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Maturation of the Ventilatory Response to CO_2 in the Newborn Piglet

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ABSTRACT. In 12 piglets aged 0 to 1.5 d, we assessed the contribution of the peripheral and central chemoreceptors in mediating the ventilatory response to CO_2 and the apneic threshold during normoxia (arterial O_2 tension, 13 kPa) using the dynamic end-tidal forcing technique. With this technique, the ventilatory response is separated into a peripheral and a central component using a two-compartment model. Each component is described by a CO_2 sensitivity, a time constant, a transport delay time, and an apneic threshold. The means of the estimated parameters per piglet were compared with those obtained in a previous study in piglets aged 2 to 11 d (Wolsink JG, Berkenbosch A, DeGoede J, Olievier CN: J Physiol (Lond) 456:39–48, 1992). The ratio of the peripheral CO_2 sensitivity to the total CO_2 sensitivity was found to be significantly lower in the younger group of piglets (0.14 ± 0.10 *versus* 0.29 ± 0.10), whereas the apneic threshold was significantly higher (2.52 ± 1.12 kPa *versus* 1.06 ± 1.46 kPa). We conclude that the peripheral chemoreceptors are responsive to CO_2 shortly after birth. However, the ventilatory response to CO_2 maturates in the first few days after birth by an increase in the relative contribution of the peripheral chemoreceptors to the total ventilatory response and a decreasing apneic threshold. (*Pediatr Res* 34: 485–489, 1993)

Abbreviations

DEF, dynamic end-tidal forcing
$PETCO_2$, end-tidal partial CO_2 pressure
$PETO_2$, end-tidal partial O_2 pressure
S, CO_2 slope, the slope of the ventilatory response to CO_2
S_p, peripheral ventilatory CO_2 sensitivity
S_c, central ventilatory CO_2 sensitivity
S_p/S, ratio of peripheral CO_2 sensitivity to (total) CO_2 slope
B, apneic threshold
PaO_2, arterial O_2 tension
$PaCO_2$, arterial CO_2 tension

The control of breathing in pre- and postnatal periods of mammals has been the subject of much research mostly directed toward the role of the peripheral chemoreceptors with little emphasis on the central chemoreceptors. Biscoe and Purves (1) reported that the carotid chemoreceptors are fully mature at birth in the lamb. These authors stated that the response of the carotid chemoreceptors does not differ with age and that these chemoreceptors are concerned in both the modulation of the hypoxic drive to ventilation and the respiratory response to inhaled CO_2. This view was challenged by Blanco *et al.* (2). They demonstrated that in the newborn lamb, the carotid chemoreceptors showed no spontaneous impulse activity on the day of birth in contrast with the detected spontaneous activity and responsiveness to hypoxia in the fetal lamb. Over the next few days after birth, there is a resetting of the hypoxic sensitivity, *i.e.* the sensitivity to PaO_2 changes from the fetal toward the more adult range of blood gas tensions giving a hypoxic response curve that is shifted to the right compared with the fetus. The findings in human infants, newborn kittens, and rats that the chemoreflex response to hypoxia and hyperoxia increases postnatally (3–5) are in agreement with this concept of resetting of the hypoxic sensitivity.

The role of the chemoreceptors in the ventilatory response to hypercapnic stimulation before and after birth is not clear. In the fetal lamb, carotid chemoreceptor activity increased when CO_2-saturated saline was injected at the carotid body via the lingual artery (2). On the day of birth, peripheral chemoreceptor activity could be evoked by extreme hypercapnia, whereas no spontaneous activity existed in the newborn lamb. It is possible that the CO_2 sensitivities of the peripheral and also of the central medullary chemoreceptors are influenced by the change from the intrauterine hypercapnic to the relatively hypocapnic environment in the newborn, similarly to the hypoxic sensitivity of the peripheral chemoreceptors. It has been reported that the respiratory response to hypercapnia of premature infants and primates increases in early postnatal life (6–8). Nevertheless, studies in awake newborn lambs and anesthetized newborn piglets showed no correlation between age and CO_2 responsiveness (9, 10). However, in all these studies, the effects of central chemoreceptors in the CO_2 responses could not be isolated from those of the peripheral chemoreceptors.

With the DEF technique it is possible to assess the relative contribution of both groups of chemoreceptors in mediating the ventilatory response to a CO_2 challenge. In a previous study, this technique was applied to a group of 2- to 11-d-old piglets (11). In these piglets, we found no change with age of the ventilatory sensitivity to CO_2 of both the peripheral and central chemoreceptors and the apneic threshold during normoxia. In the present study, piglets 0 to 1.5 d old were used to investigate whether there is a maturation of the ventilatory response to CO_2 as indicated by a change in sensitivity to CO_2 of the peripheral and central chemoreceptors and the apneic threshold immediately after birth as appears to be the case for the O_2 sensitivity of the peripheral chemoreceptors.

MATERIALS AND METHODS

Experiments were carried out on 12 piglets, 0 to 1.5 d old (mean, 0.9 ± 0.5 d), with a mean body weight of 1.6 ± 0.3 kg. The use of the animals was reviewed and approved by the Ethical Committee for Animal Experiments of the University of Leiden. All animals were initially anesthetized with 5 to 10 $mg \cdot kg^{-1}$ zolazepam and tiletamine (Zoletil; Virbac Laboratories, France)

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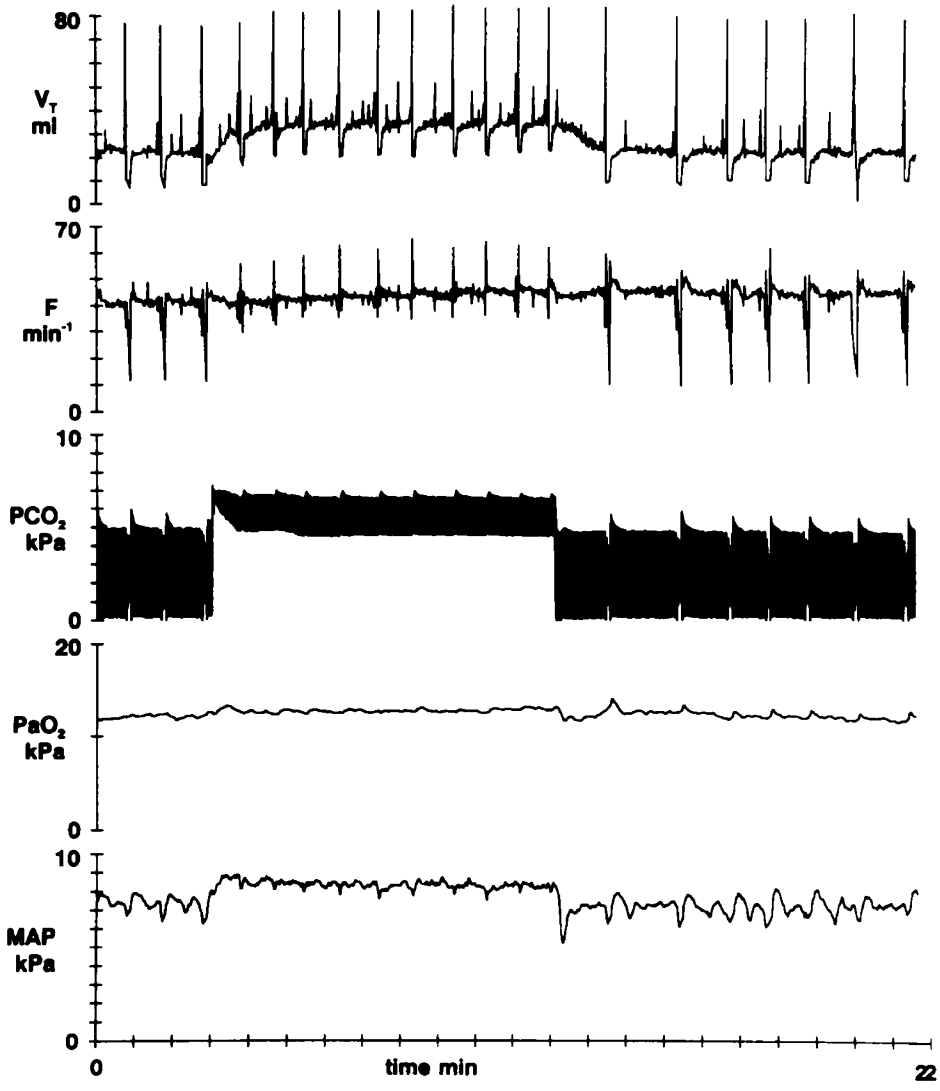

Fig. 1. Computer plot of mean arterial pressure (*MAP*), Pa_{O_2}, P_{CO_2} (in tracheal gas), respiratory frequency (F), and tidal volume (V_T) during a typical example of a DEF experiment of a 1-day-old piglet. The expiratory O_2 was held constant throughout the experiment, resulting in a Pa_{O_2} tension of about 13 kPa. The expiratory P_{CO_2} is controlled and shows a stepwise change of 2 kPa.

intramuscularly followed by halothane inhalation. After cannulation of the right femoral vein, 3 to 10 mg·kg^{-1} α-chloralose and 15 to 50 mg·kg^{-1} urethane were slowly administered i.v. and the volatile anesthetic withdrawn. Thereafter, anesthesia was maintained with a continuous infusion of a mixture of zolazepam, tiletamine, α-chloralose, and urethane at a rate of 0 to 0.8 mg·kg^{-1}·h^{-1} zolazepam and tiletamine, 0 to 1.5 mg·kg^{-1}·h^{-1} α-chloralose, and 0 to 7.5 mg·kg^{-1}·h^{-1} urethane. This regimen leads to conditions in which the level of anesthesia is sufficient to suppress the response to pain stimulation but low enough to preserve the eyelid reflex. Tracheostomy was performed for measurement of inspiratory and expiratory flow. The right femoral artery was cannulated to measure arterial pressure. An extracorporeal circuit was connected between the cannulated left femoral artery and one lumen of a double-lumen catheter in the right femoral vein.

Measurements. The airway gas flow was measured with a Fleisch No. 00 pneumotachograph, connected to a differential pressure transducer (Statham, Cambridge, MA) and electronically integrated to yield a volume signal. The CO_2 and O_2 concentrations in the tracheal gas were measured with a fast infrared analyzer (Gould Godart MK2, Bilthoven, The Netherlands) and a fast zirconium oxide cell (Jaeger O_2 test, Wuerzburg, Germany), respectively. The arterial pH, P_{CO_2} and P_{O_2} were continuously measured with electrodes in the extracorporeal circuit. Blood pressure was measured with a pressure transducer. Temperature was monitored by a rectal thermistor and maintained within 1°C by a heating pad and an infrared lamp and varied from 36.6 to 39.8°C between piglets. All signals were recorded on polygraphs, digitized (sample frequency, 100 Hz), and processed by a PDP 11/23 minicomputer. All signals were stored on a breath-to-breath basis.

Experimental protocol. The experimental protocol was similar as described earlier (11). With the DEF technique, a square wave in the $PETCO_2$ is generated at $PETO_2$ by manipulating the inspired CO_2 and O_2 concentrations under feedback control of a computer. The $PETO_2$ tension was held at a value resulting in an arterial O_2 tension of about 13 kPa (mean, 12.8 ± 0.3 kPa). Figure 1 shows the mean arterial pressure, arterial O_2 tension, P_{CO_2} in the tracheal gas, respiratory frequency, and tidal volume during a typical DEF experiment of a 1-d-old piglet.

DEF technique and data analysis. For the analysis of the breath-to-breath data obtained in the DEF runs, we used a two-compartment model (12). In this model, the ventilatory responses of the central and peripheral chemoreflex loops are described by:

$$\tau_c d\dot{V}_c/dt + \dot{V}_c = S_c[PETCO_2(t - T_c) - B] \quad (1)$$

$$\tau_p d\dot{V}_p/dt + \dot{V}_p = S_p[PETCO_2(t - T_p) - B] \quad (2)$$

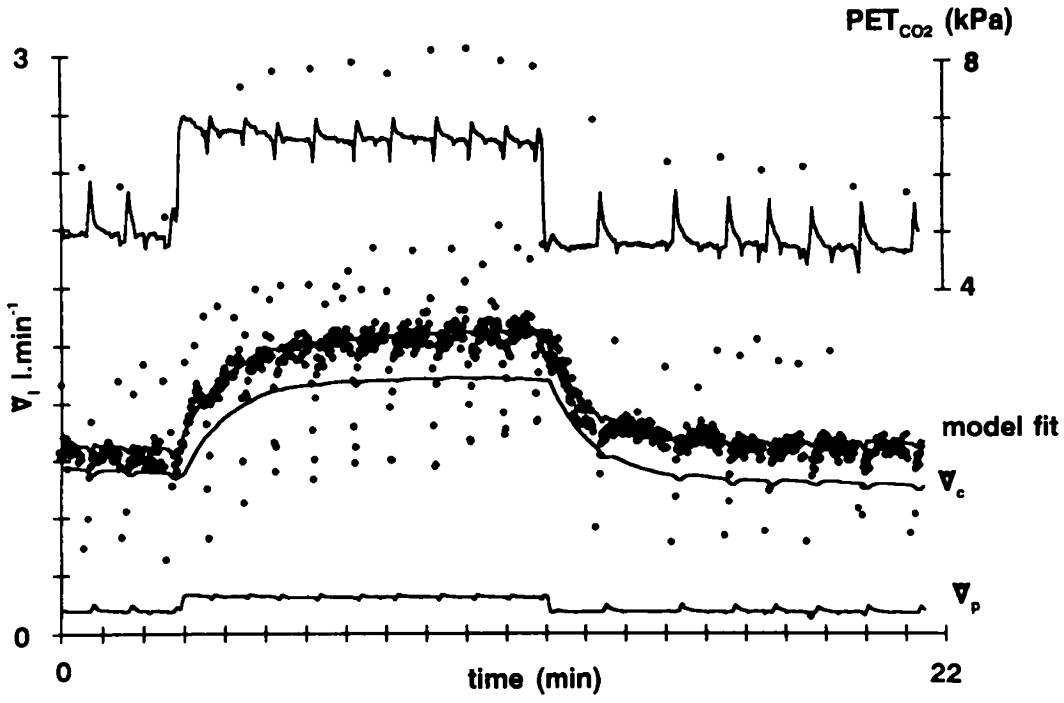

Fig. 2. Ventilatory response during normoxia and the model fit of the experiment shown in Figure 1. The *dots* represent breath-to-breath ventilation. The *curve* through the data points is the model fit, $\dot{V}_I$. It is the sum of the slow $\dot{V}_c$ and the fast $\dot{V}_p$ components and a drift term (not shown separately).

Table 1. *Mean parameters for DEF experiments**

Parameter	DEF values
B (kPa)	2.52 (1.12)
S_p (mL·min^{-1}·kPa^{-1}·kg^{-1})	25.0 (23.6)
S_c (mL·min^{-1}·kPa^{-1}·kg^{-1})	122.3 (52.9)
S_p/S	0.14 (0.10)
τ_{on} (s)	57.4 (34.9)
τ_{off} (s)	78.0 (60.3)
τ_p (s)	1.07 (1.26)
T_c (s)	6.0 (1.6)
T_p (s)	4.1 (1.2)

* Experiments included 36 runs, 12 piglets. Values are means of the means per piglet ± SD. τ_{on}, Central on-transient time constant; τ_{off}, central off-transient time constant; τ_p, peripheral time constant; T_c, central transport time; T_p, peripheral transport time.

In these equations, the time constants of the central and peripheral ventilatory responses are denoted by τ_c and τ_p, respectively. $\dot{V}_c$ and $\dot{V}_p$ are the central and peripheral parts of the response, S_c and S_p the central and peripheral ventilatory CO_2 sensitivities, and T_c and T_p the times needed to transport the P_{CO_2} disturbance from the lungs to the central and peripheral chemoreceptive structures. The offset B represents the apneic threshold or extrapolated PET_{CO_2} of the total steady state ventilatory response to CO_2 at zero ventilation, whereas the total CO_2 sensitivity S is $S_c + S_p$. To model the central time constant of the on-transient to be different from that of the off-transient, τ_c is modeled as:

$$\tau_c = \tau_{on} \cdot x + (1 - x)\tau_{off} \quad (3)$$

In this equation, x = 1 when PET_{CO_2} is high (on-transient), and x = 0 when PET_{CO_2} is low (off-transient). In most experiments, a small drift in the ventilation was present. Therefore, we included a drift term (C · t) so the total ventilation ($\dot{V}_I$) is given by:

$$\dot{V}_I(t) = \dot{V}_c(t) + \dot{V}_p(t) + C \cdot t \quad (4)$$

The parameters of the model were estimated with a least squares method. To obtain optimal time delays, a "grid search" was applied. All combinations of T_c and T_p with increments of 1 s and $T_c \geq T_p$ were tried until a minimum in the residual sum of squares was found. The minimal time delays were somewhat arbitrarily chosen to be 1 s, and τ_p was constrained to be at least 0.3 s.

Statistical analysis. The means of the means per piglet of the estimated parameters were calculated and evaluated together with the results obtained in a previous study in piglets aged 2 to 11 d (11). Pearson correlation coefficients were calculated to analyze the linear relation between the estimated parameters and age using the data from 0 to 11 d as a whole. To compare the means of the means per piglet of the estimated parameters between the two age groups, *t* tests were used. The level of significance was set at $p = 0.05$. Results are given as means ± SD.

RESULTS

A total of 36 curves (two to four per piglet) were analyzed. Figure 2 shows the computer output of the analysis of the breath-to-breath data of the DEF run shown in Figure 1. In two piglets (both 0.5 d), no peripheral component could be detected. In Table 1, the means of the means per piglet of all estimated parameters are listed with their SD. S_p and S_c were normalized to body weight and expressed per kg. We first tested for a linear correlation between the estimated parameters and age. Figure 3 shows the relation between four of these parameters (B, S_p, S_c, and S_p/S) and age. The results of the piglets aged 2 to 11 d are from a previous study (11). There was a significant linear correlation ($p = 0.025$) between S_p/S and age. No correlation was found between any of the other parameters and age. Due to the appreciable scatter and the possible nonlinear relationships between the quantities and age (especially that of S_p/S and age), the linear correlation coefficients tend to be somewhat low. The previous study in 2- to 11-d-old piglets (11) showed no correlation between any of the parameters and age within this range of age. Therefore, we also compared the 0- to 1.5-d-old and the 2- to 11-d-old piglets as two groups using *t* tests. It was found that the mean value of B of the 0- to 1.5-d-old piglets was significantly larger ($p = 0.004$) compared with the mean value of the 2- to

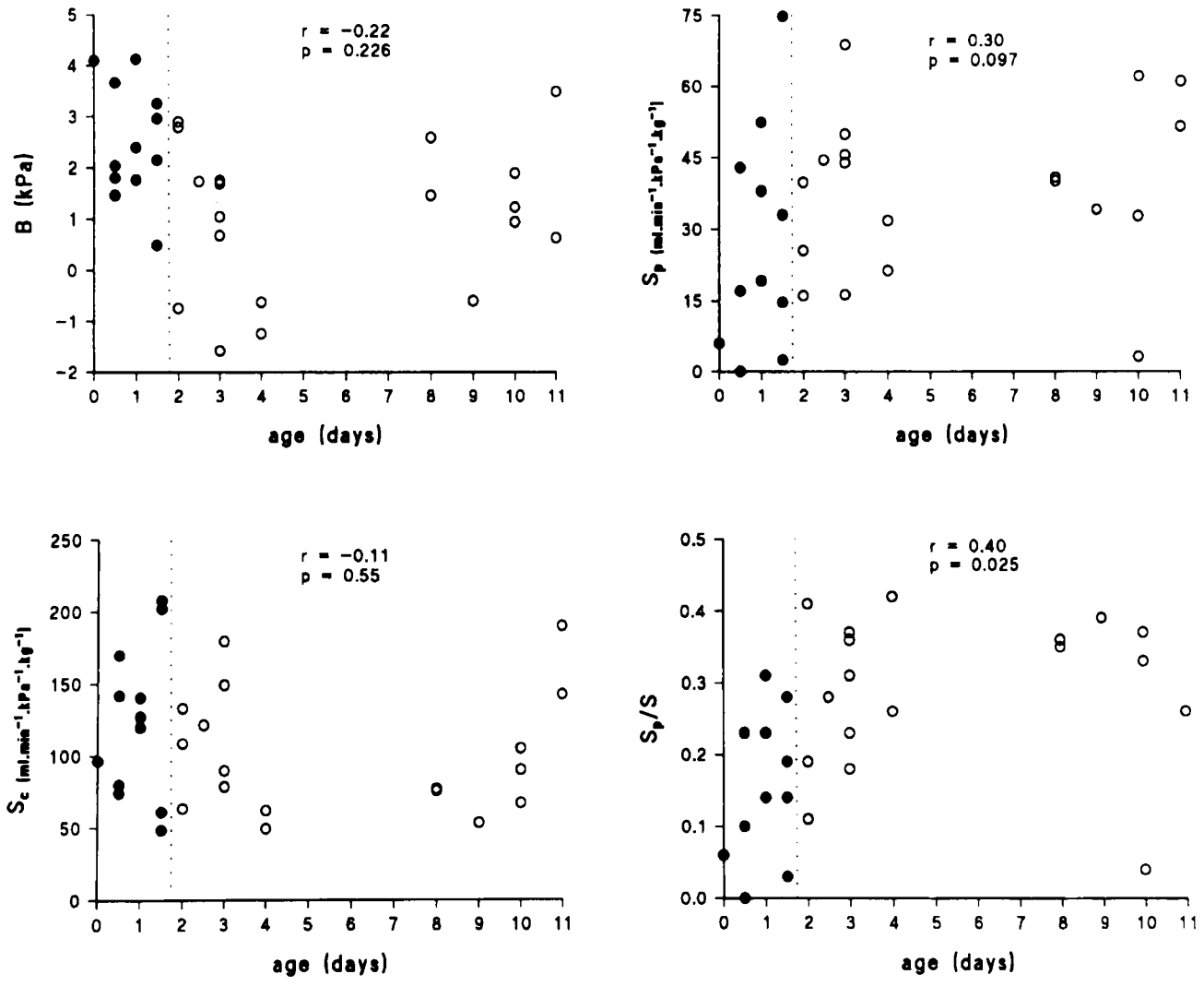

Fig. 3. The relationship between B, S_p, S_c, S_p/S, and age. There was a significant correlation between S_p/S and age. The correlation coefficients and p values are shown. *Black circles* denote the results of the present study in 0- to 1.5-d-old piglets; *open circles* are data from a previous study in 2- to 11-d-old piglets (11).

11-d-old animals (mean ± SD, 2.52 ± 1.12 kPa *versus* 1.06 ± 1.46 kPa). There were no significant differences in the absolute peripheral and central CO_2 sensitivities between the two age groups, whereas S_p/S was significantly lower ($p = 0.001$) in the group of 0- to 1.5-d-old piglets (0.14 ± 0.10 *versus* 0.29 ± 0.10).

DISCUSSION

Studies in various animal species and in the human infant have shown that the hypoxic sensitivity of the carotid and aortic chemoreceptors undergoes a resetting postnatally, from the fetal toward the more adult range of blood gas tensions (2–5). The shift of the stimulus response curve to the right leads to a greater discharge at any Pa_{O_2} and an increase in sensitivity of the receptors to changes in Pa_{O_2} with age. Studies about resetting or maturation of the CO_2 sensitivity of the peripheral and central chemoreceptors postnatally are sparse.

We used the DEF technique to obtain information about the role of both the peripheral and central chemoreceptors in mediating the ventilatory response to CO_2 in newborn piglets. With this technique the ventilatory response to CO_2 is separated into a fast peripheral and a slow central component without disrupting neuronal pathways. In previous studies, we have shown that the DEF technique can be applied with success to conscious humans and anesthetized young piglets and satisfactorily estimates the sensitivities to CO_2 of the central and peripheral chemoreflex loops with the apneic threshold (11–14).

In a previous study in a group of 2- to 11-d-old piglets, no maturation of the CO_2 sensitivities of either the peripheral or the central chemoreceptors could be detected over the age range studied using the DEF technique (11). In that study, the Pa_{O_2} was kept at 13 kPa by manipulating the PET_{O_2}. Because the group of 0- to 1.5-d-old piglets also was kept at a Pa_{O_2} of 13 kPa in the present study, the results of these two studies were tested for age-related effects. In another study in 2- to 12-d-old piglets (13), the PET_{O_2} was fixed at 15 kPa. Due to appreciable PET_{O_2}-Pa_{O_2} gradients, especially in the younger animals, the Pa_{O_2} differed between animals from 9 to 13 kPa. To avoid the possibility of introducing an artificial age-related effect because the peripheral CO_2 sensitivity was estimated at a somewhat lower Pa_{O_2} and therefore might be augmented by positive interaction between hypoxia and hypercapnia, we preferred not to use this group of animals in a comparison. If an age-related effect does exist between the younger and older groups, an effect of age should be found that is even more significant when the results of the present study are compared with those in which the Pa_{O_2} was not kept constant. By calculating the Pearson correlation coefficient we found this was indeed the case.

We found no significant differences in the absolute CO_2 sensitivities between the two age groups. This seems to be in agree-

ment with the findings of Canet *et al.* (15) who did not find a postnatal resetting of peripheral CO_2 sensitivity in the lamb within the first 2 wk of life. However, they used the immediate ventilatory response within 15 s after a CO_2 step-change as a means of quantifying peripheral chemoreceptors sensitivity in awake newborn lambs at less than 24 h and 14 d of age. The time delay required for the central chemoreceptors to produce a ventilatory response lies almost certainly within those 15 s so it is doubtful whether they would have adequately separated the peripheral ventilatory response from the central one. From studies in the anesthetized piglet (9), it was concluded that breathing responses to hypercapnia exhibit no maturation in magnitude but no separation in peripheral and central contribution was made and piglets were studied between 2.5 and 34 d of age.

To obtain an adequate level of anesthesia in our study, the younger piglets needed a smaller amount of anesthetics per kg body weight than the older ones. Therefore, the depressive effects of anesthesia on the ventilatory sensitivities to CO_2 may have been larger in the older group. Because we have shown that S_p/S is not sensitive to changes in the level of anesthesia (13), this quantity would be a more appropriate parameter to compare different age groups. Further, S_p/S is independent of the manner in which the CO_2 sensitivities are normalized with respect to changes in body weight. In the younger group of piglets, we found S_p/S to be significantly lower than in the older ones. This indicates there is a change in the relative contribution of both groups of chemoreceptors in mediating the total ventilatory response to CO_2 during normoxia. Although it cannot be excluded, it is unlikely that the CO_2 sensitivity of the central chemoreceptors would decrease during development. From results in the anesthetized piglet, Segal *et al.* (9) suggested that the central respiratory drive is already mature in the first postnatal week and does not change in magnitude over the next few weeks. Jansen *et al.* (16) found that in the newborn chemodenervated lamb, the CO_2 sensitivity of the central chemoreceptors is fully developed by 2 d of age. Marchal *et al.* (17) found that the carotid chemoreceptor response to hypercapnia in the kitten is already developed but weak at birth and continues to develop further during first weeks of postnatal life. Therefore, the increase in S_p/S in our study probably reflects an increase in the peripheral CO_2 sensitivity.

We also found that the apneic threshold B was higher in younger piglets compared with the older ones. If the anesthetic level affects the value of B, then it is to be expected that higher amounts of anesthetics needed for the older ones would have resulted in higher B values in the older piglets compared with the younger ones, although this effect of anesthesia on B remains speculative. We found a lower apneic threshold in the older piglets that would suggest a leftward shift of the CO_2 response curve in the older ones. This would be in agreement with the findings in newborn monkeys and kittens in which the response curve to CO_2 of younger animals was displaced to the right of that of the older ones (7, 16). However, in the human infant, the CO_2 response curve lies to the left of that of adults (18).

We conclude that in the newborn piglet the peripheral chemoreceptors are responsive to CO_2 shortly after birth and that their contribution in mediating the ventilatory response to CO_2 during normoxia increases with age, whereas the apneic threshold of the ventilatory response to CO_2 was found to be higher shortly after birth. This indicates a maturation of the ventilatory response to CO_2 occurring in the very first days after birth, corresponding to the time necessary for the resetting of the peripheral hypoxic sensitivity.

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