Gasping and autoresuscitation in the developing rat: effect of antecedent intermittent hypoxia

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In the last decade, the incidence of sudden infant death syndrome (SIDS) has decreased primarily as a result of public campaigns aiming to reduce prone sleeping position in babies (27). However, SIDS still remains the major cause of death in apparently healthy infants. Although the precise mechanisms underlying SIDS remain undefined, one of the leading hypotheses posits that recurrent hypoxemia may precede the fatal event and affect gasping and autoresuscitation mechanisms (15, 16, 22).

When exposed to asphyxic conditions, most mammalian species will develop a transient hyperpnea, then apnea, and, within a variable period of time (gassing latency), gasping respiratory activity will emerge and continue until terminal apnea and death (2). Our laboratory has previously shown that, in developing Sprague-Dawley rats, a triphasic gasping response occurs in response to anoxia and that such response becomes monophasic at ~20 days postnatally (14). Using a similar approach, Fewell et al. (4, 5) showed that prenatal exposure to nicotine modifies anoxia-induced gasping in an age-dependent fashion and that autoresuscitation is also adversely affected. However, whether intermittent fetal or postnatal hypoxia modifies gasping activity in the young postnatal rat and has detrimental effects on its ability to autoresuscitate.

METHODS

Time-pregnant Sprague-Dawley rats were purchased from Charles River and used for all experiments. The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. For prenatal intermittent hypoxia exposures, pregnant rats were placed from day 5 of pregnancy until delivery in alternating 6:00 AM to 6:00 PM. Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., 6:00 AM to 6:00 PM). Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., 6:00 AM to 6:00 PM). Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., 6:00 AM to 6:00 PM). Gas was circulated around each of the chambers, attached tubing, and other units at 60 l/min (i.e., 6:00 AM to 6:00 PM).

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gas was also circulated through a molecular sieve (type 3A, Fisons, Cheshire, UK) so as to remove ammonia. Humidity was measured and maintained at 40–50% by circulating the gas through a freezer and silica gel. Ambient temperature was kept at 22–24°C. Control animals were exposed to circulating normoxic gas in one of the chambers.

**Respiratory measurements.** Respiratory signals elicited by displacement of the chest were acquired in the restrained animals placed in a size-appropriate head-out plethysmographic chamber (Buxco Electronics, Troy, NJ) by using the barometric method (1,21), such that gas mixtures could be rapidly changed as needed. For all experiments, environmental temperature was maintained constant, slightly below the thermoneutral range for rat pups (30°C). Pressure changes in the chamber due to the inspiratory and expiratory displacements were measured by using a high-gain differential pressure transducer (model MP45-1, Validyne), displayed on the computer screen, and continuously digitized and stored onto a Macintosh Personal Computer System at 125-Hz sampling frequency by using MacLab Digital Acquisition Software (ADInstruments, Castle Hill, Australia) for subsequent offline analysis.

**Experimental protocol.** After a short period of acclimatization to the chamber (~15–20 min), 5-day-old pups from either experimental group were exposed to asphyxic gas (95% N₂-5% CO₂), and gasping was allowed to continue until death. The temporal delays in achieving a complete gas switch were ~10 s. In a subset of animals, room air was rapidly introduced during the second minute of the characteristic third phase of gasping as previously described (11,13,14). This phase is easily recognizable from the increased frequency of gasps (>2 gasps/min) and the exclusive presence of inspiratory efforts (6,13,14). The ability to autoresuscitate was documented (6,10,11,13,14,17,18,23,24).

**Analysis.** Data are shown as means ± SD. The gasping variables quantitated included gasp latency, i.e., the period of time from onset of primary apnea and animal immobility to the first gasp, the duration of gasps, and the gasping frequency as previously described (14). Differences between the various treatment groups were compared by two-way analysis of variance and the Newman-Keuls multiple-range test for multiple comparisons. For comparisons of autoresuscitation rates, one-way analyses of variance and χ² analyses were conducted followed by Fisher exact test as appropriate. A P value of <0.05 was considered to achieve statistical significance.

**RESULTS**

IHRA pups were smaller at birth (5.4 ± 0.4 g) compared with RARA (7.2 ± 0.3 g; P < 0.001) and RAIH pups (7.0 ± 0.3 g; P < 0.001 vs. IHRA; P = not significant vs. RARA). At postnatal day 5, IHRA pups were still smaller (8.6 ± 0.5 g), and RAIH pups showed significant decreases in weight gain (7.7 ± 0.5 g) compared with RARA pups (10.7 ± 0.5 g; P < 0.001 vs. IHRA and RAIH). However, brain weights were similar for all three groups at all postnatal ages (P = not significant).

The triphasic gasping pattern occurred in all experimental groups (Fig. 1). Mean gasp latencies were similar in RAIH (32 ± 6 s), IHRA (34 ± 8 s), and RARA pups (30 ± 7 s; n = 20/group; P = not significant). However, the mean total gasp duration was significantly reduced in both IHRA and RAIH compared with RARA pups (18.6 ± 3.1, 15.6 ± 2.9, and 24.7 ± 4.6 min, respectively; Fig. 2; P < 0.001). Similarly, the overall number of gasps was diminished in IHRA and RAIH pups (Fig. 2; P < 0.001). Indeed, the mean total number of gasps was 31.5 ± 5.2 in IHRA, 26.2 ± 5.1 in RAIH, and 44.2 ± 7.3 in RARA pups. The mean gasp frequency was not modified during each of the gasping phases (Fig. 2).

The ability to autoresuscitate from gasping during phase 3 was assessed in thirty 5-day-old pups from the various litters corresponding to each experimental group. Significantly fewer IHRA (21 of 30) and RAIH (19 of 30) pups successfully autoresuscitated compared with RARA pups (28 of 30; Fig. 3; P < 0.03 RARA vs. IHRA and P < 0.01 RARA vs. RAIH respectively).

**DISCUSSION**

In this study, we show that intermittent hypoxia occurring either prenatally or during the initial days of life adversely affects gasp generating mechanisms and that the ability to autoresuscitate during the late phase of gasping is markedly curtailed.

The neural substrate for gasp generation has been assigned to a discrete region within the caudal brain stem, the lateral tegmental field of the medulla (8,9,25,26). This concept has been further expanded to incorporate the possibility that the critical regions for respiratory rhythmicity may dynamically reconfigure to generate gasping (19,25,26). Studies from our laboratory have previously shown that N-methyl-D-aspartate glutamate receptors are critically involved in
particular components of gasp maturation (11), specifically in the early phases of respiratory activity that follow primary apnea. In addition, our laboratory has shown that increased neuronal nitric oxide synthase expression and activity occur with increasing postnatal age within the neural sites responsible for gasp generation and underlie some of the characteristic developmental changes in gasp activity (12, 13). The overall changes in N-methyl-D-aspartate receptor and neuronal nitric oxide synthase expression associated with intermittent hypoxia during fetal and early postnatal life are currently unknown.

The overall reduction in gasping duration and total gasp number as well as the increased failure rates during autoresuscitation attempts could reflect deficient glycogen stores. IHRA pups were smaller at birth, and, although they demonstrated some catch-up growth after birth, they remained smaller than controls. Similarly, RAIH pups showed lesser weight increments than RARA, suggesting that fuel tissue reserves may be compromised in both experimental groups. Relative depletion of cardiac glycogen has been proposed as a potential mechanism underlying significant differences in the autoresuscitation of SWR compared with BALB/c weanling mice (3). Similarly, depletion of cardiac glycogen with recurrent autoresuscitations was advanced as one of the mechanisms potentially leading to autoresuscitation failure in the usually successful autoresuscitating BALB/c mice (3). However, external fuel delivery as brought about by glucose supplementation before the asphyxic exposure exerts divergent effects on gasping and autoresuscitation (28). Indeed, Yuan and colleagues (28) have shown that hyperglycemia will prolong the overall duration of gasping but reduce the frequency of autoresuscitation. Yuan et al. (29) also examined the role of adrenergic receptors, and found that adrenalectomy shortened gasping duration in both 1-day-old and 8-day-old rats, whereas the nonselective α-receptor antagonist phentolamine reduced the duration of gasping in 1-day-old rats but prolonged this duration in 8-day-old rats, with similar effects on gasping by the nonselective β-receptor antagonist propranolol. Thus these investigators concluded that gasping requires intact adrenal function and primarily involves α-adrenergic receptors. However, no significant differences were found in α-adrenergic receptor expression within the putative gasping centers of infants who succumbed to SIDS (20).

An interesting analogy to intermittent hypoxia can be drawn with prenatal nicotine exposures. Continuous administration of nicotine via an osmotic pump to pregnant rats from day 6 of pregnancy was associated with decreased gasp duration and with reduced ability to autoresuscitate until postnatal age 5–6 days but not thereafter (4, 5, 21). The adverse effect of perinatal nicotine was further shown by its acute administration to neonatal piglets at doses leading to serum levels similar to those found in infants of smoking mothers (7). In these experiments, laryngeal stimulation not

Fig. 2. Overall mean number of gasps (A), gasp duration (B), and gasp frequency (C) in 5-day-old rat pups exposed to an asphyxic challenge (95% O2-5% CO2) and born after intermittent hypoxia during pregnancy (IHRA), exposed to postnatal intermittent hypoxia (RAIH), or controls (RARA). Values are means ± SE; n = 20/group. *P < 0.001, IHRA vs. RARA. ‡P < 0.001, RAIH vs. RARA.

Fig. 3. Percentage of successful (+) autoresuscitations (%AR) after reintroduction of room air gas in IHRA, RAIH, or RARA pups. n = 30/group. * P < 0.03, IHRA vs. RARA. ‡P < 0.01, RAIH vs. RARA.
only induced more severe central apneas in nicotine-treated piglets but also was associated with reduced ventilatory compensation after induced apnea (7). Thus both prenatal and early postnatal exposure to nicotine lead to impaired protective responses of rat pups that may sustain life during exposure to asphyxia. It should be emphasized, however, that the potentially deleterious effects of prenatal nicotine on gasping have not been consistently found (23). Whether these discrepant findings reflect the differences in dosages used or in the susceptibility of particular rat substrains remains unclear.

Although environmental temperature can modify gasping duration and the degree of successful autoresuscitation (24), we cannot a priori ascribe a role to this particular factor by virtue of our strict standardization of environmental temperature during all of our experiments. Similarly, all animals were studied at the same postnatal age, such that it is unlikely that there were marked differences in their developmental stages so as to induce the relatively robust changes in gasping and autoresuscitation characteristics associated with increasing postnatal age (6, 14, 18).

In summary, perinatal intermittent hypoxic exposures are associated with altered defense mechanisms against asphyxia, manifesting both as decreased total survival and as a relative inability to autoresuscitate. Although the implications of such increased susceptibility are unknown, we postulate that it may play a role in conditions such as SIDS.

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