AMPA glutamate receptors and respiratory control in the developing rat: anatomic and pharmacological aspects

GINA M. WHITNEY,1 PATRICIA J. OHTAKE,2 NARONG SIMAKAJORNBOON,3 YING-DAN XUE,1 AND DAVID GOZAL1,3,4
Departments of 1Pediatrics and 3Physiology and 4Interdepartmental Neuroscience Program, Constance S. Kaufman Pediatric Pulmonary Research Laboratory, Tulane University School of Medicine, New Orleans, Louisiana 70112; and 4Department of Physical Therapy, Exercise and Nutrition Sciences, State University of New York at Buffalo, Buffalo, New York 14214

WHEN MAMMALS ARE EXPOSED to an hypoxic environment, brisk increases in minute ventilation (Ve) occur and are primarily mediated by increased carotid chemoreceptor afferent activity (21). The ventilatory response to hypoxia (HVR) in the neonate is disproportionately smaller compared with the adult and undergoes significant developmental changes during the first 2 wk of life, after which adultlike responses occur (11, 16).

The nucleus of the solitary tract (NTS) is a longitudinal structure in the dorsomedial portion of the medulla oblongata, where it extends from the spinomedullary junction caudally to the level of the caudal region of the facial motor nucleus in the rostral medulla. In the context of the HVR, the portions of the NTS structure caudal to the obex provide the first central relay to cardiorespiratory afferent inputs, such that lesions of this area will result in marked attenuation or abolition of HVR (13, 19). At this level of the caudal brain stem are also located the nucleus ambiguus (NA) and the hypoglossal nucleus (XII), which have well-defined roles in respiratory control.

Although multiple neurotransmitters and their receptors have been implicated in HVR, glutamatergic pathways have been primarily implicated in the early component of cardiovascular and ventilatory responses to hypoxia (5). In adult rats, N-methyl-D-aspartate (NMDA) glutamate receptors within the NTS are critical to such responses, and therefore it was expected that the density of NMDA receptors would account for developmental changes in the magnitude of HVR. However, in a previous study in our laboratory, administration of NMDA blockers did not modify HVR in the neonatal rat, thereby suggesting a potential role for other ionotropic glutamate receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors (29).

Ionotropic non-NMDA receptors (GluR) include both kainate receptor subunits GluR5-GluR7 and KA1 and KA2 (2). AMPA receptors are most abundantly expressed in the brain and consist of hetero-oligomers made up of five subunits of different combinations of GluR1-GluR4 (40). It is now becoming increasingly clear that non-NMDA receptors play a major role in central pathways modulating baroreceptor inputs (8, 20).

The developmental role of AMPA glutamate receptors in respiratory regulation remains undefined. To study this issue, minute ventilation (Ve) was measured in 5-, 10-, and 15-day-old intact freely behaving rat pups using whole body plethysmography during room air (RA), hypercapnic (5% CO2), and hypoxic (10% O2) conditions, both before and after administration of the non-N-methyl-D-aspartate (NMDA) receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamido disodium (NBQX; 10 mg/kg ip). In all age groups, Ve during RA was unaffected by NBQX, despite reductions in breathing frequency (f) induced by increases in both inspiratory and expiratory duration. During hypoxia and hypercapnia, Ve increased in similar in both NBQX and control conditions in all age groups. However, tidal volume was greater and f lower after NBQX. To determine if AMPA receptor-positive neurons are recruited during hypoxia, immunostaining for AMPA receptor (GluR2/3) and c-fos colabeling was performed in caudal brain stem sections after exposing rat pups at postnatal ages 2, 5, 10, and 20 days, and adult rats to room air or 10% O2 for 3 h. GluR2/3 expression increased with postnatal age in the nucleus of the solitary tract (NTS) and hypoglossal nucleus, whereas a biphasic pattern emerged postnatal age in the nucleus of the solitary tract (NTS) and NA. GluR2/3 expression was enhanced by hypoxia at all postnatal ages in the NTS and NA and also demonstrated a clear maturational pattern. However, colocalization of GluR2/3 and c-fos was not affected by hypoxia. We conclude that AMPA glutamate receptor expression in the caudal brain stem is developmentally regulated. Furthermore, the role of non-NMDA receptors in respiratory control of conscious neonatal rats appears to be limited to modest, albeit significant, regulation of breathing pattern.

hypoaxia; hypercapnia; normoxia; nucleus of the solitary tract; caudal brain stem
18, 25). However, the data on the potential role of AMPA glutamate receptors in respiratory control are rather limited. In a recent study, Borday et al. (4) showed that blockade of non-NMDA receptors with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX) did not affect ventilatory output in adult conscious mice or cats, but induced marked respiratory depression in neonatal or anesthetized animals. In addition, Vardhan and colleagues (39) demonstrated that microinjection of the non-NMDA receptor antagonist 6,7-dinitro-quinoxaline-2,3-dione within the NTS of anesthetized adult rats attenuated the ventilatory response to carotid body stimulation. However, the role of non-NMDA receptors in the developmental characteristics of respiratory patterning during normoxia and in the responses to hypoxia and hypercapnia has not been systematically explored.

Therefore, on the basis of the hypothesis that AMPA receptors would provide the neuronal substrate underlying the HVR during early postnatal life, the ventilatory responses to hypoxia and hypercapnia (HCVR) were measured in rat pups at various postnatal ages before and after administration of the non-NMDA receptor antagonist NBQX. In addition, the developmental patterns of AMPA GluR2/3 receptor and c-fos protein expression were examined in the caudal brain stem during normoxia and hypoxia.

MATERIALS AND METHODS

Experimental protocols were approved by the Institutional Animal Care and Use Committee. Time-pregnant Sprague-Dawley rats were obtained from a commercial breeder (Charles River), and delivery times were recorded.

Ventilatory Responses

For assessment of the ventilatory response to NBQX and for hypoxic and hypercapnic ventilatory studies, pups were randomly selected from every litter at postnatal days 5, 10, or 15. At least six animals were studied per treatment group. These ages have been shown to be representative of maturational changes in the biphasic response to hypoxia (11, 16).

Protocol

Ventilatory challenges with 10% O2 (balance N2) or 5% CO2 (balance air) were initially performed in each rat pup after intraperitoneal administration of 0.2 ml normal saline (control). After a 30- to 45-min period of acclimatization, each pup was exposed to either 10% O2 or 5% CO2 for 30 min. Gas switches were performed by rapidly bleeding the premixed gas mixture into the recording chamber. Animals were then allowed to recover with their mother in room air for at least 60 min and were then injected with the non-NMDA glutamate receptor antagonist NBQX (RBI, Natick, MA; 10 mg/kg ip). Thirty minutes after NBQX administration, ventilatory challenges were repeated.

Ventilatory Recordings

Respiratory measures were continuously acquired in the freely behaving, unrestrained animal placed in a previously calibrated 0.5-liter barometric chamber (Buxco Electronics, Troy, NY), using the methods described by Bartlett and Tenney (1) and Pappenheimer (31). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size, in which temperature was measured using a T-type thermocouple, was used. In addition, as previously recommended by Epstein and colleagues (12), a correction factor was incorporated into the software routine to account for inspiratory and expiratory barometric asymmetries. Environmental temperature was maintained within 29–32°C, which corresponds to usual temperatures recorded in the dam. A calibration volume of 0.5 ml of air was repeatedly introduced into the chamber before and on completion of recordings. At least 30 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (90% relative humidity) warmed at 30°C was passed through at a rate of 2 l/min, using a precision flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes (9) were measured using a high-gain differential pressure transducer (Validyne, model MP45–1). Analog signals were continuously digitized and analyzed online by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Tidal volume (VT), inspiratory duration (Ti), expiratory duration (Te), respiratory frequency (f), and (Ve) were computed and stored for subsequent off-line analysis.

For ventilatory challenges, early and late components of HVR and HCVR were assessed as the average of the first and last 3-min periods of each 30-min challenge, whereas baseline ventilation was defined as the average of the 3 min immediately preceding the gas switch. Although the initial 3 min of a hypoxic challenge period may not always correspond to the peak Ve response in a particular animal, they are primarily representative of the peripheral chemoreceptor-mediated component with little contamination from central sources and were therefore selected for comparative analyses. Differences in data among the various age groups or within each age group for the saline and NBQX treatments were compared by ANOVA (2-way ANOVA for repeated measures) and the Newman-Keuls post hoc test. A P value of <0.05 was considered to achieve statistical significance.

Immunocytochemistry

To determine the developmental pattern of AMPA receptor expression and the topography of neuronal recruitment in response to hypoxia, double-label immunocytochemistry was performed for the GluR2/3 subunits of the AMPA receptor and for the early gene product c-fos (23).

Rat pups (ages 2, 5, 10, and 20 days) and adult rats were studied after 3-h exposures to either room air or hypoxia (10% O2, 5% CO2). For all pups, exposures were conducted in the presence of the mother to prevent separation stress and while maintaining environmental temperatures at ~28–30°C. Five animals were studied per experimental group. At the end of the exposures, rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and perfused transcardially with 30–200 ml PBS at ambient temperature and then with 2.5% paraformaldehyde in cold PBS containing 5% sucrose, pH 7.4. The brain was removed immediately from the skull after perfusion and placed overnight in a fixative containing 1% paraformaldehyde in PBS and 30% sucrose at 4°C. Coronal sections (30 μm) were cut on a freezing microtome and divided into two series. One series was stained for Nissl substance with thionine, and the other was processed immunocytochemically. Sections were washed extensively in PBS and incubated in 0.4% Triton X-100 in PBS containing 1.5% normal goat serum (Vector, Burlingame, CA) for 1 h. Sections were then incubated with anti-c-fos (Santa Cruz Biotechnol-
ogy, Santa Cruz, CA, sc-52, 1:10,000 dilution) and with an antibody to the AMPA glutamate receptor subunits 2 and 3 (23, 40) raised against the carboxy terminus peptide of rat GluR2 conjugated to BSA with glutaraldehyde (Chemicon, Temecula, CA; catalog no. AB 1506; 1:1,000). The sections were then washed extensively in PBS, incubated in biotinylated anti-rabbit IgG (Vector) diluted in 0.4% Triton X-100 in PBS for 1 h, washed three times in PBS, incubated for 1 h in avidin-biotinylated horseradish peroxidase (Vectastain Elite kit, Vector) diluted in 0.4% Triton X-100 in PBS, rinsed three times in Tris (pH 7.6), and incubated in 50 mg/100 ml diaminobenzidine tetrahydrochloride (Sigma) and 0.005% H2O2 (Sigma) diluted in Tris pH 7.6 for variable intervals until appropriate staining was achieved. The reaction was stopped in PBS, and the sections were mounted from sodium acetate onto slides coated with gelatin chrom-alum. The resulting double-labeled neurons were easy to identify because c-fos was localized to the nucleus, whereas the AMPA receptor marker was found in the cytoplasm or plasma membrane. Control experiments with either one or both antibodies were done to determine whether the primary or secondary antibodies produced false positive results.

Sections were assessed using a light microscope, and the distribution and number of cells containing c-fos, GluR2/3, or c-fos-GluR2/3 immunoreactivities were indicated on camera lucida drawings and maps of the NTS using the atlases by Paxinos and colleagues (32, 33). At least five sections were examined per animal. The cytoarchitectural boundaries of the various rostral medullary nuclei were defined by superimposing the adjacent thionine-stained sections with the camera lucida drawings. We primarily analyzed the cellular patterning within the three major nuclei of interest in the medulla oblongata, namely the NTS, XII, and NA. In every nucleus, at least 1,000 cells were counted for each animal. Data were tabulated, and responses were compared using two-way ANOVA (postnatal age and hypoxia) followed by the Newman-Keuls post hoc test. A P value of <0.05 was considered to achieve statistical significance.

RESULTS

Ventilatory Responses

Effects of NBQX on resting ventilation. With advancing postnatal age, progressive increases in V̇e during room air breathing (baseline) were observed (P < 0.0001), primarily as a result of increases in V̇t (P < 0.0001; Fig. 1). Respiratory frequency was similar in 5- and 10-day-old pups, whereas in 15-day-old pups, f had decreased 15% (P = 0.007). Inspiratory time in-

![Fig. 1. Mean (±SE) ventilation (V̇e), respiratory drive (V̇t/Ti), tidal volume (V̇t), respiratory frequency (f), and inspiratory (Ti) and expiratory (Ṫe) time during room air breathing before (open bars) and after (hatched bars) 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX) administration in 5-, 10-, and 15-day-old rat pups.*Significantly different from 5-day values; **significantly different from 10-day values; ***significantly different between treatment groups (control vs. NBQX).](http://ajpregu.physiology.org/)
creased in 15-day-old pups (P < 0.0001), paralleling the change in f, whereas T_E was not different across all ages. Ventilatory drive, determined as V_T/T_I, increased progressively with postnatal age (P < 0.0001).

Administration of NBQX was without effect on baseline V_E, V_T, and V_T/T_I at all postnatal ages (Fig. 1). However, in 5- and 10-day-old pups, NBQX administration resulted in a reduction in f to levels similar to 15-day-old animals (P < 0.0001). Increases in T_I were observed after NBQX administration at all ages (P < 0.01), but in particular in 5- and 10-day-old pups. Similarly, T_E prolongations occurred at ages 5 and 10 days (P = 0.012). Interestingly, the lowering of f and lengthening of T_E after NBQX observed at ages 5 and 10 days were not detected in 15-day-old pups.

Effects of NBQX on the hypoxic ventilatory response.
Exposure to hypoxia was associated with a brisk increase in ventilation in all ages, followed by a reduction in ventilation (Fig. 2), and this response was not altered by NBQX administration. Before NBQX administration, early increases in V_T were associated with increases in f (P < 0.003), whereas V_T remained relatively unchanged. After NBQX, alterations in breathing pattern during the early hypoxic ventilatory response occurred at all ages, such that f was lower (P < 0.0005) and V_T tended to be greater (significant only at 5 days postnatally; P < 0.02) than in the pre-NBQX condition.

The magnitude of the ventilatory decline during hypoxia (late phase of the hypoxic ventilatory response) varied with age, the youngest animals decreased ventilation to levels below baseline, whereas the V_E decline in 15-day-old pups was smaller and remained above baseline, similar to the characteristic adult response (Fig. 2). The decline in V_E during the late phase of the hypoxic ventilatory response was attributable primarily to reductions in f (P < 0.0001). During the late phase, NBQX administration resulted in lower f than in the pre-NBQX condition at all ages (P < 0.004).

Effects of NBQX on the hypercapnic ventilatory response. To determine the contribution of non-NMDA receptors to the ventilatory response to hypercapnia in developing animals, the effect of NBQX administration on the ventilatory response to 5% CO2 was examined in a separate group of pups (Fig. 3). Exposure to hypercapnia was associated with increases in V_E at all ages, with the magnitude of the response increasing with advancing postnatal age. Within 3 min of exposure to hypercapnia, V_E increased by ~16% (not significant), 21% (P < 0.0001), and 36% (P < 0.0001) in 5-, 10-, and 15-day-old pups, respectively. In 5-day-old pups, early increases in V_T (P < 0.001) without changes in f were observed. In the older animals, increases in f accounted for the initial rise in ventilation (P < 0.005). After 30 min of hypercapnia, V_E remained elevated, being ~40–60% greater than baseline levels (P < 0.0002).

The ventilatory response to hypercapnia at any age was not altered by administration of NBQX. However, alterations in breathing pattern were again observed.

![Fig. 2](http://ajpregu.physiology.org/). Mean (±SE) ventilatory response to hypoxia (10% O2) in 5-, 10-, and 15-day-old pups before (closed symbols) and after (open symbols) NBQX administration. *Significantly different from baseline values; **significantly different from early values; ***significantly different between treatment groups (control vs. NBQX).
VT was greater at all time points after NBQX administration in 10- and 15-day-old pups (P < 0.03), with a trend toward larger VT present in the 5-day-old pups. Conversely, NBQX administration resulted in lower f at all time points in 5- and 10-day-old animals (P < 0.03) and was without effect on f responses in the 15-day-old group.

**Immunocytochemistry**

With advancing postnatal age, significant increases in GluR2/3 immunoreactivity were present in the NTS and XII (Figs. 4 and 5). In the NA, a biphasic pattern of GluR2/3 expression emerged such that increases occurred until postnatal day 10, after which the GluR2/3 immunoreactivity decreased until it reached adult levels (Fig. 5). Hypoxia induced increased c-fos labeling at all postnatal ages in both the NTS and NA but not in the XII. However, the magnitude of c-fos increase was particularly prominent in older animals (Figs. 4 and 5). The colabeling of c-fos and GluR2/3 increased with postnatal development in the NTS. However, at younger ages (2 and 5 days old), decreases in the percentage of AMPA-positive cells displaying c-fos immunoreactivity occurred with hypoxia in the NTS (Fig. 5, right). Similar patterns of decreased c-fos labeling among AMPA immunoreactive cells were observed in the NA and persisted at later stages of development.

**DISCUSSION**

In this study, we showed that systemic administration of the non-NMDA receptor antagonist NBQX elicits changes in f with reciprocal changes in VT such that VE is preserved across all postnatal ages in the developing rat. In addition, NBQX was not associated with altered VE responses to either hypoxic or hypercapnic challenges. We further demonstrate that significant changes in AMPA receptor expression occur within several nuclei of the caudal brain stem, such as the NTS, XII, and NA, and that with advancing maturation, marked enhancements in c-fos nuclear immunoreactivity are induced by hypoxia. However, as would be expected from the physiological recordings, the relative proportion of cells demonstrating double labeling for GluR2/3 and c-fos was not modified by hypoxia in the NTS and NA, and, at younger ages, the percentage of c-fos-positive cells among cells with AMPA immunoreactivity decreased.

During the first 2 wk of life, maturational changes were observed in resting ventilation and the ventilatory responses to hypoxia and hypercapnia. Specifically, room air ventilation, VT, and respiratory drive more than doubled, whereas respiratory frequency decreased during this time interval, and these findings concur with previous studies (11, 16). HVR and HCVR also underwent changes with advancing postnatal age.
Between 10 and 15 days postnatally, the late HVR changed from one of profound hypoxic ventilatory depression to one more characteristic of that observed in the adult animal, where the level of ventilation during the late phase remained above baseline values. The evolution of both early and late phases of HVR in this study closely agrees with that reported by Eden and Hanson (11) and Gozal et al. (16). Similarly, the responsiveness to 5% CO₂ appeared to increase between 10- and 15-day-old animals. Although CO₂ sensitivity was not specifically measured, the percent change in \( V_E \) was greater in 15-day-old pups than in the younger animals. These findings suggest that significant maturation of the ventilatory control system is occurring between postnatal age 10 and 15 days in the rat. An additional developmental feature in HCVR involved the differing \( \dot{f} \) recruitments in 5- and 10-day-old animals compared with older rats. Indeed, \( \dot{f} \) decreased in the youngest animals during both the early and late time points of the hypercapnic challenge, whereas in 10-day-old pups, \( \dot{f} \) decreases occurred only later in the course of the challenge, and in 15-day-old pups, \( \dot{f} \) increased throughout the challenge. Because this pattern was not affected by NBQX treatment, it is likely
that the maturational process of respiratory frequency
currents during central chemoreceptor stimula-
tion is dependent on other neurotransmitter systems.

Before addressing the potential significance of our
findings, it is important to emphasize that major
differences in the responses observed during pharmaco-
logical manipulation of NMDA or non-NMDA glutam-
ate receptors occur between the waking and anesthe-
tized state (4, 6, 24, 37). In addition, caution should be
applied in the extrapolation of responses obtained from
the in vitro brain stem preparation to those measured
during in vivo experimental conditions (30). In our
experiments, maintenance of resting, hypoxic, and
hypercapnic ventilation after systemic administration
of a non-NMDA receptor antagonist suggests that
neuronal pathways controlling ventilation in the awake
developing rat in these conditions do not require non-
NMDA receptor-mediated mechanisms. Despite the
overall level of ventilation being unaffected by the
non-NMDA receptor antagonist NBQX, small but sig-
ificant alterations in breathing pattern were observed
after NBQX administration. Specifically, in 5- and
10-day-old pups, respiratory frequency was reduced
after AMPA receptor antagonism in all conditions stud-
ied. Similar results have been observed in neonatal
mice (4), nonanesthetized fetal sheep (3), and anesthe-
tized adult rats (37). Interestingly, by 15 days postna-
tally, the NBQX-induced reduction in frequency was
not observed during room air or hypercapnic exposure
and was only modestly present during the hypoxic
exposure. However, this is not surprising, because by
this age, significant maturation of the respiratory
system has already occurred and the lack of consider-
able effect of NBQX on respiratory frequency in this age
group is consistent with similar findings in adult mice
and cats (4). Thus it appears that NBQX administra-
tion particularly impacts respiratory function in ani-
mals before maturation of the ventilatory responses to
chemical stimuli. Consistent with these findings in the
conscious rat pup, data obtained using a neonatal rat

---

Fig. 5. Mean (±SD) percentages of c-fos, GluR2/3, and of c-fos-labeled cells in positive GluR2/3 cells in NTS (A) and
NA (B) in rat pups ages 2, 5, 10, and 20 days and adult rats. Open bars indicate room air conditions, whereas
crosshatched bars represent hypoxic exposures (n = 5 for each treatment group). Significant increases in c-fos
occurred with hypoxia at all postnatal ages in NTS and in NA (*room air vs. hypoxia: P value < 0.01).
Age-dependent increases in GluR2/3 occurred in the NTS (**P < 0.01 2-way ANOVA).
brain stem slice preparation have demonstrated that AMPA receptor activation is crucial for respiratory rhythm generation in neurons within the pre-Bötzinger complex (14). Taken together, these results indicate that AMPA receptor mechanisms modulate components of respiratory pattern generation in the immature, but not in the mature, rat.

The location and magnitude of c-fos immunoreactivity enhancements in the developing and adult animals studied herein are in close agreement with those previously reported by White et al. (41), Haxhiu et al. (17), and Teppema et al. (38) after similar hypoxic exposures. In addition, we have extended our experiments to younger postnatal ages and document on the existence of marked developmental pattern of c-fos enhancements in both the NTS and NA in response to hypoxia. Our findings suggest that with maturation, the NTS assumes an increasingly preponderant role in the processing of neural afferent input from peripheral chemoreceptor afferents as well as the cellular activation elicited by the hypoxic stimulus. Such trend was not as prominent in the NA, and because systematic exploration of all brain stem structures was not performed, it is possible that other brain regions may display similar developmental changes in hypoxic neuronal recruitment. Another interesting observation pertains to the decreases in colabeled cells during hypoxia occurring in both the NTS and NA in the younger animals. Although the exact function of the AMPA-positive cells remains undetermined, it is possible that they may play a role in the tonic regulation of blood pressure or of other cardiovascular functions (8, 18, 25) and that they may undergo lessened recruitments during hypoxic conditions.

We employed an antibody that recognizes the most widely expressed AMPA receptor subunits in the brain stem and confirmed the extensive distribution of these receptor subunits in the NTS of the adult rat (23, 40, 42). In addition, we further confirm previous reports on the distribution of AMPA receptor immunoreactivity throughout somata and proximal dendrites of neurons located within the ventral respiratory group as well as in the NTS (22, 36). The overall number of neurons expressing GluR2/3 immunoreactivity increased with advancing postnatal age in nearly all the caudal brain stem regions that were examined in this study. Previous investigators clearly demonstrated that AMPA glutamate receptor expression undergoes substantial developmental changes, which vary according to the brain region of interest (10, 20). Our findings are in close concordance with those recently reported by Rao and colleagues (35), who demonstrated that a postnatal increase in AMPA receptor number occurs between birth and postnatal day 9 in both the NTS and ventral medullary nuclei. Incidentally, a biphasic pattern in GluR2/3 expression was apparent in the NA, such that decreased immunoreactivity occurred in 20 day and older animals. Thus increased expression of AMPA GluR2/3 receptors occurs postnatally in brain stem nuclei, but their functional role remains to be determined. On the basis of our physiological experiments, there is little evidence to suggest that AMPA glutamate receptors play a major role in the modulation of hypoxic and hypercapnic ventilatory responses and that their role in respiratory control may actually be limited to regulation of timing mechanisms and/or to selective adaptation processes (43). Indeed, a very recent study by Petralia and colleagues (34) suggests that AMPA receptor expression may be needed to mobilize NMDA glutamate receptors to become synaptically active. It is also possible that this family of glutamate receptors may preferentially underlie cardiovascular functions, such as components of the baroreceptor reflex arc or modulation of vagal chronotrophic efferent output originating in pathways involving both the NA and NTS (7, 8, 15, 27, 28). Obviously, exploration of such possibilities is clearly beyond the scope of the present study.

In summary, we showed that although marked developmental changes occur in GluR2/3 expression within caudal brain stem regions traditionally associated with cardiorespiratory functions, such maturational processes are not immediately correlated to functional determinants of overall ventilatory output or responsiveness to chemical stimulation. Instead, the apparent role of non-NMDA receptors in respiratory control appears to be limited to modest, albeit significant, regulation of $V_r$-f interactions.

Perspectives

The central neural mechanisms underlying the developmental characteristics of the acute HVR in mammals remain elusive. Although the glutamatergic hypothesis for the HVR emerges as viable in the mature rat, the present study further indicates that extrapolation from neural pathways operative in the adult animal does not always find validation in the neonate. Further studies are needed to examine three major conceptual frameworks that derive from the results presented herein: 1) the neural processing of afferent input from peripheral chemoreceptors during hypoxia takes place within different brain stem regions dependent on the maturational stage; 2) the neurotransmitter(s) and respective receptors underlying such neural processing undergo radical changes during postnatal development; and 3) AMPA receptor expression and activation within regions such as the NTS and NA are required to elicit the conversion of NMDA receptors from synaptically silent to functionally active (34).

This study was supported by grants from the American Lung Association (CI-002-N), the National Institutes of Health (HD-01072 and HL-65270), and the Maternal and Child Health Bureau (MCJ-229163).

Address for reprint requests and other correspondence: D. Gozal, Dept. of Pediatrics, Univ. of Louisville School of Medicine, 571 S. Floyd St., Ste. 300, Louisville, KY 40202-3830 (E-mail: d0goza01@gwise.louisville.edu).

Received 26 Jan 1999; accepted in final form 17 October 1999.

REFERENCES


McManigle, J. E., A. M. Tavera DaSilva, K. L. Dretchen, and R. A. Gillis. Potentiation of MK-801-induced breathing impairment by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof(F)qui


