymes and spine density in the vagal nuclei and found differences between SIDS and controls, we found no differences in $\alpha_2$ receptor binding using $[^3\text{H}]$PAC. The reasons for the discrepancy are unknown, but may reflect compensatory changes in receptor number and/or affinity in response to a decrease in neurotransmitter level or altered spine density. While our study does not support the idea that the defect in SIDS is related to an altered level of $\alpha_2$ receptor number or affinity, the possibility remains that an intrinsic defect in this receptor or 1 of its subtypes is involved in the pathophysiology of SIDS. The use of other techniques, such as in situ hybridization to receptor subtypes or assays of $\alpha_2$-adrenergic signal transduction, is needed to further examine the role of catecholamines in SIDS and especially in the IRZ.

Neurotransmitters and Gasping

We selected $[^3\text{H}]$PAC because it is a reliable marker of the IRZ, as determined by us in our developmental study of the human fetal and infant brainstem (20). The complex and heterogeneous nature of the neurochemical and morphological characteristics of IRZ neurons are well recognized in both the rat and human (6, 28), and accounts for the identification of the “gasping center” as a zone rather than a nucleus. We are not aware of any physiologic correlates to the known anatomic localization of catecholaminergic neurons (6) and/or $\alpha_2$-receptor binding to this region. There are only a few studies that implicate specific neurotransmitters in the modulation of gasping. Gozal et al (29) have shown that the pharmacological inhibition of nitric oxide synthase prolonged gasping duration and reduced gasping frequency in the postnatal anoxic rat, suggesting a role for nitric oxide in the modulation of gasping in developing rats. Fewell et al (30) demonstrated that perinatal exposure to nicotine impaired the ability of newborn rats to autoresuscitate when challenged with repeated hypoxia. A recent study by Panigrahy et al (13) also showed decreased serotonergic binding in SIDS victims in the IRZ and the arcuate nucleus as well as several other medullary nuclei involved in the control of respiration. These results support the hypothesis of a putative gasping defect in SIDS and suggest some interrelationship between chemosensory (arcuate

Fig. 1. Scatter plots showing $[^3\text{H}]$PAC binding values in the (A) IRZ, (B) DMX, and (C) NTS.
nucleus) and gasping deficits in the etiology of SIDS. Of note, neurotransmitters involved in the function of gasping may also be maturationally regulated. This concept is of special relevance to the study of SIDS, where a putative gasping defect would presumably be related to specific developmental, neurochemical or physiologic characteristics in the IRZ. Gozal et al (31) showed that in newborn rats (<25 days old), gasping exhibits a triphasic pattern with phase I (period of strong and frequent inspiratory efforts preceded and followed by expiration), phase II (period of relative respiratory silence with interspersed phase I gasps), and phase III (period of frequent inspiratory efforts without expiration) occurring sequentially during autoresuscitation. They further demonstrated that this phasic pattern disappears in older rats (≥25 days old) and is replaced with a monophasic gasping pattern of shorter duration and increased frequency. In a subsequent experiment by Gozal et al (32), intraperitoneal administration of MK801 (N-methyl-D-aspartate receptor channel antagonist) in newborn rats abolished type I gasps in 2 to 5-day-old, but not 10 to 15-day-old rats, suggesting that gasping is regulated by very specific patterns of age-dependent neurotransmitter function and interactions.

Conclusions

In conclusion, our results suggest that the putative gasping defect in the IRZ in SIDS victims is not related to \(^{3}H\)PAC binding to \(\alpha_2\)-adrenergic receptors. This study further contributes to the series of experiments carried out in the past 2 decades to examine the relationship between catecholamines and SIDS.

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