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Influence of Repeated Upper Airway Obstruction on the Arousal and Cardiopulmonary Response to Upper Airway Obstruction in Lambs

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ABSTRACT. Experiments were done on five lambs to determine if repeated obstruction of the upper airway influences the arousal and cardiopulmonary response to upper airway obstruction. Each lamb was anesthetized and instrumented for recordings of electrocorticogram, electrooculogram, nuchal and diaphragm electromyograms, and measurements of arterial blood pressure and arterial hemoglobin oxygen saturation. A tracheostomy was done and a fenestrated tracheostomy tube placed in the trachea. The animals were studied after a 3-day recovery period. During a study, a 5F balloon-tipped catheter was inserted into the tracheostomy tube so that air flow could be obstructed by inflating the balloon. The balloon was inflated each time the animal went to sleep for approximately 100 consecutive epochs (17 to 30 h) and the time to arousal and the arterial hemoglobin oxygen saturation at arousal were recorded. Upper airway obstruction was terminated by deflating the balloon once the animal aroused from sleep. Arousal occurred from both sleep states during upper airway obstruction but was delayed in active sleep compared to quiet sleep. The time to arousal and the decrease in arterial hemoglobin oxygen saturation were significantly increased with repeated upper airway obstruction only during active sleep. Inasmuch as it is possible that alterations in the arousal response to respiratory stimuli play a role in sudden infant death, studies to investigate the mechanisms of the state-specific changes in the arousal response to upper airway obstruction are warranted. (*Pediatr Res* 23: 191–195, 1988)

Abbreviations

UAO, upper airway obstruction
SaO_2, arterial hemoglobin oxygen saturation
QS, quiet sleep
AS, active sleep

The arousal response from sleep, once characterized as "the forgotten response to respiratory stimuli (1)," is an important protective response that may prevent severe hypoxemia and death during an apneic episode. This is particularly evident during obstructive apnea, as restoration of upper airway patency and resumption of tidal ventilation, at least in adults, are generally preceded by arousal from sleep (2–4). Thus, any factor that increases the time to arousal during obstructive apnea could be potentially dangerous. The arousal response to a number of stimuli (e.g. acoustic stimuli) is known to habituate or to decrease with repeated stimulation (5). The present experiments were done to test the hypothesis that repeated obstruction of the upper airway influences the arousal and cardiopulmonary response to upper airway obstruction in lambs.

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METHODS

Five lambs ranging in age from 8 to 14 days were studied. Each lamb was separated from its ewe 1 to 3 days after birth and was housed in our laboratory in a plexiglas cage with continuous access to milk (Lamb Milk Replacer, Land O'Lakes, Inc., Fort Dodge, IA).

Surgical preparation. Each lamb underwent one operation before the study. The operation was done between 5 and 11 days of age when a double-lumen fiberoptic catheter oximeter (model U440 Opticath, Oximetrix, Inc., Mountain View, CA; 90% response to a step change in SaO_2 within 5 s) was inserted to the thoracic aorta via a femoral artery for continuous measurement of arterial hemoglobin oxygen saturation and blood pressure. Electrodes for the following recordings were also implanted: electrocorticogram, electrooculogram, nuchal electromyogram, and diaphragm electromyogram. A reference wire was sutured into the subcutaneous tissue of the scalp. The electrodes were made in our laboratory and were paired, Teflon-coated, multistranded stainless steel wires (AS 633, Cooner Wire Co., Chatsworth, CA). The proximal end of each wire was bared and soldered to the appropriate pin of an 18 pin electrical plug.

A tracheotomy was also performed and a fenestrated tracheostomy tube (Shiley, Inc., Irvine, CA) placed in the trachea. This tracheostomy tube allows one to select whether an animal breathes entirely through the opening of the tracheostomy tube (cuff inflated, inner cannula in place) or breathes entirely through its upper airway (cuff inflated, decannulation cannula in place). After surgery, the decannulation cannula was inserted into the tracheostomy tube so that airflow during tidal respiration would be through the upper airway. The lambs were allowed to recover from surgery in a Shor-Line intensive care unit for small animals (Schroer Manufacturing Company, Kansas City, MO) and were then placed in a plexiglas study cage in our sleep laboratory but were not studied before the 3rd postoperative day. The lambs received antibiotics (procaine penicillin G 100,000 U/kg; gentamicin sulfate 2 mg/kg) for 5 days beginning on the day of surgery.

Conditions of observations. Our sleep laboratory consists of a large room (12 × 26 ft) which contains two sound-attenuating chambers (W 4 ft, H 6.5 ft, L 6.5 ft). Each chamber has a one-way viewing mirror as well as a closed circuit video system to observe the lambs. Temperature, sound, and lighting can be precisely controlled in each chamber. Our recording equipment is kept in the room adjacent to the chamber. Before a study, a partition is placed in the cage to prevent the lamb from turning around once the catheter and the optical connector and electrical plug are connected. However, the lamb can still lie down, stand up, and feed *ad libitum.*

For a study, the vascular catheter is connected to a strain gauge manometer (Gould P23ID, Gould, Inc., Oxnard, CA) using rigid pressure monitoring tubing and the optical connector is connected to the optical module of the oximeter processor; the strain gauge manometer is placed at the approximate level of the heart when the animal is lying down. A 5F balloon-tipped catheter is inserted through the decannulation cannula into the tracheostomy tube so that air flow can be obstructed by inflating the balloon. The electrical plug is connected to four differential high impedance probes; a heavy duty cable connects the differential high impedance probes to A.C. preamplifiers (model 7P5 Wide Band A.C. EEG pre-amplifier, Grass Medical Instruments, Quincy, MA) in the adjacent room. The electrophysiological signals are high-pass filtered using the ½ amplitude low frequency response control on the A.C. preamplifiers (electrocorticogram 1.0 Hz, electrooculogram 0.3 Hz, and electromyograms 3 Hz).

The following electrophysiological criteria were used to define behavioral state once the animal was lying down. During quiet wakefulness, the electrocorticogram shows a fast wave-low voltage pattern; there are occasional eye movements and there is tonic activity on the nuchal electromyogram. During QS, the electrocorticogram shows a slow wave-high voltage pattern; there are no eye movements and there is tonic activity on the nuchal electromyogram. During AS, the electrocorticogram shows a fast wave-low voltage pattern, there are rapid eye movements on the electrooculogram, and there is no activity on the nuchal electromyogram and there are occasional fast ear, facial, and limb twitches. Each lamb was allowed to cycle through at least one epoch of QS before the experiment actually began so that we could determine the amplitude of the integrated electrocortical activity and set strict criteria for defining QS.

The following electrophysiological criteria were used to define arousal from sleep. During QS, the point of arousal was determined by a change in the electrocorticogram from a high voltage-slow wave pattern to a low voltage-fast wave pattern. During AS, the point of arousal was determined by a return of tonic activity on the nuchal electromyogram.

Experimental protocol. During the study, systemic arterial blood pressure, SaO_2, and electrophysiological signals were recorded on a Grass model 7 polygraph (Grass Medical Instruments) and the lambs were monitored on a closed-circuit video system. The upper airway was obstructed each time the lamb went to sleep for approximately 100 epochs. For each epoch, measurements were made during a 30-s control period of normal tidal respiration and during the experimental period of UAO. Airway obstruction was terminated during the experimental period by deflating the balloon once the animal aroused from sleep. Because the mean epoch lengths of QS and AS of chronically instrumented lambs during this age range are 6 to 11 min and 3 to 4 min, respectively (Johnson P, unpublished data), control measurements were made approximately 2 min after the lamb entered QS and 30 s after the lamb entered AS. Experiments began between 0800 and 1000 h and continued until sufficient data were collected.

Statistical analysis. For every animal, we determined an average value for each variable during the control period and during the experimental period immediately preceding arousal (heart rate and blood pressure—five cardiac cycles; respiratory frequency and integrated diaphragm activity—five respiratory cycles if possible) for QS and AS. For statistical analysis, data from the first five UAO in each sleep state were compared to the data from the last five UAO in each sleep state to determine the effect of repeated UAO. To analyze the data statistically, we performed a three-factor analysis of variance for repeated measures of the same variable to determine if state (*i.e.* QS *versus* AS), order (*i.e.* first five UAO *versus* last five UAO), or period (control *versus* experimental) affected SaO_2, blood pressure, heart rate, or respiratory rate (6). A two-factor analysis of variance for repeated measures of the same variable was done to determine if state or order affected the time to arousal or the percent change in diaphragm activity (6).

RESULTS

As we have previously found (7–9), arousal occurred from both sleep states during UAO but was delayed ($p < 0.05$) during AS compared to QS (Table 1). The time to arousal was increased ($p < 0.05$) and the SaO_2 at arousal was decreased by repeated UAO, but only in AS (Table 1). This decrease in SaO_2 at arousal that occurred after repeated UAO in AS appeared to be more closely related to total elapsed time during an experiment than to time between epochs during an experiment (Fig. 1).

Heart rate decreased during UAO before arousal in QS and AS (Table 2). This effect was accentuated by repeated UAO in AS. There were no significant changes in blood pressure.

Respiratory rate tended to decrease during UAO before arousal in both QS and AS (Table 3). The increase in diaphragm activity that occurred during UAO before arousal was accentuated after repeated UAO, again, only in AS.

DISCUSSION

Our study provides new information about the arousal response from sleep to UAO in young lambs. Arousal occurred from both sleep states during UAO but was delayed in AS compared to QS (Table 1). Furthermore, only during AS was the time to arousal increased and the SaO_2 at arousal decreased by repeated UAO.

Although it is known that the arousal response from sleep habituates to some environmental stimuli [*e.g.* acoustic stimuli (5)], we are unaware of other investigators who have provided direct evidence that the arousal response from sleep to a respiratory stimulus changes after repeated exposure to the stimulus. The fact that the arousal response was altered only during AS may be related to the different mechanisms initiating arousal from QS and AS during UAO in lambs (7, 9). Arousal from QS during UAO in lambs occurs rapidly before any significant changes in the SaO_2 (7) (Table 1); the arousal response is not delayed by increasing the inspired oxygen tension before UAO

Table 1. *Influence of repeated UAO on the arousal response from sleep to UAO in lambs**

	QS		AS		
	First 5 epochs	Last 5 epochs	First 5 epochs		Last 5 epochs
Time to arousal (S, O) (s)	7 ± 1	6 ± 2	18 ± 6	†	28 ± 3
SaO_2 (%) (S, O, P)					
Control	93 ± 1	92 ± 2	92 ± 2 †		92 ± 2 †
Arousal	91 ± 2	90 ± 3	80 ± 7	†	62 ± 9

* Values are means ± 1 SD for $n = 5$. Significant differences by multivariate analysis of variance ($p < 0.05$) are indicated as follows: state (S), order (O), period (P).

† Significant differences by Duncan's multiple range test are indicated by a dagger.

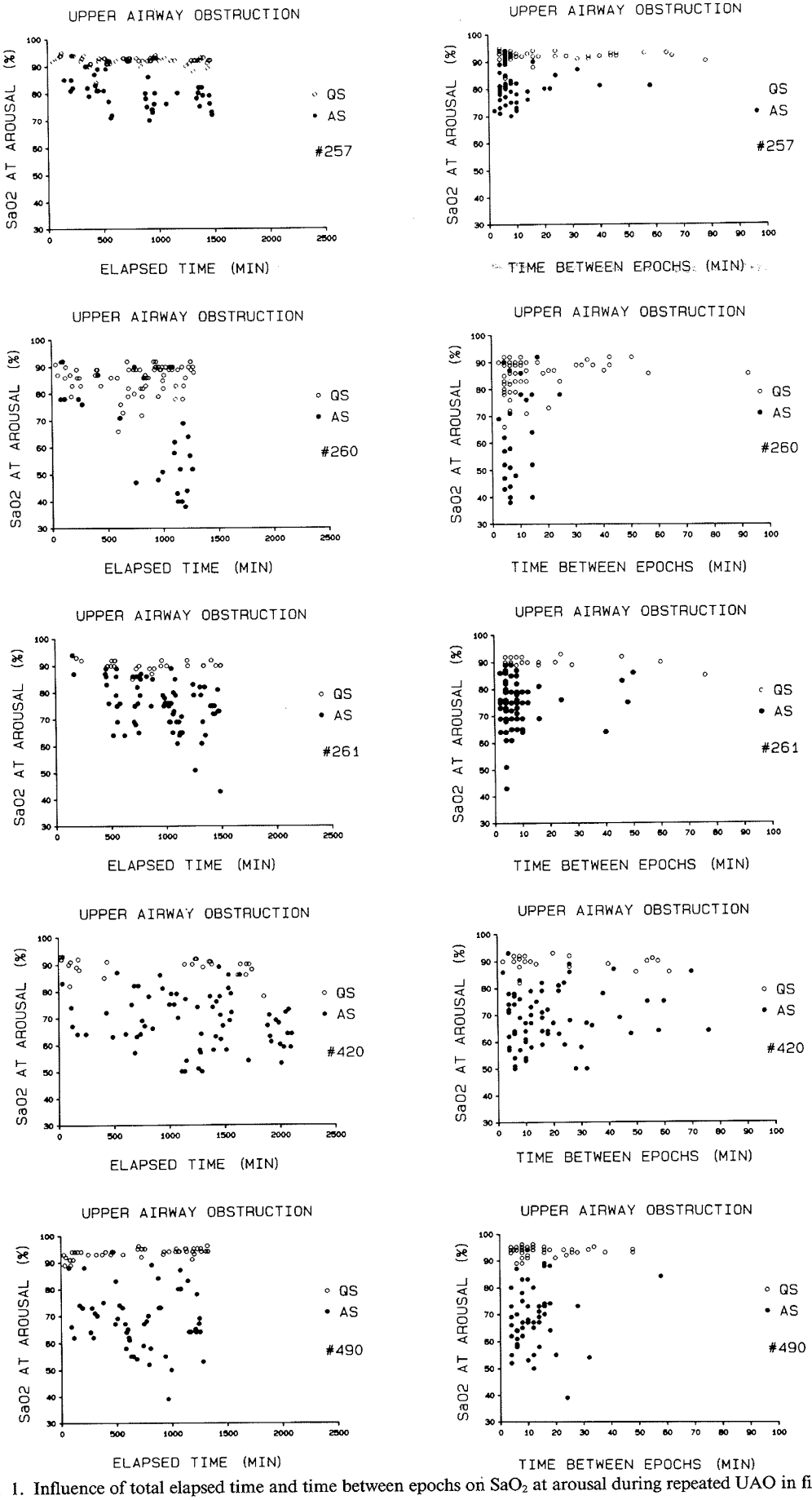

Fig. 1. Influence of total elapsed time and time between epochs on SaO_2 at arousal during repeated UAO in five lambs.

Table 2. *Influence of repeated UAO on the cardiovascular response to UAO in lambs**

	QS		AS	
	First 5 epochs	Last 5 epochs	First 5 epochs	Last 5 epochs
Blood pressure (mm Hg)				
Control	97 ± 5	96 ± 7	98 ± 4	100 ± 8
	74 ± 11	72 ± 8	74 ± 8	73 ± 5
Arousal	99 ± 5	98 ± 8	99 ± 5	100 ± 9
	74 ± 11	72 ± 8	70 ± 5	64 ± 14
Heart rate (bpm) (S, P)				
Control	180 ± 19 †	180 ± 15 †	158 ± 15 †	151 ± 14 †
Arousal	167 ± 22	167 ± 14	116 ± 26 †	100 ± 16

* Values are means ± 1 SD for $n = 5$. Blood pressures are presented as systolic/diastolic in mm Hg. Significant differences by multivariate analysis of variation ($p < 0.05$) are indicated as follows: state (S), order (O), period (P).

† Significant differences by Duncan's multiple range test are indicated by a dagger.

Table 3. *Influence of repeated UAO on the respiratory response to upper airway obstruction in lambs**

	QS		AS	
	First 5 epochs	Last 5 epochs	First 5 epochs	Last 5 epochs
Respiratory rate (breaths/min) (S, P)				
Control	41 ± 4	37 ± 9	60 ± 3 †	63 ± 30 †
Arousal	38 ± 2	34 ± 7	45 ± 7	37 ± 22
Diaphragm activity at arousal (%) (S)	+46 ± 27	+30 ± 25	+51 ± 31 †	+97 ± 13

* Values are means ± 1 SD for $n = 5$. Significant differences by multivariate analysis of variation ($p < 0.05$) are indicated as follows: state (S), order (O), period (P).

† Significant differences by Duncan's multiple range test are indicated by a dagger.

(9). In contrast, arousal from AS during UAO in lambs occurs after a longer period of time and significant decreases in the SaO_2 do occur (7) (Table 1); the arousal response is significantly delayed by increasing the inspired oxygen tension before UAO (9). Thus, it appears that the arousal response from AS is primarily mediated by the peripheral chemoreceptors in response to hypoxemia, whereas the arousal response from QS during UAO may be mediated by lung or chest wall mechanoreceptors. The former mechanism appears to change after repeated stimulation, whereas the latter does not. In addition, we have recently found evidence of an arousal response decrement from both QS and AS in response to rapidly developing hypoxemia (*i.e.* hypocapneic hypoxemia, FIO_2 0.05) after repeated exposure to rapidly developing hypoxemia in young lambs (10). These data, along with the data from the present study, allow one to suggest that the arousal response decrement is not sleep state dependent, but rather, dependent on the stimulus initiating arousal.

Although our study was not designed to determine the mechanism of the arousal response decrement after repeated UAO, our results allow us to speculate on the mechanism. We do not believe that the arousal response decrement after repeated UAO is due to sleep fragmentation per se, as sleep fragmentation, produced by intense auditory stimuli over a 36- to 42-h time period, does not significantly affect the arousal response from QS to AS to UAO in lambs (8). Response decrement after repeated stimulation can occur through a number of mechanisms including sensory adaptation, nerve accommodation, effector fatigue, and habituation (5). If the arousal response from AS during UAO is, indeed, initiated by the peripheral chemoreceptors in response to hypoxemia, then the response decrement may be due to sensory adaptation (*i.e.* a resetting of the peripheral chemoreceptors). It is possible that a resetting of the peripheral chemoreceptors occurs after multiple episodes of hypoxemia and that this is responsible for the arousal response decrement after repeated UAO. However, our cardiovascular data would allow one to suggest that the peripheral chemoreceptor response to hypoxemia is not attenuated following repeated UAO. The primary heart rate response to carotid chemoreceptor stimulation, in the absence of lung inflation, is bradycardia (11, 12). We observed a decrease in heart rate during UAO in AS that was accentuated with repeated UAO (Table 2). This would suggest that the chemoreceptor response is not attenuated after repeated UAO. It also seems unlikely that the response decrement is due to effector fatigue as a novel stimulus, such as opening the door of the study chamber, would readily arouse the lambs before and after repeated UAO. At present, we believe the response decrement observed during AS in response to UAO is best explained as being due to habituation of the arousal response to a specific stimulus (*i.e.* chemoreceptor stimulation by hypoxemia). The specific site(s) in the central nervous system as well as the specific mechanism(s) responsible are presently unknown.

The arousal response from sleep has been suggested to be an important protective response that may prevent severe hypoxemia and death during an apneic episode (1). Although apnea occurs to some extent in almost all preterm (13) and term infants (14), little is known about the mechanism(s) that terminate an apneic episode. Read and Henderson-Smart (13) have observed that prolonged apnea (*i.e.* apnea of 20 s or more) occurs in the majority of babies less than 30 wk gestation, in about 50% of babies at 30–32 wk gestation, and in about 7% of babies at 34–35 wk gestation (13). Furthermore, Southall *et al.* (14) have presented data showing that 34 of 50 (*i.e.* 68%) randomly selected, healthy term-infants studied between 1 and 15 days postnatally had apneic episodes of 10 s or more and that the 95th percentile extended up to 18 s. Guntheroth (15) has suggested that these infants do not die because of an intact arousal response and has hypothesized that the crucial area of abnormal physiology in sudden infant death syndrome is arousal after apnea. The importance of the arousal response has been suggested to be at least 2-fold. First, wakefulness per se is a potent stimulus to breathing. Second, arousal permits the initiation of behavioral and ventilatory response to the stimulus; arousal is generally thought to precede resumption of tidal ventilation during apnea (2–4).

Two recent studies have indeed provided evidence of an abnormal arousal response to hypoxemia in infants who have had an apparent life-threatening event (16, 17). McCulloch *et al.* (16) found that only one of 11 infants who had had an apparent life-threatening event aroused in response to progressive alveolar hypoxia (FIO_2 0.15) compared to 14 of 22 normal infants. Subsequently, van der Hal *et al.* (17) found that nine of nine control infants aroused in response to more pronounced alveolar hypoxia (FIO_2 0.11) compared to only 19 of 50 infants who had had an apparent life-threatening event. Although these data might support the hypothesis that an abnormal arousal response to hypoxemia plays a role in the final pathway to the sudden infant death syndrome, one also has to implicate failure of other "backup" mechanism(s) (*e.g.* gasping or circulatory failure) because these infants did not die (18).

The results of our studies may have implications for the sudden infant death syndrome. If the final event is apnea, as has been hypothesized (15, 19), our previous data would allow one to speculate that if the rate of change of arterial oxygen is great enough during apnea in AS, arousal may fail to occur before electrocortical signs of cerebral hypoxia and primary apnea occur

(20). In addition, data from our present study would allow one to speculate that if an infant is repeatedly exposed to hypoxemia—either as a result of multiple apneic episodes or hypoxemia during sleep as a result of gas exchange abnormalities—that the arousal response to apnea might be impaired. If in addition there is a deficit in the gasping mechanism or if the circulation fails before the onset of gasping, death could quickly ensue.

REFERENCES

1. Phillipson EA, Sullivan CE 1978 Arousal: the forgotten response to respiratory stimuli. Am Rev Respir Dis 118:807–809
2. Gastaut H, Tassinari CA 1966 Polygraphic study of the episodic diurnal and nocturnal (hypnic and respiratory) manifestations of the Pickwickian Syndrome. Brain Res 2:167–186
3. Walsh RE, Michaelson ED, Harkerload LE, Zichelboim A, Sackner MA 1972 Upper airway obstruction in obese patients with sleep disturbances and somnolence. Ann Intern Med 76:185–192
4. Remmers JE, DeGroot WJ, Sauerland EK, Anch AM 1978 Pathogenesis of upper airway occlusion during sleep. J Appl Physiol 44:931–938
5. Sharpless S, Jasper H 1956 Habituation of the arousal reaction. Brain 79:655–680
6. Winer BJ 1971 Multifactoral experiments having repeated measures on the same elements. In: Statistical Principles in Experimental Design. McGraw-Hill Book Company, New York, pp 514–599
7. Fewell JE 1985 Arousal and cardiorespiratory responses to upper airway obstruction during sleep in lambs. In: Jones CT, Nathanielsz PW (eds) The Physiological Development of the Fetus and Newborn. Academic Press, London, pp 217–222
8. Fewell JE 1987 Influence of short-term sleep fragmentation produced by intense auditory stimuli on the arousal response to upper airway obstruction in lambs. J Dev Physiol (9:409–418)
9. Baker SB, Fewell JE 1987 Effect of hyperoxia on the arousal response to upper airway obstruction. Pediatr Res 21:116–120
10. Fewell JE, Konduri GK 1988 Does repeated exposure to rapidly developing hypoxemia influence the interaction between oxygen and carbon dioxide in causing arousal from sleep in lambs? In: CT Jones (ed) Fetal and Neonatal Development, Perinatology Press, Ithaca, NY (in press)
11. Daly M de B, Scott MJ 1962 An analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors in the dog. J Physiol 162:555–573
12. Daly M de B, Scott MJ 1963 The cardiovascular response to stimulation of the carotid body chemoreceptors in the dog. J Physiol 165:179–197
13. Read DJC, Henderson-Smart DJ 1984 Regulation of breathing in the newborn during different behavioral states. Ann Rev Physiol 46:675–685
14. Southall DP, Richards J, Brown DJ, Johnston PGB, DeSwiet M, Shinebourne EA 1980 24-hour recordings of ECG and respiration in the newborn infant with findings related to sudden death and unexplained brain damage in infancy. Arch Dis Child 55:7–16
15. Guntheroth WG 1983 Arrhythmia, apnea or arousal? In: Tildon WT, Rolder LM, Steinschneider A (eds) Sudden Infant Death Syndrome. Academic Press, London, pp 263–269
16. McCulloch K, Brouillette RT, Guzzetta AJ, Hunt CE 1982 Arousal responses in near-miss sudden infant death syndrome and in normal infants. J Pediatr 101:911–917
17. van der Hal AL, Rodriguez AM, Sargent CW, Platzker ACG, Keens TG 1985 Hypoxic and hypercapneic arousal responses and prediction of subsequent apnea in apnea of infancy. Pediatrics 75:848–854
18. Hunt CE, Brouillette RT 1987 Sudden infant death syndrome: 1987 perspective. J Pediatr 110:669–678
19. Steinschneider A 1972 Prolonged apnea and the sudden infant death syndrome: clinical and laboratory observations. Pediatrics 50:646–654
20. Fewell JE, Baker SB 1987 The arousal response from sleep to rapidly developing hypoxemia in lambs. Pediatr Res (22:471–477)