FMRI Responses to Hyperoxia in Congenital Central Hypoventilation Syndrome

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ABSTRACT

Congenital Central Hypoventilation Syndrome (CCHS) patients show partial retention of peripheral chemoreception despite impaired ventilatory responses to CO₂ and hypoxia. The condition allows examination of central responses to hyperoxia, which minimizes afferent traffic from peripheral chemoreceptors. We used functional magnetic resonance imaging to assess blood oxygen level-dependent signals over the brain during a baseline and subsequent 2-min hyperoxia (100% O₂) period in 14 CCHS and 15 control subjects. After partitioning gray matter and correcting for global effects, the images were analyzed using volume-of-interest time trends followed by repeated-measures ANOVA and conventional cluster analyses. Respiratory rates initially (first 20 s) fell in CCHS, but rose in control subjects; CCHS heart rate increased in the first minute, and then decreased in the second minute, as in controls, but with muted rise and extent of decline. Multiple sites within the cerebellum, midbrain, and pons responded similarly to the challenge in both groups. Response patterns differed early in the right amygdala, paralleling initial respiratory pattern deficits, and late in the right insula, concomitant with cardiac rate differences. Signals also differed between groups in the medial and anterior cingulate, hippocampus, head of caudate, and lentiform nuclei, as well as pontine and

midbrain structures and regions within the superior temporal and inferior frontal cortical gyri. The findings emphasize that structures that can alter respiratory timing, such as the amygdala, and modulate sympathetic outflow, such as the right insula, are deficient in CCHS. Medullary and pontine areas targeted by PHOX2B expression are also affected. (*Pediatr Res* 57: 510–518, 2005)

Abbreviations

BOLD, blood oxygen level dependent
CCHS, Congenital Central Hypoventilation Syndrome
EPI, echo planar imaging
fMRI, functional magnetic resonance imaging
FOV, field of view
MNI, Montreal Neurological Institute
MRI, magnetic resonance imaging
NTS, nucleus of the solitary tract
RMANOVA, repeated measures ANOVA
SE, standard error
TE, time to echo
TR, repetition time
VOI, volumes of interest

In addition to impaired ventilatory responses to CO₂ and deficient breathing drive during sleep (1), patients with CCHS show inadequate breathing and cardiovascular responses to elevated or lowered levels of O₂ (2–5). The mechanisms involved in altered sensitivity to O₂ in CCHS remain unclear, because some degree of both central and peripheral chemoreception apparently is retained in the syndrome. Respiratory rates increase to hypoxia and decrease to hyperoxia (3,5,6), although both the respiratory and cardiovascular response patterns to challenges are distorted from the normal condition.

Determining the neural mechanisms underlying response deficits to differing O₂ levels in CCHS is a significant issue for reasons other than the obvious respiratory implications: CNS responses to hypoxia or hyperoxia recruit the sympathetic nervous system to, among other cardiovascular actions, preferentially alter perfusion of the periphery. CCHS patients show substantial deficits in both sympathetic and parasympathetic components of autonomic nervous system control, which are manifested by such signs as extreme reduction in respiratory-related heart rate variability during sleep (7), diminished blood pressure “dipping” during the night (8), syncope to relatively mild provocation (9), a high incidence of cardiac arrhythmia (10), intolerance to heat and cold, and poor control of sweating (11). The autonomic deficits have the potential to modify compensatory cardiovascular changes and organ perfusion to alterations in O₂ levels. Respiratory and cardiac rate and

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variability responses to hyperoxia in CCHS suggest deficiencies in both early and late physiologic responses, with particularly profound effects on rapid-variation aspects, as reflected in breath-by-breath heart rate changes (respiratory sinus arrhythmia).

A principal consequence of administering 100% O₂ is an effective suppression of peripheral chemoreceptor input (12). If peripheral chemoreception is at least partially intact in CCHS, the suppression of afferent chemoreceptor activity by hyperoxia potentially removes the remaining source of afferent chemoreceptor information in the syndrome. Neural sites responsive to hyperoxia should reveal areas mediating central processing to chemoreception free of input from peripheral signaling. The findings will complement data from hypoxia and hypercapnia challenges (13,14).

The objective of this study was to examine neural responses mediating the normal slowing of heart rate and changes in breathing that accompany exposure to high levels of O₂, and alterations in these patterns that may mediate the aberrant respiratory and cardiac responses in CCHS. Because hyperoxia is associated with both immediate and late-developing physiologic changes, it was important to follow time courses of neural patterns. We used fMRI procedures to measure changes in BOLD signals to hyperoxic (100% O₂) challenges, because the BOLD signals indirectly relate to neural activity (15). We hypothesized that structures mediating respiratory timing early and late in the challenge would show deficient responses temporally matching the deficits in breathing and heart rate during hyperoxia in CCHS, and that brain areas regulating sympathetic outflow would be similarly affected in the syndrome.

METHODS

Fourteen children with a diagnosis of CCHS (7 male, 7 female) and 15 controls (8 male, 7 female) participated. Fourteen pairs were age- and gender-matched; the remaining control subject was matched with one CCHS case (age in years for each group: mean, 11; range, 8–15; SD 2). Diagnosis was based on standard criteria (16). Patients were ventilated *via* tracheostomy only during sleep, and not during waking, and showed a clear reduction in ventilatory responses to hypercapnia. Patients with Hirschsprung's disease were excluded, as were patients with additional cardiac, pulmonary, or neuromuscular disorders.

Subjects lay supine in an MRI scanner and breathed spontaneously through a two-way nonrebreather valve. Tracheostomy openings were closed throughout the studies. Masking tape across the forehead and foam pads on either side of the head were used to minimize head movement. Measurements of airflow and the ECG were recorded simultaneously with the fMRI signal (3). Each subject underwent two scanning periods, the first consisting of a 150-s baseline and the second a 30-s baseline followed without pause by a 120-s challenge. A 2-min period was chosen as sufficiently long to elicit early central and peripheral chemoreceptor responses (which occur within the first minute), and to allow certain slower responding changes to occur (second minute). More lengthy challenge periods would have been desirable, but were precluded because of technical limitations with the scanner (newer MRI scanning technology resolves such issues for future studies). The hyperoxia challenge (100% O₂) was delivered *via* the inspiratory arm of the valve throughout the 120-s challenge period.

Images were collected using a 1.5 Tesla scanner (General Electric Signa, GE Medical Systems, Milwaukee, WI). For each 150-s scanning period, 24 volumes of 20 oblique image slices were collected using a gradient echo EPI protocol (TR = 6 s, TE = 60 ms, flip angle = 90°, FOV = 30 × 30 cm, no interslice gap, and voxel size 2.3 × 2.3 × 5 mm). The EPI protocol used the BOLD intrinsic contrast to highlight changes in neural activity during the challenge. Conventional spin echo T1-weighted images (TR = 500 ms, TE = 9 ms, FOV = 30 × 30 cm, no interslice gap, voxel size 1.2 × 1.2 × 5 mm)

were collected at the same location and orientation to aid in anatomical identification.

The images were preprocessed using the statistical parametric mapping package SPM (17), and custom software. EPI volumes were adjusted for slice timing, motion corrected, and spatially normalized. Segmentation was performed on the spatially normalized images to create a gray matter mask (18). This image mask was applied to the spatially normalized images, which were then smoothed. Global changes were removed (19), and the images were subsequently smoothed and analyzed for significant signal changes.

Two types of analyses were performed on the preprocessed images: 1) VOI analysis using custom routines, and 2) cluster analysis using SPM. VOI analysis allowed the investigation of the response of various structures without assumptions regarding the pattern of response. Additionally, because each VOI was outlined on a subject-by-subject basis, the VOI time trends were not affected by inevitable discrepancies in spatial normalization. The cluster analysis allowed a whole-brain search for regions not selected as VOI, but with the restriction that the response pattern be generally increasing or decreasing during the challenge period.

VOI analysis used *a priori* defined regions (examples from one subject in Fig. 1), within which the voxel intensities were averaged for each subject. RMANOVA was used to assess both differences from baseline for each group, as well as response differences between the groups (20).

Cluster analysis was performed over the entire brain on a voxel-by-voxel basis, comparing the time course of each voxel to a parametric boxcar model [step function from baseline (off), to challenge (on)], convolved with a standard hemodynamic response function. The fit of such a model was calculated for each subject, with resulting estimates of the contribution of the boxcar pattern at each voxel recorded. Clusters of voxels where group differences between CCHS and control subjects approximately matched this boxcar pattern were identified by performing a two-sample *t* test of the boxcar estimates at each voxel (from the fMRI literature, a population or random effects analysis). Cluster analysis was used to provide an overview of areas in which response patterns differed between groups. Statistical criteria of *p* < 0.05, corrected for multiple comparisons (false discovery rate), were used for an overview of response sites. For selected clusters, the time courses of all voxels within that cluster were extracted and plotted for the two groups.

RESULTS

Physiology. Physiologic characteristics to the breathing challenge have been described earlier (3). Briefly, respiratory rates initially fell in CCHS patients, but rose in control subjects over the first 20 s of the challenge, after which they returned to baseline levels until 60 s, when the CCHS patients showed a transient increase *versus* a transient decrease in the control subjects. Heart rate changes were more muted in CCHS patients both early (first minute increase: 0–40 s in CCHS, 10–50 s in controls) and later (second minute decline: from 60 s in CCHS, 70 s in controls) to the challenge. Respiratory-related heart rate variation was especially reduced in CCHS, and remained well below control levels throughout the challenge.

Global BOLD signal. The global BOLD signal increased in both groups during the later portion of the challenge period (45 s onwards), but initial (first 20 s) values declined in the control group, a trend not found in the CCHS patients (21). Detrending removed all global effects (19).

VOI analysis. Table 1 and Figure 2 show the responses in selected VOI. Cerebellar regions, including the vermis and deep nuclei of the dentate and fastigial areas, increased signal throughout the challenge in both groups, but no group differences appeared. The amygdala response was lateralized, with a transient signal decline in the right amygdala in the control group, but an increase in CCHS patients that was coincident with a marked decline in respiratory rate in the patients. The head of the caudate showed a signal decline in control subjects, but a transient increase in CCHS patients. Lentiform nuclei

Figure 1. Examples of VOI areas for one subject in white, overlaid onto an individual T1 image, from which time trends were calculated. A subset of these images was shown earlier for another challenge in a previous publication (34).

showed a transient decline that was more extensive in control than CCHS subjects; both groups returned to baseline after approximately 40 s, with a later decline in both groups, al-

Table 1. Summary of VOI analysis

Area name of VOI	Control n = 15	CCHS n = 14	Group difference
Basal ganglia			
Head of caudate	—	+	*
Lentiform	—	—	*
Limbic regions	0	0	
Amygdala (left)	0	—	
Amygdala (right)	—	+	*
Hippocampus	+	+	
Insula (left anterior)	+	+	
Insula (right anterior)	+	0	*
Insula (left posterior)	++	+	*
Insula (right posterior)	+	0	*
Brainstem regions			
Dorsal midbrain	+	—+	*
Ventral midbrain	+	+	
Dorsal pons	+	+	
Ventral pons	+	+	
Dorsal medulla	++	+	*
Medial medulla	+	0	*
Ventral medulla	+	0	*
Cerebellar regions			
Dentate nucleus	+	+	
Fastigial nucleus	+	+	
Culmen of the vermis	+	+	

Significance was tested using RMANOVA ($p < 0.05$). Significant signal increase (+), or decrease (—), or no significant difference over more than one time point is indicated for each group, relative to baseline. Group differences are indicated by an asterisk (*). Where both groups increased and a group effect was noted, the group with the greater extent of signal change is indicated by “++”; similarly, “—” is used for greater extent of decrease, and “—+” for a decline followed by an increase. 0 indicates no significant change from baseline.

though the decline was again more extensive in the control subjects. The hippocampus and insular cortex increased signal throughout the challenge in both groups; however, during the last minute of the challenge in the posterior insula, the control subjects' signal continued to increase to a significantly higher extent than in the CCHS patients. Both groups showed signal increases in the brain stem, with greater enhancement in medullary areas for control subjects, and an initial transient difference in the dorsal midbrain. Midbrain and pontine regions showed initial transient increases in signal.

Cluster analysis. Table 2 shows regions of signal increase throughout the challenge in both groups. Areas within the insula, cerebellar cortex, anterior and medial cingulate, and hippocampus all showed signal increases, together with the dorsomedial nucleus of the thalamus. The region of signal increase was especially extensive in the quadrangular lobule of the cerebellar cortex.

Table 3, and Figures 3 and 4 show regions of group difference in response. Control subjects showed greater signal responses in the medial and anterior cingulate, insula, and caudal hippocampus, along with smaller regions on the right side in the cerebellar cortex, superior temporal gyrus, and inferior frontal gyrus. Regions of greater signal response in CCHS compared with control subjects emerged in the temporal gyrus, pons and midbrain, and left insula and putamen.

Correspondence with physiology. Figure 5 illustrates fMRI signal intensity changes for the right amygdala and posterior

Figure 2. Time trends of VOI from control ($n = 15$) and CCHS ($n = 14$) subjects during baseline and challenge periods, with vertical SE bars. Time points of significant group difference are indicated by * above the plots (RMANOVA, $p < 0.05$). Time points of significant signal increase or decrease relative to baseline within each group are indicated by horizontal bars above or below plots (key at bottom of figure). Baseline series are plotted on the left of the graphs, and preceded the challenge series by several minutes.

insula overlaid with breathing or heart rate traces for CCHS and control groups. These traces highlight the concurrent alterations in breathing rate with amygdala BOLD signal changes, especially during the first 30 s of the challenge. The heart rate patterns follow the posterior insula signals, in particular, during the second minute of the challenge.

DISCUSSION

Hyperoxia elicited distinct response patterns in limbic structures involved in respiratory timing and autonomic outflow, basal ganglia recruited in motor coordination, and pontine and medullary areas targeted by PHOX2B gene expression, mutations of which are suspected of causing autonomic deficits in

Table 2. Clusters of voxels with a significant signal increase throughout the challenge in control and CCHS groups ($p < 0.05$, corrected for multiple comparisons)

Area name of cluster (significant increase in all voxels)	MNI co-ordinates (t-statistic maximum)	Significance (t-statistic maximum)	Cluster size (voxels)
Regions of overlap			
Insula right			
Superior temporal gyrus right	40 -18 0	5.8	758
Brodman 42 right			
Insula left			
Inferior frontal gyrus left	-44 -12 -4	5.6	677
Brodman 42 left			
Cerebellar cortex (quadrangular lobule)			
Vermis, Fastigial nucleus	30 -54 -30	5.3	5943
Dorsal pons			
Superior temporal gyrus left			
Insula left	-40 8 -20	3.9	106
Brodman 42 left			
Superior temporal gyrus left	-54 2 -2	3.6	74
Brodman 32 left			
Hippocampus left	-18 -20 -18	3.5	46
Anterior cingulate	4 22 20	3.5	76
Medial cingulate	0 -20 30	3.4	18
Inferior frontal gyrus right			
Insula right	38 20 10	3.1	101
Brodman 42 right			
Thalamus right (dorsomedial nucleus)	2 -20 10	2.9	12
Hippocampus right	20 -20 -16	2.8	21
Fornix	-2 0 0	2.7	17

All voxels in these regions exhibited significant signal increases relative to baseline, at the $p < 0.05$ level. The t-statistic maximum is an indication of the robustness of the increase at the “best-fit” voxel within the region, or cluster.

CCHS (22–24). Specific response differences temporally coincided with breathing and cardiac rate responses to the challenge.

Interpretation of BOLD signal changes. The BOLD signal is usually interpreted as an indirect indication of the functional processing performed by regions where local signal changes independently of the global signal. An increase in signal reflects increased input and intercellular processing, as opposed to just an increase in neuronal firing rate (25). The BOLD signal is influenced by global cerebral blood flow, blood volume, and oxygenation independently of localized changes resulting from neural activity-related oxygenation demands. The previously described difference in initial global BOLD signal response was indicative of a reduced cerebral autoregulatory capacity in CCHS subjects (21). However, local BOLD signal changes occur in an additive fashion (26), and procedures were implemented to remove all global effects (19). A consequence of the global effects procedure is that any neural signal pattern matching the global pattern would also be removed.

Comparable control and CCHS group responses. The cerebellar responses were similar in both groups and involved an increased signal throughout the challenge, as shown by the VOI and cluster analysis. The similarity of the cerebellar response in both groups is notable, inasmuch as cerebellar

Table 3. Clusters of voxels with a significant difference in response between groups ($p < 0.05$, corrected for multiple comparisons)

Area name of cluster	MNI co-ordinates (t-statistic maximum)	Significance (t-statistic maximum)	Cluster size (voxels)	Control vs CCHS
Regions of overlap				
Medial and anterior cingulate	2 4 32	3.2	64	>
Insula left	-44 -4 -2	3.1	115	>
Brodmann 42 left				
Cerebellar cortex	12 -40 -6	3.1	21	>
Parahippocampal gyrus right				
Insula right	44 4 -8	3.0	70	>
Superior temporal cortex right				
Insula right				
Inferior frontal gyrus right	42 18 6	2.9	76	>
Brodmann 42 right				
Insula left	-34 22 0	2.8	38	>
Parahippocampal gyrus left	-18 -32 -4	2.6	43	>
Temporal gyrus right	56 -58 -2	3.6	304	<
Temporal gyrus left	-40 -74 -10	3.5	291	<
Pons				
Midbrain	6 -32 -30	2.7	51	<
Putamen				
Insula left	-32 -12 -6	2.7	35	<

structures are remarkable in showing different response patterns to a range of ventilatory challenges in other sleep-disordered breathing cases (27), and to cold pressor and expiratory loading challenges in CCHS (28, 28a). Cerebellar sites normally participate in mediating respiratory challenges (29) and are frequently recruited during large changes in blood pressure rather than conditions of minor change (30). The ventilatory and blood pressure challenges in other CCHS studies are associated with substantial elevation of blood pressure. The cardiovascular component in the current study, however, was minimal in extent, and perhaps less likely to reveal cerebellar group differences. The dorsal medial thalamus showed similar response patterns in both groups; a portion of that region projects to ventral respiratory group neurons in the rat (31).

CCHS patients earlier showed significant areas of deficits to hypercapnia and hypoxia in the dorsal medial thalamus and cerebellar cortex and deep nuclei (13,14). The dorsal thalamus shows *c-fos* expression to hypoxia (32), and must be intact for the normal biphasic ventilatory response to hypoxia in the sheep fetus (33). The cerebellar cortex shows deficits to multiple ventilatory and pressor challenges, including hypercapnia (13), hypoxia (14), expiratory loading (28), and cold pressor challenges (28a). The classic role attributed to cerebellar function has been to mediate extremes of afferent input; the structure normally does not regulate momentary blood pressure changes, for example, but is involved in restorative action to extreme hypo- or hypertension (30). The cerebellum may play similar compensatory roles in hypercapnia and hypoxia, both of which are life-threatening. In extreme blood pressure and ventilatory challenges, the cerebellar role may be one of compensatory respiratory motor or other somatomotor action to restore perfusion or appropriate ventilation. Hyperoxia is not immediately life-threatening, and we speculate that cerebellar structures may not serve comparable regulatory roles for high O_2 stimulation. The dorsal thalamic role may serve comparable survival functions, but from arousal processes. Both hypercap-

nic and hypoxic challenges elicited a near-continuous column of deficient responses from the thalamus to pontine regions near the locus coeruleus (13,14), the latter a site with widespread alerting processes (36). We speculate that dorsal thalamic responses to hypercapnia and hypoxia, which show deficits in CCHS patients to these two challenges but not to hyperoxia, reflect a survival-related chemosensitivity response.

Initial responses—group differences. The amygdala responses in CCHS were unique in their timing and lateralization. These responses were inverse to those of control subjects and paralleled the opposite respiratory rate pattern between groups found early in the challenge. The timing of response pattern differences raises the possibility of a role for amygdala structures in mediating the slowing of respiratory rate in CCHS over the increased rate in control subjects during that initial period. The amygdala is not normally considered a “respiratory-related” structure, but can modify respiratory rate, because the central amygdala nucleus projects heavily to respiratory phase-switching areas of the dorsal pons (37), and, on single-pulse stimulation, can pace the respiratory rhythm, a dependency that declines with entry into sleep (38); projections from the amygdala/hippocampal region have also been demonstrated to the ventral respiratory region in the rat (31). A role for the amygdala in breathing control in CCHS is especially of interest, inasmuch as CCHS patients typically do not readily perceive “air hunger” from increasing CO_2 (39) or potential asphyxia, and do not show signs of emotional distress to the condition; affected patients must be encouraged to voluntarily breathe when very relaxed, despite extreme O_2 desaturation. The reduced anxiety in CCHS patients extends to other behavioral stimuli, as assessed by neuropsychological examination (40). Amygdala structures have long been known to mediate distress or negative emotion (for overview, see 41), and has been implicated, together with cingulate, cerebellar, and insular structures, with dyspnea (42,43), and the structure has considerable potential to enhance switching to inspiratory effort on activation. Aberrant function in this site to the chal-

Figure 3. Regions of significant group difference ($p < 0.05$) in response overlaid onto mean anatomical images, with regions of increased signal in control over CCHS subjects color coded in yellow-red scale, and regions of decreased signal in control compared with CCHS subjects color coded in blue-green (key at bottom of figure).

lenge, manifested as an increase in activity rather than the normal transient decline, suggests a failure to appropriately process information on the hyperoxic stimulus.

The amygdala signal response differences emerged only on the right side. A lateralization in multiple brain structures, including the amygdala, has been noted in normal brain responses to both ventilatory and autonomic challenges (44,45).

Remarkably, the role of the amygdala in mediating aversive conditioning appears to be preferentially lateralized to the right side in rodents (46,47), and at least partially on the right side in humans (48). It is unclear whether the differential CCHS response to hyperoxia reflects deficiencies in the normal lateralized function for this structure, or whether influences from other related areas (e.g. right insula, right cingulate) may contribute to the findings.

Several of these sites, especially the dorsal medulla and dorsal pons, are targets for PHOX2B gene expression; a mutation of PHOX2B is found in a substantial proportion of CCHS patients (22,24). The gene is especially involved in autonomic nervous system development, and particularly in a sensory structure involved in afferent detection of O_2 levels, the NTS, and an area involved in respiratory phase-switching and blood pressure control, the dorsal pons (23). The dorsal pontine differences consisted of higher responses in CCHS patients. Both white matter damage and diminished cell density have been noted for medullary structures in the syndrome (49,50).

Sustained group differences—clusters. Several areas showed patterns of differing responses between groups that emerged early, and were sustained throughout the entire challenge. These areas included the posterior temporal cortex, insula, medial cingulate, and caudal and dorsal hippocampus (Figs. 3 and 4). The response pattern was typically one of no or little change in the CCHS group, and an increase or decline in the control subjects, although in the hippocampus, a late rise emerged in CCHS patients over an initial transient response in control subjects, and transient patterns in the opposite direction developed in the dorsal pons. The data illustrate the extent of “unreactive” areas in CCHS patients, despite substantial signal increases in other areas.

Longer-latency, first- or second-minute group response differences. The lentiform nuclei showed an initial response that was greater in control than CCHS subjects, followed by a later signal decline in both groups. Again, the response was more extensive in the control than the CCHS patients. Response patterns also differed in the head of the caudate nucleus. Both areas within the basal ganglia are involved in motor coordination, presumably affecting respiratory muscle patterning.

Remarkable among rostral brain areas, the anterior and posterior insula showed much-reduced responses in CCHS late in the challenge over control findings. The data reinforce a central role for insular structures to mediate autonomic outflow, a relationship earlier outlined by others (51–53), with lateralization of sympathetic modulation by the right insula, and parasympathetic action by the left side. Portions of the insula exert modulatory control over baroreflexes (54). The absence of a response in CCHS relative to control subjects was reflected as a more pronounced difference on the right (sympathetic) side, and was particularly enhanced late in the challenge (Fig. 4). Heart rate slowing was prominent late in the response in control subjects, and minimized in CCHS patients (3). Because the right insula can exert an inhibitory influence on sympathetic outflow (55), unresponsiveness in that site would lead to the physiologic response found here.

Control > CCHS

CCHS > Control

Figure 4. Time trends for control ($n = 15$) and CCHS ($n = 14$) groups from selected clusters of significant group difference ($p < 0.05$), with SE bars; overlays follow the same conventions as Figure 2.

As with respiratory rate responses, the fMRI signal patterns differed between the first and second minutes of the challenge in several regions. After initial transient increases, signals in the dorsal and ventral pons increased only late in the challenge, and presumably reflect the slowing of heart and respiratory rate in both groups. No group difference in pontine signals emerged, despite the significant late physiologic differences in CCHS cases.

Other considerations. The CCHS patients were all ventilator dependent during sleep but not during waking, and thus were a subset of the entire CCHS population, some of whom require continuous ventilation and presumably have more se-

rious neural injury. The findings here might be more extensive or differ in other fashions in more seriously affected patients, and that possibility should be explored. The potential for more extensive damage to elicit additional deficits cautions against extension of these findings to the broader CCHS population.

Baseline neural conditions cannot be measured using the technique used, because fMRI procedures provide an index of change rather than absolute measures of functional integrity in particular areas. Neural structures mediating cardiac and respiratory integration are clearly deficient in waking, baseline conditions, as shown by earlier evaluation of respiratory effects on heart rate variation (3,56). The presence of damage in white

Figure 5. fMRI signal intensity time trends overlaid with breathing (right amygdala) and heart rate (posterior insula) traces for control and CCHS groups; key at bottom. The rate curves are a moving mean (10-s window). The vertical gray dashed line indicates the onset of the challenge. Each graph has a signal intensity scale (left) and a rate scale (right). The breathing and heart rate trend lines are from a previous publication (3).

matter and diminished cell density (49,50) may contribute both to the waking deficits and sleep-related pathology. The current findings add support to evidence that breathing control mechanisms in CCHS are impaired in the waking state as well as during sleep.

Several of the areas affected differentially in CCHS cases by hyperoxia, including sites within the insula and caudate nucleus, are not structures described as targeted by PHOX2B gene expression. In addition to hyperoxia, these areas consistently show deficits to other ventilatory and pressor challenges (13,14,27,28a). It is unclear why these areas are affected in the syndrome; some of the structures, such as the basal ganglia, are susceptible to intermittent hypoxia, but others are not. It may be the case that PHOX2B expression extends to structures in addition to autonomic ganglia, medullary, and pontine sites originally described (23). Undetected mutations, other than PHOX2B, may also be operating in CCHS, but this possibility remains speculative.

Processes. The nature of the processes by which structures show deficits to hyperoxia could follow several mechanisms. The defects could be primarily peripheral, at the receptor or afferent pathway level. However, CCHS patients show at least partially intact peripheral chemoreceptor responses (6), thus the response deficits likely result from failure of central reaction to high levels of O_2 . Hyperoxia should suppress peripheral chemoreceptor activity. The absence of chemoreceptor traffic constitutes as much of a signal to central neural structures as differing levels of input, and presumably results in the recruitment of neural responses found in control subjects and common areas in multiple sites of CCHS patients. The failure of select central sites, or the failure to send appropriate information to multiple more-rostral areas, particularly those mediating cardiovascular regulation, such as the insula, frontal cortex, and insula, appear to underlie the most likely defective processes. The response deficits to hyperoxia, therefore, are complex, and appear to reflect selective interactive failure of signaling between structures.

SUMMARY

Hyperoxia elicited comparable responses in CCHS and control subjects in multiple areas of the brain, and distinctly different patterns in other regions. The response differences

emerged both early and late in the challenge, and in sites that affect timing of respiratory switching, such as the amygdala, or respiratory and cardiovascular concomitants of the challenge, such as the insula, cingulate, and dorsal and ventral medulla. In several of these areas, including the insula, the response pattern was largely “no change” in CCHS cases, whereas signals increased or declined in control subjects. The findings are remarkable in demonstrating apparent significant roles for limbic sites in mediating respiratory and cardiovascular patterns to hyperoxia, and an apparent absence of cerebellar response differences in CCHS patients, unlike other challenges that elicit larger blood pressure effects.

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