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Birth-Related Activation of Preprotachykinin-A mRNA in the Respiratory Neural Structures of the Rabbit

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ABSTRACT. The perinatal ontogeny of preprotachykinin-A gene expression was assessed in central respiration-related structures. On the day of birth, there was an enhanced expression of preprotachykinin-A mRNA in the region of the nucleus tractus solitarii, the primary relay station for respiratory and cardiovascular reflexes. This increased expression was also seen in the pups delivered by cesarean section and allowed to breathe for a couple of hours as compared with their littermates, which were not allowed to breathe at all. On the basis of this finding, we suggest that the commencement of continuous breathing at birth, unlike the episodic breathing of fetal life, is associated with the enhanced expression of preprotachykinin-A mRNA in the nucleus tractus solitarii. (*Pediatr Res* 29: 369–371, 1991)

Abbreviations

DRG, dorsal respiratory group
nTS, nucleus tractus solitarii
PPT-A, preprotachykinin-A
STR, striatum
E31, 1 d before expected delivery
P0, day of birth
P1... P8, number of days after birth

Birth is a momentous event for the fetus. The ability to cope in the new environment requires the establishment of effective continuous respiration. Although the fetus demonstrates breathing movements, these are episodic in nature (1). Respiration is partially inhibited at this stage and poorly controlled by metabolic needs. This inhibition has been postulated to be due to a dominance of inhibitory neuromodulators in fetal life (2, 3). At birth, there is an increase of excitatory neuroactive agents (4). Among the latter is the tachykinin, substance P, which is known to stimulate respiration (5). We have earlier shown that substance P causes a more pronounced increase in ventilation in the youngest animals as compared with older ones (6).

Substance P, first isolated by von Euler and Gaddum (7), belongs to a family of structurally related peptides termed the tachykinins. It is abundantly present both in the central and peripheral nervous systems (8). The other mammalian tachykinins are neurokinin-A and neurokinin-B. Substance P and neurokinin-A are encoded by the same gene, PPT-A. PPT-A gene undergoes alternative splicing to give rise to three forms, of which α PPT-A mRNA encodes only for substance P, whereas the β- and γ-PPT-A mRNA encode for both substance P and neurokinin-A (9, 10). A recent study demonstrated the presence of PPT-A mRNA-containing cells in the nTS and none in the cerebellum (11). Significant quantities of substance P-like immunoreactivity was found to be localized in the nTS (12), which plays an important role in the reflex transmission of visceral afferent inputs.

The region of the nTS has been shown to receive multiple inputs from peripheral sources such as respiratory, cardiovascular, gustatory, and gastrointestinal systems via the V, VII, IX, and X cranial nerves (13, 14). Substance P, which is abundant in nTS, is known to mediate baro- and chemoreceptor inputs to the nTS; hypoxic provocation results in an increased release of substance P in the region of nTS (15). Denervation of the IX and X cranial nerves decreases the immunoreactivity for substance P in the nTS (16). Thus, substance P has been suggested to have a significant physiologic role in chemo- and baroreceptor transduction mechanisms.

Two distinct populations of substance P-immunoreactive neurons have been described in the ventrolateral medulla: *1*) a rostral group situated ventral to the facial nucleus and *2*) a caudal group lying ventrolaterally spanning the rostro-caudal length of the inferior olivary nucleus (17). In a developmental study on substance P immunoreactivity in the rabbit nTS using the technique of RIA, higher levels have been shown in younger animals than in adults (18). However, the time points just around birth are lacking in this study. Therefore, a study on the ontogenetic expression of PPT-A mRNA was undertaken especially around the period of birth in rabbit pups. Rabbits, which are known to be precocial developers, closely resemble human infants with regard to the maturation of sleep-wakefulness cycles (19).

MATERIALS AND METHODS

Animals. Rabbit pups of various ages (E31, P0, P1, P3, P8, and adult) were killed with an overdose of pentobarbital. The following regions, depicted in Figure 1, were rapidly dissected out, frozen on dry ice, and stored at −70°C until processed further: *1*) the DRG containing the region of the nTS (however, it should be noted that this area does not specifically contain only nTS and such a resolution could have been obtained by combining with *in situ* hybridization) where the chemoreceptor afferents terminate; *2*) the ventral medullary surface structures where the central chemoreceptors are thought to be located; *3*) STR as a hybridizing control; and *4*) cerebellum as a negative control area.

In the second set of experiments, pregnant rabbits were anes-

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thetized and pups were delivered by cesarean section a day before estimated delivery. Half the pups in the litter were immediately killed, whereas the rest of the pups were placed in a warm environment and allowed to breathe for 2 h.

Prior permission from the Animal Ethical Committee, Stockholm, was obtained for carrying out these experiments.

RNA preparation and blot analysis. The dissected regions from four to six pups were pooled for each age. Frozen tissue samples were homogenized with a Polytron in 4 M guanidine isothiocyanate, 0.1 M β-mercaptoethanol, 0.025 M sodium citrate, pH 7.0. Each homogenate was layered over a 4-mL cushion of 5.7 M CsCl in 0.025M sodium citrate, pH 5.5, and centrifuged at 15°C in a Beckman SW41 rotor at 35 000 rpm for 21 h (20). The recovery of RNA was quantified spectrophotometrically before use in RNA blot analysis. RNA from each sample (30–50 μg) was electrophoresed in 1% agarose gels containing 0.7% formaldehyde and transferred to nitrocellulose filters as previously described (21). The filters were prehybridized in 4 × SSC (1 × SSC = 0.15M NaCl, 0.015M sodium citrate pH 7.0) 40% formamide, 1 × Denhardt's solution, 10% dextran sulphate, and 150 μg/mL of denatured sheared single-stranded salmon sperm DNA for 1 h at 43°C to block nonspecific binding sites on the membrane. The filter-bound RNAs were then hybridized overnight with a 300-bp *Bgl*II-*Bst*EII fragment from a rat PPT-A cDNA clone (22). The fragment was labeled with α-[^{32}P]-deoxycytidine 5′-triphosphate by nick-translation to a sp act of approximately 5×10^8 cpm/μg. The filters were washed at reduced stringency (0.5 × SSC containing 0.1% SDS) at 45°C and exposed to Kodak X-AR5 film at −70°C. Autoradiograms were quantified using a Shimadzu (Tokyo, Japan) CS-9000 densitometer. The filters were subsequently boiled for 5 min in 1% glycerol and then probed with a nick-translated 1.5-kb *Pst*I fragment encoding mouse α-actin (23). The level of PPT-A mRNA in each sample was then normalized relative to the level of actin mRNA.

RESULTS

In the region of the DRG, a 380% increase was seen in the level of PPT-A mRNA on P0 when compared with E31 (Fig. 2*A* and *B*). However, on P1 there was a decrease by 25% and a further decrease was seen on P3. Although the PPT-A mRNA expression increased on P8, the adult levels were lower than postnatal values, which agrees with RIA studies in the rabbit (18) and immunohistochemical findings in the rat (24). A different ontogenetic pattern was seen in the ventral medullary surface structures. Here the prenatal expression was higher than on the day of birth, then decreased until P1, and thereafter gradually increased until adulthood (fig. 2*A* and *B*). In the striatum, there was only a 50% increase on P0 when compared with E31 (Fig. 2*A* and *B*). In agreement with previous *in situ* hybridization studies (11), no PPT-A mRNA was detected in the cerebellum.

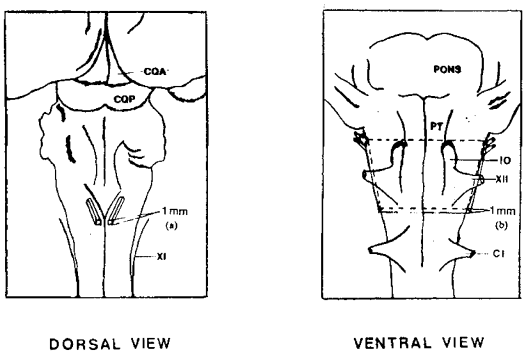

Fig. 1. Drawing of the dorsal and ventral surfaces of the brain stem. The *outline marked as 1 mm* was the area and thickness of this tissue removed for Northern blot analysis. Dorsal view: *CQA,* corpora quadrigemina anteriora; *CQP,* corpora quadrigemina posteriora; *XI,* nervus accessorius; (a), DRG. Ventral view: *PT,* pyramidal tract; *IO,* inferior olivary nucleus; *XII,* nervus hypoglossus; *CI,* nervus cervicalis primus; (b), ventral medullary surface structure.

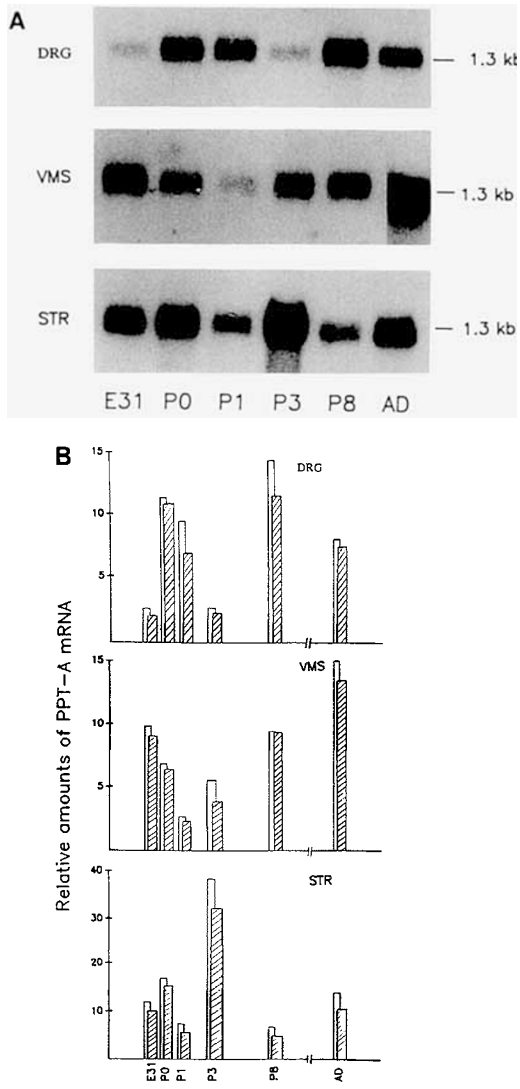

Fig. 2. Ontogenetic pattern of expression of PPT-A mRNA in DRG, ventral medullary surface structure (*VMS*), and STR. *A*, total RNA prepared from the above areas, at the indicated developmental ages, was subjected to electrophoresis in a formaldehyde-containing agarose gel followed by transfer to a nitrocellulose filter. The filter was hybridized to a ^{32}P-labeled rat PPT-A cDNA probe using 2.5×10^7 cpm of the probe (50 ng) followed by washing and autoradiography. AD, adult. *B*, developmental pattern of relative amounts of PPT-A mRNA in DRG ventral medullary surface structure (*VMS*) and STR. Densitometric scannings were performed from two independent experiments as depicted above, one of which is shown in *A*. In each experiment, the amount of PPT-A mRNA was normalized to the level of actin mRNA. *DRG:* An enhanced expression is seen on P0 as compared with E31. *VMS:* Prenatal expression is higher than on the day of birth. *STR:* The increased expression seen at P0 as compared with E31 is lower as compared with the DRG region.

The level of PPT-A mRNA in the region of DRG from pups delivered by cesarean section and not allowed to breathe was closely similar to the level of PPT-A mRNA observed at E31 in the ontogenetic study reported above. However, the DRG region from the pups that had breathed for 2 h showed a 370% increase in the level of PPT-A mRNA as compared with their nonbreathing littermates (Fig. 3). This is similar to the 380% increase seen at P0 compared with E31 in the ontogenetic study of the DRG region (Fig. 2*B*). No difference between the two groups was seen in the STR (Fig. 3).

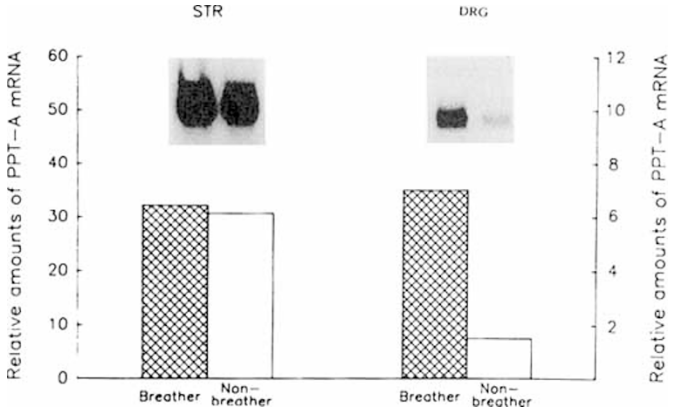

Fig. 3. Breathing-induced changes in PPT-A mRNA expression at birth. Total RNA was prepared, electrophoresed, and transferred to nitrocellulose filters as described in Fig. 2*A*. Densitometric scannings were performed as described in Fig. 2*B*. *DRG:* The level of PPT-A mRNA was enhanced approximately 4-fold in the breathers as compared with the nonbreathers. *STR:* There was no change in the level of PPT-A mRNA between the two groups.

DISCUSSION

The regulation of respiration involves complex interactions of various brainstem nuclei. Conventionally, the major neural structures concerned with the regulation of breathing are located in the medulla in the regions of the nTS, retrofacialis, para- and retroambigualis nuclei, the paragiganto-cellular nuclei involving the structures bordering the ventral surface of the medulla and also the pontine parabrachial area (13).

The results presented here are the first evidence that the expression of mRNA encoding a neuromodulator is enhanced in the first few hours after birth. Earlier studies suggest that the substance P-neuron system develops during the prenatal period of ontogeny (25), long before the establishment of normal synaptic transmission in this area (26). In a study of the developmental characteristics of substance P immunoreactivity within specific brainstem nuclei in the rabbit (18), it was shown that substance P immunoreactivity is highest in the nTS. Higher levels of substance P immunoreactivity were demonstrated postnatally, with a peak at P7 as compared with 4 d before expected delivery in the nTS (18), but the time points just around the period of birth were not studied. Immunohistochemical studies demonstrate only the level of neuropeptides, which are the net results of synthesis and processing. On the other hand, by analyzing mRNA levels we can directly follow the changes in gene activity that may be the underlying mechanism for the changes in peptide levels.

The peripheral chemoreceptors are active in the fetus in late gestation, but it is interesting to note that peripheral chemodenervation does not affect the incidence of fetal breathing movements (27). The control mechanisms behind the switch from intermittent fetal breathing to continuous postnatal breathing have not yet been elucidated. There appears to be a preponderance of inhibitory transmitters during fetal life, but their effect may be overcome at birth by excitatory neuromodulators like substance P. The question of whether or not the increased expression of the gene for substance P is essential for the control of breathing at birth cannot yet be answered. The fluctuating phenomena seen in the expression of the PPT-A gene, especially in the areas of DRG and STR, suggest either the inhibition of operation of negative feedback loops or the lack of precision of negative control by the known inhibitory neuromodulators of respiration (opioids, dopamine) after birth. However, the fluctuation seen at birth in the area of DRG has been more closely examined and an enhanced expression of PPT-A mRNA was shown to occur 2 h after birth (Fig. 3). The enhanced expression of the PPT-A gene in DRG at the transition from intrauterine to extrauterine life may also be associated with either an increase in PO_2 levels or an enhancement in sensory inputs from various peripheral sensors, including lung receptors.

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The Natural History of the Appearance of Apnea of Prematurity

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ABSTRACT. Twenty healthy preterm infants of less than 34 wk gestation were studied with continuous recordings, commencing within 8 h of birth, for up to 1 wk of age to determine the usual time course of the appearance of apnea and to classify apnea types. Airway occlusion studies were also performed on a regular basis to determine whether apneic spells were preceded or followed by a reduction in central respiratory drive. Apneic spells of greater than 15 s duration accompanied by hypoxia or bradycardia occurred in all infants before 24 h of age. The frequency of apneic spells was highest in the first 24 h after birth with a mean frequency of 0.9/h and gradually reduced thereafter, falling to 0.2/h by 5 d of age ($p < 0.01$). Apneic spells were more likely to be obstructive in the first 2 d of life than thereafter ($p < 0.05$). Central apnea was proportionately significantly less frequent during this time period. Reduced respiratory drive, as demonstrated by airway occlusion pressures, was associated with more frequent apnea and was evident at the first occlusion study, which frequently preceded the first significant apnea. (*Pediatr Res* 29: 372–375, 1991)

Abbreviations

SpO_2, pulse oximeter-derived saturation
$P_{0.1}$, airway pressure 0.1 s after initiation of first occluded breath
ANOVA, analysis of variance

It is commonly stated that idiopathic apnea of prematurity develops after the first 24 to 72 h of life and apnea that is noted early requires vigorous investigation (1). The factual basis of this statement is uncertain. Henderson-Smart (2) previously demonstrated that apnea (more than three spells of greater than 20 s duration) developed on the 1st or 2nd postnatal d in 77% of preterm infants. He appears to have used impedance monitoring only and it is uncertain whether any recordings were made for independent confirmation of apnea (3). Carlo *et al.* (4), utilizing 60-min recordings, showed that apneic spells of greater than 5 s duration occurred on d 1 in eight of 10 preterm infants without lung disease.

There appears to be no published information regarding the usual frequency and nature of significant apneic spells in an unselected population of healthy newborn preterm infants who have undergone prolonged recordings.

Our main objective was to ascertain the usual age of the appearance of apneic spells and the relative frequency of obstructive, mixed, and central apnea during the 1st wk of life in preterm infants without respiratory disease. Our secondary objective was to describe the development of respiratory drive, by determining the postnatal progression of occlusion pressures, in infants with and without apnea.

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SUBJECTS AND METHODS

Twenty infants of less than 34 wk gestation with birth weights above the 5th percentile for gestational age were studied. Infants were all born in the Royal Alexandra Hospitals and were entered into the study as soon after birth as possible. We entered consecutive infants who were clinically determined to be free of acute medical problems; in particular, they were without significant respiratory disease. The criteria for this were an oxygen requirement of no more than 30%, respiratory rate less than 70/min, and no clinical suspicion of pneumonia or hyaline membrane disease. Infants requiring any supplemental oxygen had chest radiographs, which were required to be normal. Ultrasound examination of the head was performed in all infants before 4 d of age and again at approximately 2 wk of age. Any infant with intraventricular hemorrhage that distended the ventricles or evidence of intracerebral hemorrhage or periventricular leukomalacia on either examination was eliminated from the study.

After the initial clinical examination and assessment to ensure that the infants were stable, each was monitored with a combination of impedance pneumogram, for the detection of chest wall movement, ECG, pulse oximetry (SpO_2), and end-tidal CO_2 monitoring; some infants were monitored with transcutaneous Po_2 in addition. The analogue outputs of each of these monitors was digitized by a Data-translation DT2800 analogue to digital convertor board (Data Translation, Inc., Marlborough, MA) and then analyzed on a Compaq 286 personal computer (Compaq Computer Corp., Houston, TX), using programs that we wrote using the Asyst programming language (Asyst Software Industries, Rochdale, NY).

At this point, parental consent was sought for the airway occlusion studies and for the continuance of noninvasive monitoring. In the event of parental refusal of the occlusions, consent for continued monitoring alone was requested. This protocol was approved by the clinical investigation committee of the Royal Alexandra Hospitals.

After obtaining consent, airway occlusions were performed as previously described (5). A face mask was applied and the absence of leaks was confirmed. Airflow was monitored by means of a Fleisch 00 pneumotachograph and integrated electronically to give tidal volume. At end-expiration the airway was occluded and during the next breath the resultant airway pressures were recorded. The $P_{0.1}$ was recorded. Three occlusion responses were obtained on each occasion and averaged. This process was performed as soon as possible after birth and then every 12 h for

the next 72 h. Thereafter, occlusions were performed daily until the study terminated.

The study continued until the infant was 1 wk old or until therapy (theophylline) for idiopathic apnea of prematurity was prescribed by the attending physician. Thus, all of the results presented herein are from infants who were not receiving methylxanthines or any other therapy for apnea. The clinical staff had no knowledge of the results of the recordings and clinical diagnosis of apnea was according to the usual unit practice of examination of the nursing records.

At the termination of the study, the recordings were analyzed by means of a semiautomated process (6). The program used will flag an epoch that is considered to contain a potential apneic spell by looking for a combination of any two of the following: loss of end-tidal CO_2, fall in heart rate, and fall in SpO_2. The epoch may then be visually inspected to determine whether a significant apneic spell was present. The criteria for the diagnosis of a significant apneic spell were as follows: lack of nasal airflow for 15 s accompanied by either a fall in heart rate 20% below the previous mean heart rate or a fall in SpO_2 of 10 percentage points. When a spell was identified, its duration and time of occurrence were noted and it was classified into central, obstructive, or mixed. Central spells were those in which there were no obstructed breaths during the apnea. The presence of one or more obstructed respiratory efforts prompted the diagnosis of a mixed or obstructive apneic spell, which were further differentiated by the presence of a period of absent chest wall movement of at least 3 to 4 s duration.

Apnea frequency was calculated on a daily basis. For the first 24 h, the apnea frequency was calculated as the number of apneas noted before 24 h of age divided by the number of hours of recording performed. Thereafter, the apnea frequency was calculated by dividing the daily number of apneas by 24.

Statistical methods included a two-way ANOVA to compare frequencies of apnea with respect to apnea types and postnatal age, with a protected Fisher's least significant difference being used as the post hoc test to define intergroup differences in the event of a significant ANOVA. Pearson correlation coefficient was used to determine the significance of the relationship between apnea frequency and occlusion pressures.

Some data from 10 of these infants has been previously reported (6) (incidence of periodic breathing and relationship between periodicity and apnea).

RESULTS

The 20 infants were studied commencing at a mean age of 3.8 h (range 2 to 8). Their mean birth weight was 1012 g (SD 322) and mean gestation was 29.7 wk (SD 2.7). Thirteen infants were delivered by the vaginal route and seven by cesarean section. The first head ultrasound revealed six infants with subependymal hemorrhages. Two of these infants progressed to small intraventricular hemorrhages. Three infants required supplemental oxygen when first entered into the study to maintain an SpO_2 of greater than 92%; all were in room air by 12 h of age.

All infants in the study experienced significant apneic spells before 24 h of age. The mean age of the first recorded apnea was 8.5 h (SD 3.6, range 3 to 18), which was a mean of 4.7 h (SD 4.0) after initiating the recording. The daily distribution of apneic spells is shown in Figure 1. When the daily apnea rates were compared, there was a significant difference in total apnea frequency between d 1 and d 4 to 7 (ANOVA, $F_{6,133} = 3.2$, $p < 0.01$) Table 1. Obstructive and mixed apneas were significantly more frequent on d 1 than on d 4 to 7 ($p < 0.05$). Central apneic spells were not significantly different in frequency between any 2 d; thus, the proportions of types of apneic spells were significantly different between d 1 and d 4 to 7 (χ^2 $p < 0.05$).

Thirteen of the infants were diagnosed as having clinical apnea during the 1st wk of life and all were treated with theophylline. Seven of the infants therefore had recordings for the entire 1st wk of life. None of the infants were removed from the study before d 4, three were excluded on d 5, seven on d 6, and three on d 7. There were no significant differences in apnea frequency or apnea subtype distribution between d 4 and d 7.

We wished to determine whether there was a threshold number, or duration, of apneic spells that would trigger clinical recognition of apnea and subsequent treatment. On d 4, 5, and 6 of the study there were, therefore, a group of infants who received therapy the next day (and were then removed from the study) and a group of infants that were not treated next day. On each of the 3 d, the "not treated next day" infants included the seven who never received therapy (Table 2). Apnea frequency was therefore compared between the groups, using a t test with Bonferroni correction. There were no significant differences on d 4, 5, or 6. Eight of the 13 treated infants had a work up for sepsis that included a complete blood count, blood culture, and spinal tap. All cultures were negative.

Airway occlusion studies were performed in 16 infants. The first study was performed at a mean postnatal age of 7 h and preceded the first recorded apnea in 12 of the infants. There was a progressive nonsignificant increase in the $P_{0.1}$ from the first to the last day of the study. There was a significant negative association ($r = -0.57$, $p < 0.05$) between the apnea frequency over the first three days and $P_{0.1}$ measured at the time of the first occlusion study, but a very wide scatter prevents this from being a useful predictive test (Fig. 2).

DISCUSSION

There was a 100% incidence of significant apnea in this study during the first 24 h of life. This contrasts with studies that used either brief (4) recordings or none at all (2) and emphasizes the importance of both continuous recordings and airflow monitoring for studies on apnea. Our findings imply that there is a 95% probability that 85% or more of clinically healthy infants of less than 34 wk gestation will have apneic spells on the first day.

We have performed a number of comparisons of our unique, purpose written programs with paper recordings at each state of development. These evaluations have demonstrated that significant apneic spells are not missed and many artefacts that a fully automated method could record as apneas are eliminated. The use of a single nasal detector could lead to problems if mouth opening or nasal blockage occurred; however, the requirement for hypoxia or bradycardia, in addition to the loss of nasal airflow, to diagnose an apnea makes this an unlikely cause for confounding of our results.

There is no generally accepted definition of what constitutes a significant apneic spell; investigators in recent years have accepted spells of 10 (7), 15 (8, 9), 20 (10), or 30 (11) s duration regardless of the occurrence of other physiologic changes. It is also frequently stated that shorter apneas were considered to be significant if associated with either hypoxia or bradycardia. Our definition of apnea requires both a duration of at least 15 s and either hypoxia or bradycardia. If we had simply required a duration of 15 s, regardless of hypoxia or bradycardia, we would have reported an even higher incidence of early apneic spells.

The initial objective of the study was to compare the early occlusion pressures of infants who developed apnea to control infants who did not. However, in view of the absence of nonapneic infants, we revised our planned analysis of the results. We demonstrated that occlusion pressures were lower in the infants with the most frequent apneic spells before the occurrence of the first recorded apnea. This suggests that apneic spells and reduced occlusion pressures may be caused by similar factors. Previous data from adults indicate that reduced respiratory drive may occur as a result of multiple apneic episodes, because respiratory drive gradually improves after tracheostomy (12, 13). Our data would not support the idea that occlusion pressures in premature infants are reduced secondarily to the physiologic effects of apneic spells.

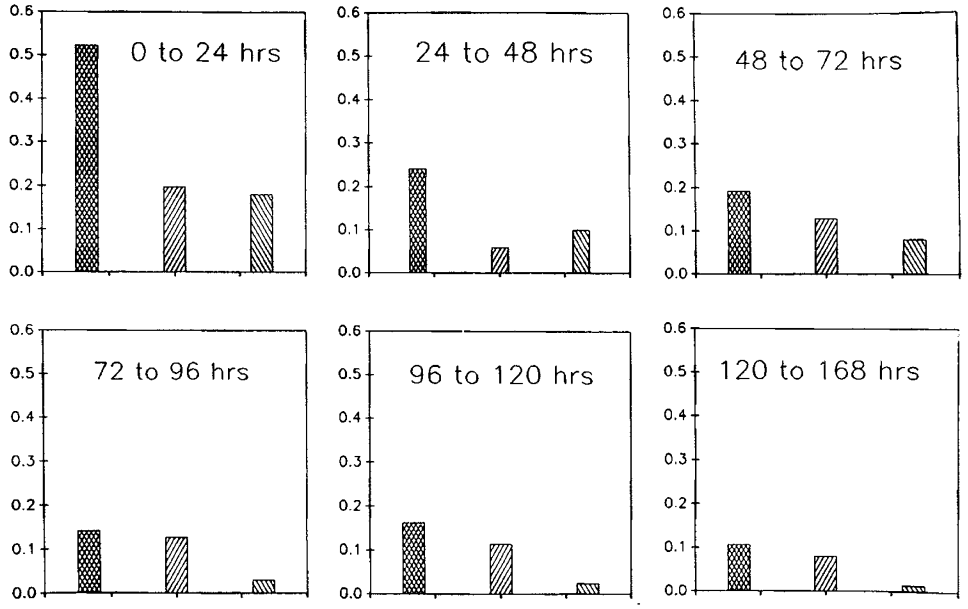

Fig. 1. Apnea frequency (/h) during consecutive periods after birth in 20 premature infants. Mixed apneas ▩, obstructive apneas ▧, and central apneas ▨.

Table 1. *Mean and range of daily total apnea frequencies and apnea durations*

	Day						
	1	2	3	4	5	6	7
Apneas >15 s (/h)	0.9	0.4	0.4	0.3	0.3	0.3	0.2
Maximum frequency (/h)	2.1	2.5	1.6	2.6	1.1	1.8	1.7
Minimum frequency (/h)	0.21	0.13	0.08	0.04	0.08	0.08	0.04
Mean duration (s)	24.2	25.4	25.4	25.7	29.8	28.5	24.2
Maximum duration (s)	49.1	68.2	50.0	43.2	49.5	53.1	32.0
Apneas >20 s (/h)	0.6	0.3	0.2	0.2	0.1	0.2	0.1

Table 2. *Apnea frequency (/h) of infants treated and not treated for apnea of prematurity*

	Day 4			Day 5			Day 6		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Treated next day	3	0.47	0.23	7	0.35	0.19	3	0.24	0.22
Not treated next day	17	0.26	0.20	10	0.28	0.21	7	0.31	0.11
Never treated	7	0.32	0.14	7	0.29	0.18			

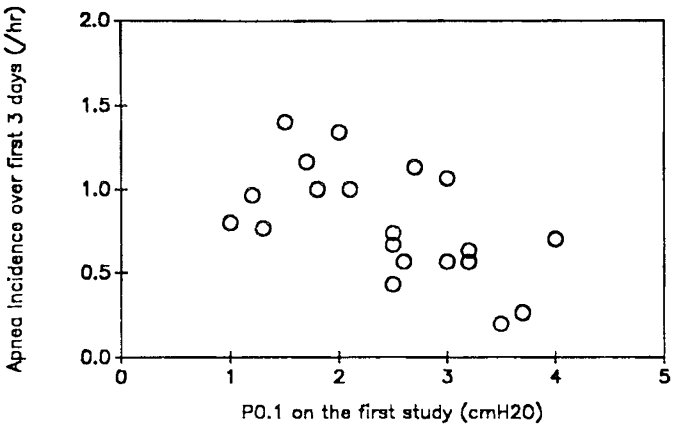

Fig. 2. Relationship between occlusion pressure ($P_{0.1}$) and apnea frequency during the first 3 d of life.

Airway occlusion pressures, although commonly used as an index of respiratory center output, also reflect the state of contractility of the respiratory muscles (14); the association between occlusion pressure and apnea severity could, therefore, be due to an effect of respiratory muscle contractility. Indeed, there may well be an association between fatigue of the diaphragm and apneic spells (15). We currently have no data to confirm or refute this suggestion. There is also little data on the effects of chest wall distortion on perceived occlusion pressures. It seems likely that chest wall distortion decreases measured occlusion pressures and may also be related to apneic spells because of an increase in respiratory work (16). We did not measure chest wall movement or distortion during occlusion studies.

Our finding of early apnea confirms our previous report (6) and the suggestions of Henderson-Smart (2) and Carlo *et al.* (4). The common assertion that idiopathic apnea of prematurity occurs later may be due to the use of thoracic impedance as the exclusive method of clinical apnea monitoring in neonatal nurseries. That is a method that will not detect obstructive apneas or the obstructive portion of mixed apneas. To detect the frequent obstructed breaths of preterm infants, a monitor of oronasal airflow, such as the nasal detection of expired CO_2, is required.

The frequency of early apnea suggests that deficient immediate postnatal adaptation is etiologically related to the spells. We postulate that reduced central respiratory drive is associated with both reduced occlusion pressures and apneic spells. Central respiratory drive is dependent upon afferent input from a number of sources (17). We suggest that the documented suppression of the peripheral chemoreceptor during this interval, because of the sudden increase in arterial P_{O_2} (18, 19), could well be a cause of both reduced respiratory drive and obstructive apneas. Two

convergent lines of evidence support this suggestion. First, arousal is required for termination of apneic spells in adults with obstructive sleep apnea (13). Activity of the carotid bodies is required for arousal in adult dogs (20) and newborn lambs (21) subjected to progressive hypoxia. Thus, carotid body inactivity could well lead to failure of arousal and to apneas becoming prolonged and more likely to be associated with hypoxia and bradycardia.

Second, the peripheral chemoreceptors appear to be particularly important in the maintenance of upper airway muscle activity. Evidence from both adult animals (22) and newborn infants (23) supports this and would suggest that during immediate postnatal adaptation maintenance of upper airway patency would be less efficient and obstructive and mixed apnea would be more common.

We were unable to demonstrate any significant differences between infants who were removed from the study because they were treated for apnea with methylxanthines and those who remained untreated. The clinical staff was not aware of the results of the computer analysis and the question of what prompted the clinicians to treat apnea remains unresolved. We assume that either the reporting of the apneic spells by the bedside nurse to the physician may have differed or the threshold that individual physicians used to treat apnea may have varied. Because of the lack of agreement concerning the outcome of infants who experience apnea of prematurity (24, 25), it is inevitable that such differences will persist. Further research is required to determine what severity, frequency, and type of apneic spells are associated with clinically significant impairment.

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