Ventilatory Sensitivities of Peripheral and Central Chemoreceptors of Young Piglets to Inhalation of CO₂ in Air

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ABSTRACT. In 20 piglets aged 2–12 d (mean 6.8 d) and anesthetized with α -chloralose-urethane, we investigated the contribution of the peripheral and central chemoreceptors to the ventilatory response to inhalation of CO₂ in air. For this purpose we used the dynamic end-tidal forcing technique, applying square-wave changes in end-tidal CO₂ tension of 1.5–2.0 kPa at a constant end-tidal O₂ tension of 15 kPa. Each response, measured on a breath-to-breath basis, was separated into a fast peripheral and a slow central component by fitting the sum of two exponentials to the measured ventilation. Each component was characterized by a CO₂ sensitivity, a time constant, a time delay, and an apneic threshold. The results showed that in 2- to 12-d-old piglets the peripheral chemoreceptors are responsive to CO₂ during air breathing. The contribution of the peripheral chemoreceptors in mediating the response to CO₂ averaged $30 \pm 10\%$. Within this age range we could not demonstrate a significant correlation of the parameters characterizing the dynamic ventilatory response to CO₂ with postnatal age. (*Pediatr Res* 30: 491–495, 1991)

Abbreviations

DEF, dynamic end-tidal forcing
PETCO₂, end-tidal partial CO₂ pressure
PETO₂, end-tidal partial O₂ pressure
S, CO₂-slope, the slope of the ventilatory response to CO₂
S_p, peripheral ventilatory CO₂ sensitivity
S_c, central ventilatory CO₂ sensitivity
S_p/S, the ratio of the peripheral CO₂ sensitivity to (total) CO₂-slope
B, apneic threshold
PaO₂, arterial O₂ tension

The role played by the peripheral and central chemoreceptors in the control of breathing shortly after birth has received much attention. Most studies in this field have been directed to the peripheral chemoreceptors monitored by the hypoxic and hyperoxic ventilatory response. In kittens, lambs, and rats immediately after birth, this response is weak or absent, indicating a feeble respiratory drive from the peripheral chemoreceptors. This is probably due to the rise in PaO₂ that occurs when air breathing is established (1–3). Over the next 1–3 d after birth, the peripheral chemoreceptors undergo a resetting, *i.e.* the range of hypoxic sensitivity is changed from the fetal toward the more adult range of blood gas tensions (4, 5). From that time on, they actively

control the ventilation. Studies from Belenky *et al.* (6) and Bureau and Bégin (7) suggest that in the lamb the peripheral chemoreceptors continue to mature after the resetting in the first 2 d after birth. The ventilatory response to hypoxia and hyperoxia as well as the response to potassium cyanide showed an increase until about 10 d of life. In this paper, animals in which a major part of the peripheral chemoreceptor resetting has occurred (age at least 2 d) will be called young animals to distinguish them from newborns.

In contrast to the amount of information about the hypoxic and hyperoxic ventilatory response, studies of the ventilatory response to CO₂ in young animals are sparse. The question of whether the carotid chemoreceptors in young animals contribute to the ventilatory response to CO₂ inhalation during air breathing has not been answered. There is some indirect information from steady state techniques in which the CO₂-slope is compared before and after denervation of the peripheral chemoreceptors. In lambs 1 to 10 d old (8), denervation of the carotid bodies led to a slight decrease in CO₂-slope, an increased latency time, and a slower rate of rise of ventilation in response to inhaled CO₂, suggesting that at least a part of the CO₂ response was dependent on the fast-responding peripheral chemoreceptors. Furthermore, an age-related change in CO₂-slope could not be detected. In monkeys the CO₂-slope increased between 2 and 21 d (9). Human infants showed an increase in CO₂-slope of 62% from 2 to 27 d of postnatal life (10). However, to the best of our knowledge there are no studies about to what extent the peripheral and central chemoreceptors contribute to the ventilatory response to CO₂ in young animals during air breathing. The DEF technique is probably the only way to get information on this issue noninvasively (11, 12). With this technique the end-tidal PCO₂ is forced to follow a prescribed pattern in time at a constant end-tidal O₂ tension. The ventilatory response, measured on a breath-to-breath basis, is separated into a fast peripheral and a slow central component using a two-compartment model (12, 13). The measured end-tidal CO₂ is used as input function.

The technique has been successfully applied in anesthetized adult cats (12) and awake human beings (14). It was our purpose to investigate the relative contribution of the peripheral and central chemoreceptors in newborns as well as young animals using the DEF technique. In a first approach, however, young piglets were used in which a major part of the peripheral chemoreceptor resetting would have occurred, so it is plausible to expect a sizeable contribution of the peripheral chemoreceptors to the ventilatory response to CO₂.

MATERIALS AND METHODS

Experiments were performed on 20 anesthetized piglets of either sex. The use of the animals was reviewed and approved by the Ethical Committee for Animal Experiments of the University of Leiden. Animals ranged in age from 2 to 12 d (mean 6.8 ± 3.1 d) with a mean body weight of 2.7 ± 0.6 kg. Anesthesia

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was induced with 5–7.5 mg·kg⁻¹ zolazepam and tiletamine (Zoletil; Virbac Laboratories, France) intramuscularly followed by halothane inhalation. The right femoral vein was cannulated and 7–25 mg·kg⁻¹ α -chloralose and 35–125 mg·kg⁻¹ urethane were slowly administered. Then the volatile anesthetic was withdrawn. About an hour later an infusion of a mixture of zolazepam, tiletamine, α -chloralose, and urethane was started at a rate of 0.5–5 mg·kg⁻¹·h⁻¹ each of zolazepam and tiletamine, 0.5–2 mg·kg⁻¹·h⁻¹ α -chloralose, and 2.5–10 mg·kg⁻¹·h⁻¹ urethane. The level of anesthesia was periodically evaluated by noting the eyelid reflex and the response to pain stimulation. The trachea was cannulated through a tracheostomy and connected to a T-piece. One arm of the T-piece received a flow of gas from a gas mixing system in excess of the inspiratory demand. The end-tidal CO₂ and O₂ could be set at any desired level by manipulating the inspired CO₂ and O₂ concentrations by servo control with a computer. The right femoral artery was cannulated to measure arterial pressure. In 15 piglets, after cannulation of the left femoral artery, an extracorporeal circuit was connected between the cannulated left femoral artery and one lumen of a double-lumen catheter lying in the right femoral vein.

Measurements. Inspiratory and expiratory flows were measured using a Fleisch no. 0 pneumotachograph connected to a differential pressure transducer (Statham, Cambridge, MA). The flow signal was electronically integrated to yield a volume signal. The CO₂ concentration in the respiratory air was measured with the aid of a capnograph (Gould Godart MK2, The Netherlands) and the O₂ concentration with a fast oxygen analyzer (Jaeger O₂-test, Germany). The arterial pH, arterial CO₂, and PaO₂ were continuously measured with electrodes in the extracorporeal circuit. Blood pressure was measured with a pressure transducer. The rectal temperature was monitored with a thermistor and controlled within 1.0°C with an infrared lamp and a heating pad, but varied from 37.2–40.0°C between piglets. All signals were recorded on polygraphs, digitized (sample frequency 100 Hz), processed by a PDP 11/23 minicomputer, and stored on a disk. The tidal volume, inspiratory time, expiratory time, inspiratory ventilation, respiratory frequency, end-tidal CO₂, and end-tidal O₂ were stored on a breath-to-breath basis. Details of the methods have been published previously (15).

Experimental protocol. After a period of surgical preparation and stabilization (about 4.5 h in total), the measurements were started. Each DEF experiment consisted of an episode of steady state ventilation of approximately 2 min during which the PETCO₂ was held slightly above its resting value by giving small amounts of CO₂ in the inspired air. The PETCO₂ was then elevated 1.5–2.0 kPa within one or two breaths, maintained constant for a period of 5–8 min, and then returned stepwise to the original value and kept constant for a further 5–8 min (Fig. 1). During this maneuver, the PETO₂ was held constant at 15 kPa. In each animal at least three DEF runs were performed.

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 proposed by Bellville *et al.* (13). In this model the dynamics of the ventilatory responses of the central and peripheral chemoreflex loops are described by:

$$\tau_c d\dot{V}_c/dt + \dot{V}_c = S_c[\text{PETCO}_2(t-T_c)-B]$$

and

$$\tau_p d\dot{V}_p/dt + \dot{V}_p = S_p[\text{PETCO}_2(t-T_p)-B].$$

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 CO₂ sensitivities; and T_c and T_p , the times needed to transport the PCO₂ disturbance from the lungs to the central and peripheral chemoreceptive structures. The offset B represents the apneic threshold or extrapolated PETCO₂ of the steady state ventilatory response to CO₂ at zero ventilation, and the total CO₂ sensitivity S equals $S_p + S_c$. To

model the central time constant of the on-transient to be different from that of the off-transient, τ_c is written as:

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

In this equation, $x = 1$ when PETCO₂ is high (on-transient) and $x = 0$ when PETCO₂ is low (off-transient).

In most experiments a small drift in the ventilation was present. Therefore, we included a drift term $C \cdot t$, so that the total ventilation ($\dot{V}_t$) is given by:

$$\dot{V}_t(t) = \dot{V}_c(t) + \dot{V}_p(t) + C \cdot t$$

The parameters of the model were estimated by fitting the data with a least squares method. To obtain optimal time delays, a "grid search" was applied. All combinations of T_p and T_c 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.

RESULTS

Figure 1 shows a computer plot of the PO₂ and PCO₂ in the tracheal gas and breath-to-breath ventilation in a typical example of a DEF experiment on a piglet 3 d of age. In response to the step increase in PETCO₂, the ventilation increased shortly thereafter. In 17 piglets this ventilatory increase was brought about by an increase in both tidal volume and respiratory frequency. In three piglets, only tidal volume increased after raising the PETCO₂. A total of 76 DEF runs 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 74 of the 76 runs, a fast and a slow component were obtained. The analysis of two of the three runs in one 9-d-old piglet yielded no fast component. Most animals showed at regular time intervals an augmented breath or sigh with a tidal volume of about 2 times the tidal volume of "normal breaths." The frequency of the sighs was rather high (about 2–3 per min). These sighs seem to be a characteristic feature of the ventilatory pattern in anesthetized young piglets, and we did not remove them from the data set. As a result of a sigh, the PETCO₂ of the next breath was appreciably lower especially at the low PETCO₂ level where the difference between inspired and expired PCO₂ is maximal. However, inasmuch as the model parameters were estimated using the actual PETCO₂ as input, these disturbances in PETCO₂ are taken into account, as can be seen in Figure 2.

We looked for a correlation between the estimated parameters and age. Figure 3 shows the relation between four of these parameters (S_p , S_c , B , and S_p/S) and age. No significant correlation was found between any of these parameters and age, although S_p/S tended to increase and S_c tended to decrease with age. For summarizing purposes, the means of the means per piglet of all the estimated parameters of the individual DEF runs together with their SD are listed in Table 1. S_c and S_p were normalized to body weight and expressed per kg.

DISCUSSION

A survey of the literature reveals that the relative contribution of the central and peripheral chemoreceptors in the control of breathing in young animals has not been assessed satisfactorily (16, 17). In this study we used the noninvasive DEF technique in young piglets to separate the ventilatory response to CO₂ into a peripheral and central component without the need of interrupting neural pathways as in denervation techniques. To separate the ventilatory response to inhalation of CO₂ in air (PETO₂ 15 kPa) into a peripheral and a central component, the difference in speed of response of both chemoreflex loops and the difference in transport time of the CO₂ challenge to the site of action is utilized. In previous studies (12, 14), we have shown that in awake human beings as well as in anesthetized animals the

Fig. 1. Computer plot of breath-to-breath ventilation, P_{O_2} , and P_{CO_2} (in tracheal gas) during a DEF experiment of a 3-d-old piglet. The PET_{O_2} (15 kPa) was held constant throughout the experiment by servo-control continually adjusting the inspired O_2 fraction. At the beginning and the end of the trace, PET_{CO_2} was about 5.6 kPa. The step increase was 1.5 kPa.

Fig. 2. Ventilatory response during air breathing and the model fit of the experiment shown in Figure 1. *Top panel* shows the PET_{CO_2} stimulus. The *dots* represent the breath-to-breath ventilation. The *curve through the data points* is the model fit, minute ventilation ($\dot{V}_I$). It is the sum of the slow ($\dot{V}_c$) and the fast ($\dot{V}_p$) components and a drift term, $C \cdot t$ (not shown separately). The estimated parameter values are: B , 3.36 kPa; S_c , 199 mL $\cdot$ min⁻¹ $\cdot$ kPa⁻¹ $\cdot$ kg⁻¹; S_p , 43.3 mL $\cdot$ min⁻¹ $\cdot$ kPa⁻¹ $\cdot$ kg⁻¹; central on-transient time constant, 38.7 s; central off-transient time constant, 64.9 s; peripheral time constant, 0.3 s; central transport delay time, 4 s; and peripheral transport delay time, 4 s.

estimated CO₂ sensitivity of the slow component corresponds to that of the central chemoreflex loop and the CO₂ sensitivity of the fast one to that of the peripheral chemoreflex loop.

Using the DEF technique, we found that in piglets aged 2–12 d the peripheral chemoreceptors contribute appreciably to the ventilatory response to CO₂ during air breathing. It is interesting to remark that piglets 3–9 d of age and 2-d-old lambs showed a high mortality in the days after carotid body denervation, sug-

gesting an essential role for these receptors in the control of breathing at this stage of life (18, 19). The values found for the time constants and the ratio S_p/S_c are similar to those found in adult anesthetized cats (12) and awake human beings (14). The delay times (central and peripheral transport) have values to be expected for the transmission of the disturbance in CO₂ from the lungs to the sites of peripheral and central chemoreception. The B value is lower than in cat and man and might indicate that a

Fig. 3. The relationship between four estimated model parameters and age. No significant correlation could be found between S_p , S_c , B , and S_p/S versus age. The correlation coefficients together with the p values are shown. Note that there are coinciding points. In the *left upper panel*, the upper point at 9 d; the *right upper panel*, the point at 10 d; the *lower left panel*, the point at 6 d and the upper point at 4 d.

Table 1. Mean parameters for the DEF experiments (76 runs, 20 piglets)*

Parameter	DEF values
S_c (mL·min ⁻¹ ·kPa ⁻¹ ·kg ⁻¹)	122 ± 37.3
S_p (mL·min ⁻¹ ·kPa ⁻¹ ·kg ⁻¹)	48.0 ± 17.8
S_p/S	0.30 ± 0.10
B (kPa)	1.48 ± 1.19
τ_{on} (s)	69.7 ± 23.4
τ_{off} (s)	119 ± 51.8
τ_p (s)	2.5 ± 3.7
T_c (s)	5.8 ± 1.7
T_p (s)	3.9 ± 0.7

* 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 delay time; and T_p , peripheral transport delay time.

CO₂-independent ventilatory drive in the young piglet is present. There was no change in B with age in contradistinction to the findings in 2- to 21-d-old monkeys (8). The correlation between the other estimated parameters and age was also not significant, although there was a tendency of S_p to increase and S_c to decrease.

Throughout each DEF experiment, the PETO₂ was held at a level of 15 kPa, comparable to air breathing. Continuous measurement of the PaO₂ in 11 piglets showed appreciable PETO₂-PaO₂ gradients, especially in the piglets of younger age. If there is positive interaction in the ventilatory response between peripheral hypoxia and hypercapnia like in the cat (20), the peripheral chemoreceptors may be more stimulated in younger piglets because of the lower PaO₂ values. It may well be that when the PaO₂ of the piglets are matched an age-related effect on S_p becomes more apparent. This point deserves further investigation.

The tendency of the central chemosensitivity (S_c), normalized to body weight, to decrease with age might be due to the level of anesthesia. To obtain an adequate level of anesthesia, the total amount of anesthetics per kg body weight supplied to older animals was larger than to younger ones. Anesthesia is known to depress the ventilatory sensitivity to CO₂. In studies in the cat (21, 22), we have found that, although at deeper levels of anesthesia the total slope is decreased, the peripheral and central CO₂ sensitivities are decreased to the same extent so that the ratio S_p/S was independent of the level of anesthesia. We investigated in seven piglets the effects of an additional dose of the anesthetics of 0.5 mg·kg⁻¹ zolazepam and tiletamine and 5 mg·kg⁻¹ chloralose with 25 mg·kg⁻¹ urethane. The overall ventilatory CO₂ sensitivity decreased about 50%, but like in the cat there was no systematic change in the ratio S_p/S (Fig. 4). This suggests that also in the young piglet the ratio S_p/S is independent of the level of anesthesia. The ratio S_p/S is, therefore, an interesting quantity because it is not sensitive to the level of anesthesia and, furthermore, it is independent of the way of normalization to the size of the piglet.

Studies in the lamb suggest that after the initial resetting to hypoxia in the first 2 d after birth the maturation of the peripheral chemoreceptors continues up to 10 d of life (6, 7). In our piglets we could not obtain a significant correlation between the peripheral contribution to the ventilatory response to CO₂ and age although S_p over S tended to increase with age. In one 9-d-old piglet, we could not detect a peripheral component in two of the three DEF runs. This finding might indicate nonfunctional peripheral chemoreceptors in this particular piglet. When the S_p/S value of this piglet was considered as an exception and left out of the statistical analysis, the correlation between S_p/S and age became significant ($r = 0.55$, $p = 0.01$).

This study describes the first quantitative measurements of the contribution of the peripheral and central chemoreflex loop to

Fig. 4. S_p/S at two levels of anaesthesia (light and deep).

the ventilatory response to CO₂ during air breathing in young animals. Using the DEF technique, we have shown that the contribution of the peripheral chemoreceptors to this response is appreciable in 2- to 12-d-old piglets. Within this range of age, we could not demonstrate an age-related effect on the peripheral contribution. It would be interesting to use the DEF technique in piglets of younger age to investigate whether there is a change in sensitivity to CO₂ of the peripheral and central chemoreceptors immediately after birth, as is the case for the O₂ sensitivity of the peripheral chemoreceptors.

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