Journal of Physiology (1990), **425**, *pp.* 211–225
With 7 figures
Printed in Great Britain

DEVELOPMENT OF THE ARTERIAL CHEMOREFLEX AND TURNOVER OF CAROTID BODY CATECHOLAMINES IN THE NEWBORN RAT

BY T. HERTZBERG*†, S. HELLSTRÖM‡, H. LAGERCRANTZ*† AND J. M. PEQUIGNOT§

*From the *Nobel Institute for Neurophysiology, Karolinska Institute, S-104 01 Stockholm, Sweden, †Department of Pediatrics, Karolinska Hospital, S-104 01 Stockholm, Sweden, ‡Department of Anatomy, University of Umeå, S-901 87 Umeå, Sweden and §UA CNRS 1196, Physiologie, Faculté de Médecine Grange Blanche, Lyons, France*

(*Received 9 June 1989*)

SUMMARY

1. The peripheral, arterial chemoreceptors in the carotid body are active and responsive in the fetus. At birth, when oxygenation increases, the chemoreceptors are silenced. Over the next few days the sensitivity is reset toward the adult level and the chemoreceptors influence breathing during normal conditions. In order to investigate the underlying mechanisms of this resetting we examined the strength of the chemoreflex in newborn rats and correlated this to the contents of dopamine and noradrenaline in the carotid bodies of the newborn pups and near-term fetuses. Furthermore, turnover rates of dopamine and noradrenaline were determined in newborn rats up to 1 week of age by analysis of catecholamine decreases after inhibition of synthesis with α-methyl-*p*-tyrosine.

2. Chemoreceptor influence was assessed by the method of 'physiological chemodenervation' with hyperoxia of 15–20 s duration in unanaesthetized rat pups. Relative changes in ventilation elicited by hyperoxia were determined by body plethysmography. We found no change in ventilation on the day of birth either in vaginally born rats or in near-term pups delivered by Caesarean section. After 1 day there was a significant decrease in ventilation of $-19{\cdot}4 \pm 2{\cdot}3\%$ (mean ± S.E.M.) and at 7 days of age the decrease was $-28{\cdot}8 \pm 2{\cdot}2\%$, suggesting an increasing influence from the peripheral chemoreceptors.

3. The contents of dopamine and noradrenaline were measured by high-performance liquid chromatography. Dopamine increased from $3{\cdot}7 \pm 0{\cdot}4$ pmol (pair of carotid bodies)$^{-1}$ in the fetus to a peak of $15{\cdot}9 \pm 2{\cdot}6$, 6–12 h after birth followed by a decline to $7{\cdot}1 \pm 0{\cdot}7$ at 7 days of age. Noradrenaline levels increased from $1{\cdot}3 \pm 0{\cdot}3$ in the fetus to $9{\cdot}6 \pm 1{\cdot}1$ pmol (pair of carotid bodies)$^{-1}$ after 4 days. The turnover rate of dopamine decreased from 4·4 pmol (pair of carotid bodies)$^{-1}$ h^{-1} 0–6 h after birth to 1·0 at 6–12 h of age. The turnover rate of noradrenaline also decreased over the first hours following delivery.

4. Since dopamine is an inhibitory neuromodulator in this system, we suggest that the increase in sensitivity seen after the first day of life is, at least in part, due to a decrease in the release of dopamine and thus a removal of an inhibitory mechanism.

MS 7746

INTRODUCTION

Diverging opinions regarding the function of the peripheral arterial chemoreceptors in the carotid bodies in the fetus and their role in the onset of breathing have been advanced (see Purves, 1974; Purves, 1981; and Hanson, 1986 for review). The carotid bodies were found to be silent in the fetus and activated at birth (Biscoe & Purves, 1967; Purves, 1974). In contrast to these findings, Hanson and co-workers found that the peripheral chemoreceptors in the carotid body were spontaneously active and responsive in the exteriorized fetal lamb at the lower arterial O_2 pressure (P_{a,O_2}) prevalent during pregnancy. At the onset of air breathing, the rise in P_{a,O_2} virtually abolished the afferent chemoreceptive impulses in the sinus nerve. Moreover, spontaneous activity during normoxia could be recorded again after a few days and there was a shift of the carotid body response curve to a P_{a,O_2} towards the adult range of sensitivity over the first days of life (Blanco, Dawes, Hanson & McCooke, 1984). These results suggest that the sensitivity of the peripheral chemoreceptors in the carotid body is adapted to the comparatively hypoxic environment of the fetus and then reset to the higher P_{a,O_2} of the newborn. This concept of a resetting after birth is supported by findings in kittens (Hanson, Kumar & Williams, 1987) and babies (Hertzberg & Lagercrantz, 1987) in which the sensitivity of the chemoreceptors appears to increase postnatally. The mechanism of this alteration is unknown. One possibility is a change in the expression of neurotransmitters or neuromodulators in the carotid body near birth. Several transmitter candidates mediating chemosensitivity have been proposed, for instance substance P (Prabhakar, Runold, Yamamoto, Lagercrantz & von Euler, 1984), acetylcholine, dopamine and noradrenaline (for reviews, see Eyzaguirre & Fidone, 1980; Eyzaguirre & Zapata, 1984; Pallot, 1987).

The carotid bodies of several mammalian species contain dopamine and noradrenaline (Mir, Al-Neamy, Pallot & Nahorski, 1982). The function of noradrenaline is controversial; it has been proposed to have a stimulatory role (Folgering, Ponte & Sadig, 1982) but as the effect is absent in the *in vitro* preparation (Zapata, 1975) this finding may be due to a direct effect on the carotid body vessels. Quantitatively, dopamine is the dominant catecholamine in some species investigated, including the rat, while noradrenaline is predominant in others (Fidone & Gonzalez, 1986). Since dopamine does not fulfill the classical criteria of a neurotransmitter in the carotid body (Eyzaguirre & Zapata, 1984) it is assumed that it exerts a modulatory action on carotid sinus nerve activity (Hellström, Hanbauer, Commissiong, Karoum & Koslow, 1984). Accumulating evidence suggests an inhibitory role of dopamine on carotid body afference in the cat (Docherty & McQueen, 1978; Llados & Zapata, 1978), goat (Kressin, Nielsen, Laravuso & Bisgard, 1986), lamb (Mayock, Standaert, Guthrie & Woodrum, 1983) and rat (Cardenas & Zapata, 1981).

As some observations suggest that dopamine may be involved in the acclimatization to hypoxia (Bisgard, Kressin, Nielsen, Daristotle, Smith & Forster, 1987) and carotid body chemoreceptor resetting (Dawes, Hanson, Holman & McCooke, 1984) the contents of dopamine and noradrenaline in the carotid bodies and their temporal relationship to the development of the arterial chemoreflex was inves-

tigated in rat pups. Preliminary results indicated that a resetting of the peripheral chemoreflex also occurred in rat pups and that this was preceded by marked changes in the carotid body contents of dopamine and noradrenaline (Hertzberg, Hellström, Pequignot & Lagercrantz, 1988). The aim of the present study was therefore to correlate the dynamics of dopamine and noradrenaline turnover in the carotid body with the chemoreflex. Since we wished to investigate developmental aspects, a method was needed in which the same unanaesthetized animal could be studied repeatedly and non-invasively under physiological conditions. The ventilatory response of the newborn to acute hypoxia is biphasic, rendering chemoreceptor input difficult to interpret from a hypoxic challenge. The method of 'physiological chemodenervation' of the chemoreceptors with hyperoxia was therefore used. This approach implies that the initial change in ventilation during hyperoxia reflects the tonic, 'hypoxic' drive from the peripheral chemoreceptors during normoxia that is abolished by a short-term exposure to hyperoxia (Dejours, 1962).

METHODS

Ventilatory study

The strength of the peripheral chemoreflex during normoxia was assessed by hyperoxic chemodenervation of the chemoreceptors in newborn Sprague–Dawley rats (Alab, Stockholm). The test was performed in nine pre-term rats delivered by Caesarean section 1 day before expected delivery, in eight newborn rat pups 12–24 h after birth and again in the vaginally born animals at 2, 4 and 7 days of age. The unanaesthetized rats were placed in a small body plethysmograph (Fig. 1) appropriate for the size of the animals. The smallest plethysmograph used for the newborn rats measured $48 \times 20 \times 20$ mm and was made in 3 mm Perspex. For the older pups a box measuring $60 \times 33 \times 33$ mm was used. The head end was open and after the pup was placed in the box this end was sealed with plastic film with the head emerging through a hole. Potential leaks around the neck were reduced with electrode jelly. The method is similar to the one used by Mortola (1984) and McCooke & Hanson (1985).

Flow in and out of the box was measured with a pneumotachometer coupled to a pressure transducer (Validyne, North Ridge, USA). The flow signal was electronically processed by a custom-built integrator (Modesto & daSilva, 1982) to obtain a volume-related signal. The integrator was modified to reset after a sudden offset of the baseline. This made recordings possible even in the very young rats who produced frequent movement artifacts. A disadvantage with this modification was that it made calibration by passing air into the plethysmograph with a syringe impossible. We did not regard this as essential since we were mainly interested in the relative changes in ventilation.

Temperature in the box was kept at 33–35 °C by means of a heating pad and an infra-red lamp. Air and O_2 were delivered via a Perspex hood measuring $130 \times 45 \times 52$ mm placed over the plethysmograph. Both gases were dry and delivered from cylinders at the same flow rate of $1 \pm 0{\cdot}2$ l min^{-1}. The gases were led through coils passing through a water-bath to prevent cooling of the facial area. A four-way stopcock permitted switching from one gas to another without sudden pressure changes, thus precluding tactile stimulation. Changing gas to pure O_2 yielded 45% O_2 at the pup's head within 5 s. Oxygen concentrations were continuously monitored using a zirconium cell analyser (Ametek, Pittsburgh, USA) with a 90% response time of 0·2 s. Although the tubing was kept as short as possible, a certain dead space of the apparatus was inevitable but was constant between runs. Thus there was a delay of 1·5 s from the switching of gases until the oxygen tracing started to rise. Of this delay, 1 s was accounted for by the tubing between the head-box and the oxygen analyser.

No attempt was made to determine the behavioural state of the rats. When the pup breathed regularly, air was substituted for by O_2. Ventilation was measured during 3–5 s periods before hyperoxia and again during hyperoxic breathing (Fig. 2). The aim was to asses ventilation after 5 s of hyperoxia but sometimes augmented breaths or movement artifacts required a shift in the

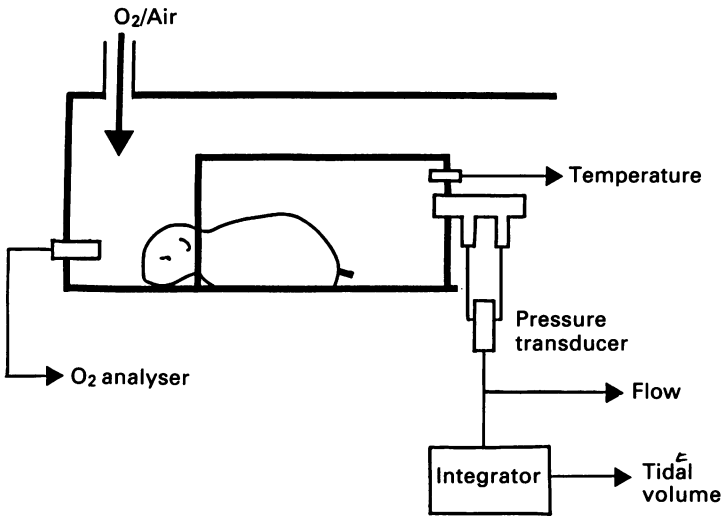

Fig. 1. Schematic drawing of equipment for ventilatory recordings. Ventilation was assessed from the flow passing in and out of the body plethysmograph via a pneumotachometer coupled to a pressure transducer. Inspired air could be switched to oxygen by means of a hood placed over the plethysmograph. Box temperature and inspired O_2 levels were continuously monitored.

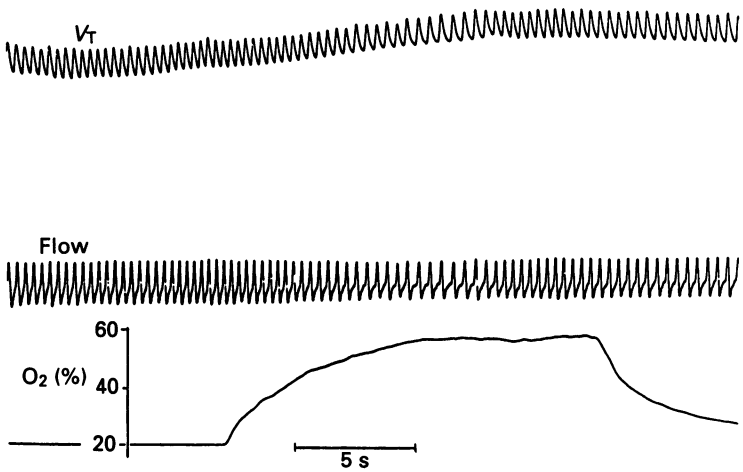

Fig. 2. Response of a 2-day-old rat to transient hyperoxia. From top to bottom: tidal volumes (V_T), tidal flow and inspired O_2 levels. Note the decrease in frequency elicited by hyperoxia.

measurement period which in extreme cases started as early as 3 s or as late as 8 s after the switching of gases. Generally, the newborn rats, whether delivered by Caesarean section or vaginally, showed an irregular breathing pattern with frequent spontaneous apnoeas, especially on the day of birth. To be sure that an apnoea was caused by hyperoxia and not spontaneous, a large number of trials had to be performed in each rat. Therefore, only recordings where respiration was fairly regular during the hyperoxic challenge were used to evaluate the chemoreflex, i.e. trials with

gross body movements or apnoeas within the measurement or control periods were discarded. The relative changes in ventilation, frequency and tidal volume were calculated. The trial was repeated three to five times in each rat and the mean response for each rat was used for statistical evaluation. In a few cases, however, only one or two acceptable tests were obtained due to restless animals. The pups were nursed by their mothers between the experiments.

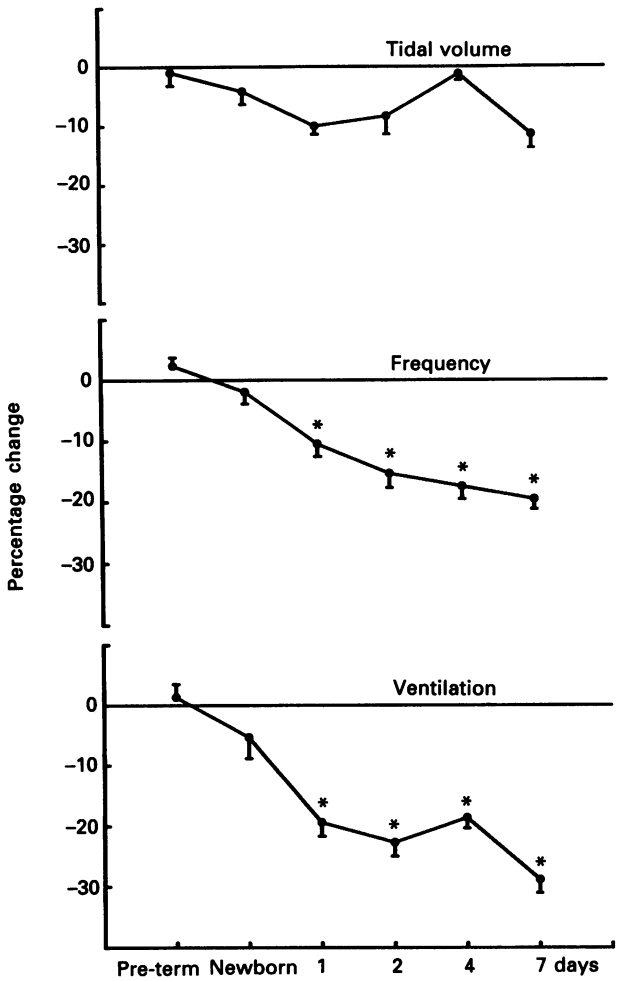

Fig. 3. Percentage changes in tidal volumes, frequency and ventilation (mean ± S.E.M.) elicited by transient hyperoxia. The test was carried out in near-term pups delivered by Caesarean section and in vaginally born rats on the day of the birth. The vaginally born group was tested repeatedly over the first week of life. * denotes significant changes relative to pre-term and newborn rats ($P < 0.05$). From 1 day of age and onwards ventilation decreased significantly, suggesting an increase in chemoreceptor activity with increasing age. The change in ventilation was mainly due to a decrease in frequency.

Biochemical study

The contents of dopamine and noradrenaline were determined in the carotid bodies of near-term fetal and newborn rats. The fetal samples were obtained by surgical removal of the intact uterus from the anaesthetized dam (pentobarbitone, 60 mg kg^{-1} I.P.) on gestational day 20. The uterus was immediately placed on ice. Fetuses were removed individually and the carotid bodies were

excised under a dissection microscope. The rats were thus never allowed to breathe. The vaginally born rats were killed with a sharp blow on the head and the carotid bodies were dissected out within a few minutes. Immediately after excision the carotid bodies were put into ice-cold perchloric acid (0·1 mol l^{-1} + Na_2EDTA, 2·7 mmol l^{-1}) and stored at −70 °C for later analysis. Some specimens were taken for morphological verification.

TABLE 1. Percentage changes in tidal volumes, frequency and ventilation (mean ± S.E.M.) elicited by transient hyperoxia

Age	*n*	Tidal volume	Frequency	Ventilation
Pre-term	9	−0·9 ± 2·2	2·4 ± 1·4	1·3 ± 2·2
Newborn	8	−4·1 ± 2·2	−2·0 ± 1·9	−5·3 ± 3·5
1 day	8	−10·0 ± 1·3	−10·5 ± 2·0*	−19·4 ± 2·3*
2 days	8	−8·3 ± 3·0	−15·3 ± 2·3*	−22·7 ± 2·3*
4 days	8	−1·2 ± 1·1	−17·4 ± 2·1*	−18·6 ± 1·7*
7 days	8	−11·4 ± 2·3	−19·5 ± 1·7*	−28·8 ± 2·2*

The test was carried out in near-term pups delivered by Caesarean section and in vaginally born rats on the day of birth. The vaginally born group was tested repeatedly over the first week of life. * denotes significant changes relative to pre-term and newborn rats ($P < 0·05$). From 1 day of age and later ventilation decreased significantly, suggesting an increase in chemoreceptor activity with increasing age. The change in ventilation was mainly due to a decrease in frequency.

TABLE 2. Contents of dopamine and noradrenaline in the carotid bodies of newborn rats at different postnatal ages and near-term fetuses

Age	Dopamine (pmol $pair^{-1}$)	$T_{\frac{1}{2}}$ (h)	Turnover (pmol h^{-1})	Noradrenaline (pmol $pair^{-1}$)	$T_{\frac{1}{2}}$ (h)	Turnover (pmol h^{-1})
Fetus	3·7 ± 0·4	—	—	1·3 ± 0·3	—	—
0–6 h	13·3 ± 2·2*	2·1	4·4	1·8 ± 0·2	5·4	0·2
6–12 h	15·9 ± 2·6*	10·6	1·0†	2·7 ± 0·3	—	0·0†
12–24 h	10·8 ± 0·7*	7·1	1·0†	4·4 ± 0·6*	93·8	0·0†
2 days	12·0 ± 1·4*	5·4	1·5†	7·1 ± 1·1*	37·7	0·1
4 days	8·1 ± 1·1	2·7	2·1†	9·6 ± 1·1*	5·3	1·3†
7 days	7·1 ± 0·7	3·1	1·6†	5·5 ± 0·5*	14·2	0·3

During the first day after birth carotid bodies were collected from newborn rats at 0–6, 6–12 and 12–24 h after delivery. The values are expressed as pmol (pair of carotid bodies)$^{-1}$ (mean ± S.E.M.). * indicates a significant difference relative to fetal values ($P < 0·05$). Note the prominent peak in dopamine during the day of birth while noradrenaline shows a more gradual increase. For vaginally born rats the half-lives ($T_{\frac{1}{2}}$) and turnover rates for the amines were calculated and expressed as hours and picomoles per pair of carotid bodies per hour respectively. † indicates a significant difference in turnover rate *versus* pups 0–6 h old ($P < 0·05$). Due to a very low turnover, no half-life could be calculated for noradrenaline in rats 6–12 h old. There was a marked decrease in dopamine turnover 6–12 h after birth compared to the immediately newborn.

Levels of dopamine and noradrenaline were determined via high-performance liquid chromatography with electrochemical detection (Eldec 102, Chromatofield, Marseille, France) as previously described (Pequignot, Cottet-Emard, Dalmaz, De Haut de Sigy & Peyrin, 1986). The sensitivity was 0·06 pmol for noradrenaline and 0·11 pmol for dopamine.

Half-lives and turnover rates were determined at 0–6, 6–12 and 12–24 h, 2, 4 and 7 days after birth. Catecholamine synthesis was blocked through inhibition of tyrosine hydroxylase with α-

methyl-*p*-tyrosine methylester (Sigma, St Louis, USA). Rat pups were either injected with α-methyl-*p*-tyrosine 250 mg kg^{-1} s.c. dissolved in 0·05 ml saline or with equal amounts of saline only, returned to their mothers and killed later. This dose is sufficient enough to inhibit the enzyme in the adult rat (Brodie, Costa, Dlabac, Neff & Smookler, 1966) and we have no reason to doubt its effect on tyrosine hydroxylase in the newborn. Drug-injected rats were killed 1, 2 or 4 h after drug

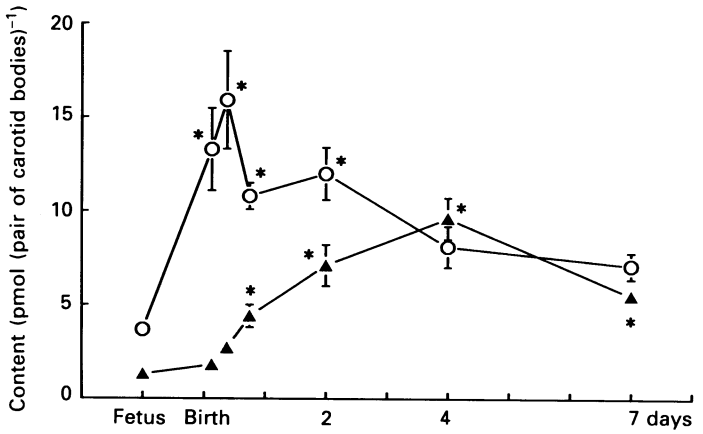

Fig. 4. Contents of dopamine (○) and noradrenaline (▲) in the carotid bodies of newborn rats at different postnatal ages and near-term fetuses. During the first day after birth carotid bodies were collected from newborn rats at 0–6, 6–12 and 12–24 h after delivery. Each point represents mean ± S.E.M. of at least five pairs. Some of the error bars are smaller than the symbols. * indicates a significant difference relative to fetal values ($P < 0·05$). Note the prominent peak in dopamine during the day of birth while noradrenaline shows a more gradual increase.

administration and the carotid bodies were excised as previously described. At 4 days of age, however, no samples were taken after 1 h due to small litter sizes. Semilogarithmic plots of amine content *versus* time were made and half-lives were calculated from the rate of noradrenaline and dopamine decrease (Figs 5 and 6). The turnover rate (pmol (pair of carotid bodies)$^{-1}$ h^{-1}) is the product of k times the mean content of the amine in carotid bodies of saline-injected rats, where k is the slope of the regression line in the semilogarithmic plot (Brodie *et al.* 1966). Half-life was calculated by $\ln(0·5)k^{-1}$.

Statistical methods

Analysis of variance was used to compare the contents of dopamine and noradrenaline and the ventilatory parameters at different ages. The rate of catecholamine decrease after α-methyl-*p*-tyrosine injection was determined by regression analysis. The turnover rates were compared by Dunnet's test for comparison of several means with one control condition. A $P < 0·05$ was considered significant.

RESULTS

Ventilatory responses to hyperoxia

The initial effects of hyperoxia are presented in Fig. 3 and Table 1. The responses in tidal volume elicited by hyperoxia did not change significantly with increasing age in the vaginally born group. There were no significant changes in frequency or ventilation on the day of birth either in pre-term rats delivered by Caesarean section

or vaginally born pups. From 1 day of age and onwards the changes elicited by hyperoxia were significant. Furthermore, all results regarding ventilation and frequency obtained from 1 day of age and onwards were significantly different from the values recorded on the day of birth.

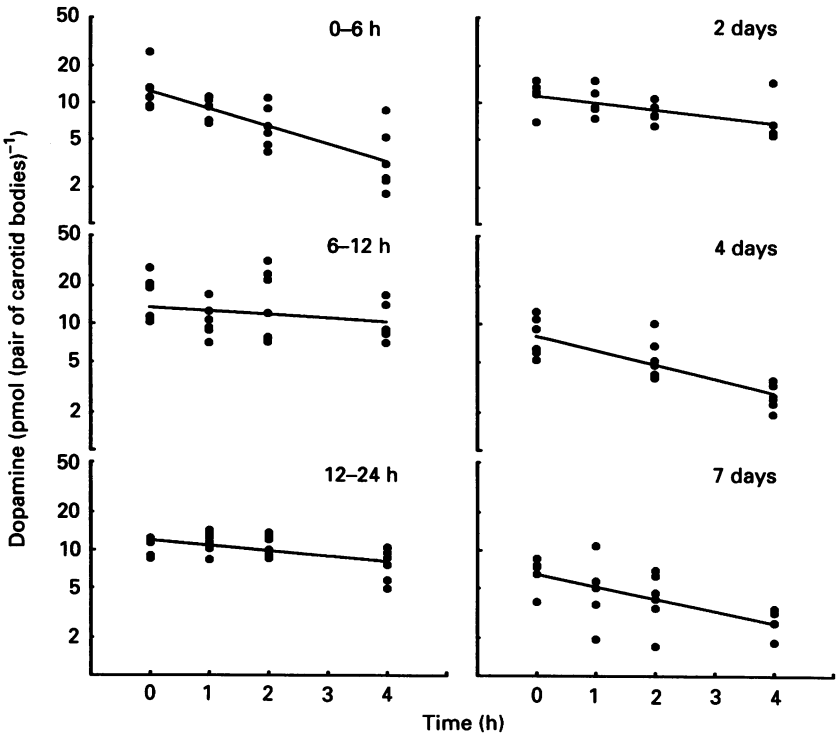

Fig. 5. Semilogarithmic plots of decrease in carotid body content of dopamine over time after inhibition of synthesis with α-methyl-*p*-tyrosine at different postnatal ages. The slopes of the fitted lines describe the time constant of the decrease. Each point represents the dopamine content of one pair of carotid bodies. Control values are results from rats injected with saline. Note the rapid decrease in dopamine content during the first hours after birth and the lower depletion rate only a few hours later.

Catecholamine contents in carotid bodies

Dopamine

Dopamine and noradrenaline contents in the carotid bodies are shown in Fig. 4 and Table 2. During the first day after a normal delivery there was a fourfold increase in dopamine within the first 6–12 h after birth. In rats 4 and 7 days of age the mean levels were no longer significantly different from fetal values.

Noradrenaline

Noradrenaline content increased during the first 2 days of life, although the peak seen in dopamine content on the day of birth was not observed. After a maximum at 4 days of age there was a slight decrease after 7 days. In rats 12–24 h and older the noradrenaline content was significantly higher than in the fetus.

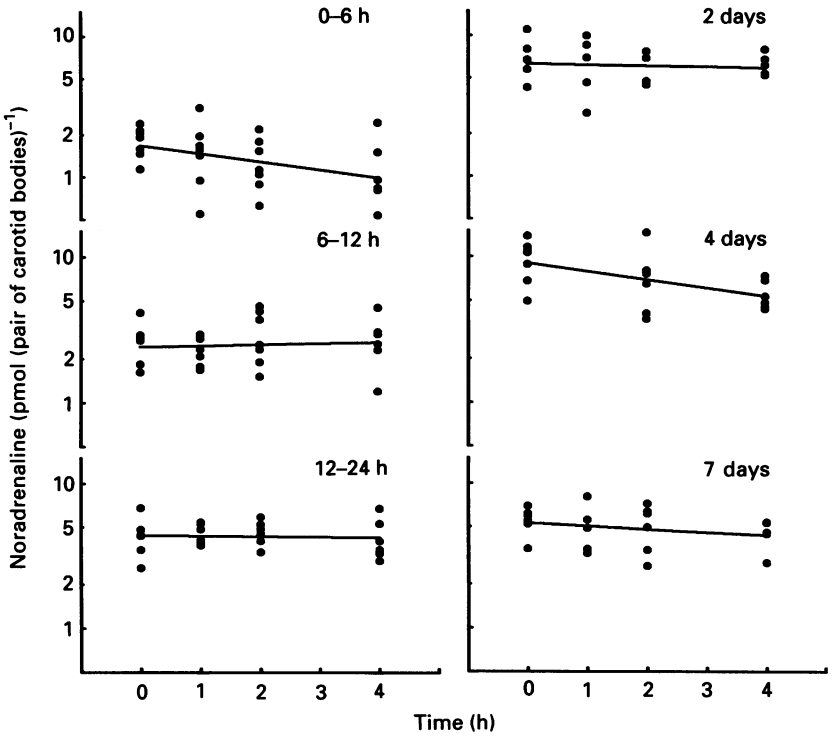

Fig. 6. Plots of noradrenaline content in carotid bodies of rats after inhibition of synthesis. For explanation, see Fig. 5.

Catecholamine turnover

Dopamine

A decrease in dopamine was found after injection of α-methyl-*p*-tyrosine (Fig. 5). The half-lives and turnover rates are shown in Table 2. The turnover rate of dopamine was 4·4 pmol (pair of carotid bodies)$^{-1}$ h^{-1} in rats less than 6 h old with a marked decrease to 1·0 in rats 6–12 h old. There was a slight increase towards the end of the first week. Turnover rates of dopamine were significantly lower in older rats compared with rats 0–6 h of age (Table 2 and Fig. 7).

Noradrenaline

The drop in noradrenaline after α-methyl-*p*-tyrosine was much slower than for dopamine, and the decrease was small after 4 h (Fig. 6). The slope of the decrease in noradrenaline after inhibition of synthesis was not significantly different from zero except during the first hours after birth and at 4 days of age, adding a large amount of insecurity to the calculations of half-lives and turnover rates. No half-life could be calculated in rats 6–12 h after birth due to a very slow turnover. There was a significant decrease in turnover at 6–12 h and 12–24 h after birth compared to the immediately newborn pups; turnover peaked at 4 days of age.

DISCUSSION

This study reports two main findings: (1) the transient response to hyperoxia is absent or weak in the newborn rat on the first day of life, indicating a weak respiratory drive from the peripheral chemoreceptors. There was no difference between near-term rats delivered by Caesarean section and term rats born vaginally in this respect. The influence of the chemoreflex becomes apparent after about 1 day in this species and develops further during the first week of life. (2) The emergence of the chemoreflex is preceded by profound changes in dopamine and noradrenaline levels in the carotid body. Especially interesting is the marked decrease in dopamine turnover rate found after 6 h following birth.

The method for determining the peripheral chemoreflex activity by transient hyperoxia or a 'single-' or 'double-breath O_2 test' is well established (Dejours, 1962). In this study a longer period of hyperoxia was used because a single breath is difficult to administer without a mask at the high breathing rates of the newborn rats. The short-term changes in ventilation elicited by the hyperoxic exposure were assumed to reflect alterations of peripheral chemoreceptor activity. If there was a tonic respiratory drive emanating from the peripheral chemoreceptors, it would have been abolished by hyperoxia resulting in a ventilatory decrease. However, these changes only constitute an indirect measure of chemoreceptor input to the respiratory centres, and other mechanisms may also influence the response.

This method provides a means for examining the same unanaesthetized rat pups at different ages under physiological conditions. It gives a good estimate of any changes in ventilation during hyperoxia, but it is not suited for determining the absolute levels of ventilation. Since the plastic film around the pup's neck was not rigid, movements of the seal probably occurred. Changes in ventilation induced by hyperoxia seemed reliable since the mechanical properties of the seal remained constant over this short period. As the plethysmograph was not tightly sealed, this method was insensitive to temperature-induced changes in pressure in the plethysmograph.

Spontaneous apnoeas were abundant in the immediately newborn rat. Our approach to discard hyperoxic tests with apnoeas may therefore have underestimated the chemoreflex in these animals since we could not differentiate between spontaneous and hyperoxia-induced apnoeas. Our results from the newborn rats are in accordance with earlier studies on newborn lambs (Blanco *et al.* 1984), kittens (Hanson *et al.* 1987) and babies (Miller & Behrle, 1954; Girard, Lacaisse & Dejours, 1960; Lahiri, Brody, Motoyama & Velasquez, 1978; Hertzberg & Lagercrantz, 1987) in that the chemoreflex is weak or absent soon after birth. Although the time of ventilatory measurement may have differed slightly between different runs, we do not think this is crucial since the response curve of chemoreceptor afference *versus* P_{a,O_2} is rather flat above normoxaemia, especially in newborn animals who have not reset their chemoreceptors (Blanco *et al.* 1984). If a tonic drive is present, a decrease in chemoreceptor afference is probably apparent already after a few seconds of hyperoxic breathing. Furthermore, to make the test more reliable the trial was repeated in each rat and the mean response was used. In the present study a decrease in breathing frequency occurred when the pups were exposed to hyperoxia, while the

newborn baby responds primarily with a decrease in tidal volume (Hertzberg & Lagercrantz, 1987). These differences may suggest that the Hering–Breuer reflex is stronger in the newborn rat than in the newborn human.

It has been shown that the carotid bodies are not necessary for initiation of breathing at birth or for the maintenance of regular breathing in the newborn

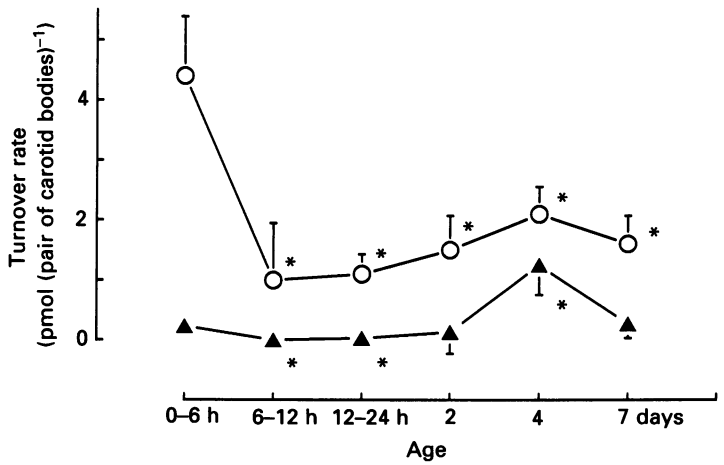

Fig. 7. The turnover rates of dopamine (○) and noradrenaline (▲) in carotid bodies at different postnatal ages. The turnover is higher for dopamine than for noradrenaline. Note the relatively high turnover rate of dopamine in the immediately newborn and the marked decrease in turnover rates only a few hours later. * indicates significant differences relative to the turnover rates at 0–6 h after birth ($P < 0{\cdot}05$). The error bars of some of the values are smaller than the symbols.

period (Jansen, Ioffe, Russel & Chernick, 1981). Factors besides hypoxia such as hypercapnia and cold stimulation are probably more important for the onset of respiration (Gluckman, Gunn & Johnston, 1983; Blanco, Martin, Hanson & McCooke, 1987). Nevertheless, the carotid bodies seem to gain increasing importance after the first few days. Transection of the sinus nerve in rats 8 to 10 days old caused severe disturbances in respiratory pattern, especially when combined with the cutting of the aortic depressor nerves. This effect became less pronounced in older animals (Hofer, 1986); Bureau, Lamarche, Foulon & Dalle (1985) found that three out of seven carotid body-denervated lambs died unexpectedly at 4–5 weeks of age, suggesting a vital role of the carotid chemoreceptors during development.

The factors determining the resetting of the peripheral chemoreceptors are unknown. It has been suggested that a rise in P_{a,O_2} is necessary, since ventilation of the fetal sheep *in utero* with a gas mixture preserving the fetal blood gas levels or 7 h of hyperoxia failed to reset the chemosensor while 27 h of hyperoxia did (Blanco, Hanson & McCooke, 1988). Thus an inflation of the lungs *per se* did not change the sensitivity of the chemoreceptors.

The finding in the present study of profound changes in dopamine turnover in the carotid body may provide a clue to the mechanism of postnatal resetting. We did not perform a turnover study in the fetus but we have found a rapid decrease in dopamine turnover paralleled by an increase in dopamine content during the first

days of life (Fig. 7). Short-term hypoxia depletes the dopamine stores of the carotid body *in vivo* (Hanbauer & Hellström, 1978), and increases the release *in vitro* (Fidone, Gonzalez & Yoshizaki, 1982*a*) while chronic hypoxia resulted in an increase in content (Hanbauer, Karoum, Hellström & Lahiri, 1981) and turnover rates (Pequignot, Cottet-Emard, Dalmaz & Peyrin, 1987) of both dopamine and noradrenaline.

The peripheral chemoreceptors are active and responsive in the fetal sheep, although at the lower P_{a,O_2} prevailing in the fetus (Blanco *et al.* 1984). From the present observations, it may be speculated that the low sensitivity before birth can, at least in part, be due to a comparatively high release of dopamine from the glomus cells of the fetus. Hypoxia increases synthesis and release of dopamine from the carotid body in a dose-dependent fashion (Fidone, Gonzalez & Yoshizaki, 1982*a*, *b*; Rigual, Gonzalez, Gonzalez & Fidone, 1986). It therefore seems reasonable to assume that hyperoxia decreases dopamine release. Furthermore, in the *in vitro* preparation of the cat carotid body, it was demonstrated that the release of dopamine was decreased when ambient O_2 levels returned to hyperoxia after a period of relative hypoxia. We found that at a few hours after birth, when P_{a,O_2} is increased, dopamine release is rapidly dropping. Moreover, as tyrosine hydroxylase is O_2 dependent (Davis & Carlsson, 1973), dopamine synthesis may increase as oxygenation improves. The result will be a pooling of dopamine within the glomus cells which is reflected in the peak of dopamine observed during the first days after birth.

As the release of dopamine is decreased, the partial inhibition exerted is abolished and the chemoreceptor becomes potentially more sensitive. The notion that the chemoreceptive afferent activity is still low or absent during the first day after birth suggests that other mechanisms also come into play. The decrease in dopamine receptor activity may also initiate other events in the carotid body or the neuronal circuit that establish the resetting. It was recently demonstrated that tyrosine hydroxylase increases markedly in the rat petrosal ganglion from the day before to the day after birth (D. Katz, personal communication). Whether this is dependent on carotid sinus nerve activity and any connection to the strength of the chemoreflex remain to be established. Furthermore, it is presently not clear whether the sensitivity acquired after birth is permanent throughout life, as some authors claim that there is a critical period within which the chemoreceptors gain their permanent sensitivity (Sørensen & Severinghaus, 1968), while other believe that the process is reversible (Edelman, Lahiri, Braudo, Cherniack & Fishman, 1970).

The changes in noradrenaline content are more difficult to interpret. Firstly, since the sampling was confined to the first 4 h after blocking of synthesis and the turnover was very low, calculation of turnover rate and half-life may be less accurate. Secondly, there are at least two pools of noradrenaline within the carotid body. One is believed to be intrinsic, and one constituting about 50% of the total carotid body contents that disappears after ganglionectomy and thus is assumed to be located in sympathetic nerve endings emanating from the superior cervical ganglion (Hanbauer & Hellström, 1978). We found a fourfold increase in noradrenaline over the first 2 days of life. After an initial decrease in turnover, there was no significant change in turnover rate until at 4 days of age (Fig. 7). Whether this reflects changes in turnover of carotid body noradrenaline content, in sympathetic innervation or activity of the

noradrenergic ganglioglomerular nerves is unknown. Since sympathetic innervation of autonomic target organs is absent or non-functional at birth in Sprague–Dawley rats (Slotkin, 1986) it is possible that part of the increase in noradrenaline is a result of ingrowth of new sympathetic nerve fibres. An increased activity in the superior cervical ganglion has been demonstrated at birth in the lamb (Purves, 1974), but important species differences may exist. Since we are dealing with different pools, an interpretation of turnover data is equally difficult. In a recent study of the cat carotid body *in vitro* it was found that 3 h exposure to hypoxia increased the synthesis of dopamine while noradrenaline production was unaffected (Rigual *et al.* 1986). During chronic hypoxia, noradrenaline turnover rate increases in the rat carotid body, but the time course is slower than for dopamine (Pequignot *et al.* 1987). In the superior cervical ganglion, increases in tyrosine hydroxylase activity and the levels of noradrenaline over the first weeks of life were demonstrated and this seemed to be, at least partially, dependent on trans-synaptic regulation (Smolen, Beaston-Wimmer, Wright, Lindley & Cader, 1985). The noradrenaline increase found in the carotid body may therefore be influenced by postnatal events in the ganglion.

Whether the action of dopamine on carotid body sensitivity in the newborn rat is similar to that in the adult is unknown. The decrease in ventilation during normoxia and hypoxia elicited by exogenous dopamine was less pronounced in lambs on the first day of life than later on (Mayock *et al.* 1983). This supports the notion that dopamine suppresses carotid body afference also in the newborn, albeit to a lesser extent than in the adult, possibly because carotid body activity is lower on the day of birth. Since there may be species differences in this respect, the action of dopamine in the newborn rat calls for further attention.

In conclusion we have found that the chemoreflex, assessed by the method of hyperoxic denervation, emerges after about 1 day in the newborn rat. The development of the arterial chemoreflex is preceded by a decrease in carotid body dopamine turnover, suggesting a lifting of an inhibition of the chemosensory mechanism.

This study was supported by Swedish Medical Research Council grant no. 05234, Expressen's Prenatal Foundation, the Swedish Society for Medical Research, General Maternity Hospital Foundation and NIH grant HL-39921. The valuable advice concerning catecholamine analysis provided by Dr J. M. Cottet-Emard is gratefully acknowledged.

REFERENCES

Biscoe, T. J. & Purves, M. J. (1967). Carotid body chemoreceptor activity in the new-born lamb. *Journal of Physiology* **190**, 443–454.

Bisgard, G. E., Kressin, N. A., Nielsen, A. M., Daristotle, L., Smith, C. A. & Forster, H. V. (1987). Dopamine blockade alters ventilatory acclimatization to hypoxia in goats. *Respiration Physiology* **69**, 245–255.

Blanco, C. E., Dawes, G. S., Hanson, M. A. & McCooke, H. B. (1984). The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *Journal of Physiology* **351**, 25–37.

Blanco, C. E., Hanson, M. A. & McCooke, H. B. (1988). Effects on carotid chemoreceptor resetting of pulmonary ventilation in the fetal lamb *in utero*. *Journal of Developmental Physiology* **10**, 167–174.

Blanco, C. E., Martin, C. B., Hanson, M. A. & McCooke, H. B. (1987). Determinants of the onset of continuous air breathing at birth. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **26**, 183–192.

BRODIE, B. B., COSTA, E., DLABAC, A., NEFF, N. H. & SMOOKLER, H. H. (1966). Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *Journal of Pharmacology and Experimental Therapeutics* **154**, 493–498.

BUREAU, M. A., LAMARCHE, J., FOULON, P. & DALLE, D. (1985). Postnatal maturation of respiration in intact and carotid body-chemodenervated lambs. *Journal of Applied Physiology* **59**, 869–874.

CARDENAS, H. & ZAPATA, P. (1981). Dopamine-induced ventilatory depression in the rat, mediated by carotid nerve afferents. *Neuroscience Letters* **24**, 29–33.

DAVIS, J. N. & CARLSSON, A. (1973). Effect of hypoxia on tyrosine and tryptophan hydroxylation in unanesthetized rat brain. *Journal of Neurochemistry* **20**, 913–915.

DAWES, G. S., HANSON, M. A., HOLMAN, R. B. & MCCOOKE, H. B. (1984). Concentrations of catecholamines and their metabolites in carotid bodies of fetal and new-born lambs. *Journal of Physiology* **346**, 97*P*.

DEJOURS, P. (1962). Chemoreflexes in breathing. *Physiological Reviews* **42**, 335–358.

DOCHERTY, R. J. & MCQUEEN, D. S. (1978). Inhibitory action of dopamine on cat carotid chemoreceptors. *Journal of Physiology* **279**, 425–436.

EDELMAN, N. H., LAHIRI, S., BRAUDO, L., CHERNIACK, N. S. & FISHMAN, A. P. (1970). The blunted ventilatory response to hypoxia in cyanotic congenital heart disease. *New England Journal of Medicine* **282**, 405–411.

EYZAGUIRRE, C. & FIDONE, S. J. (1980). Transduction mechanisms in carotid body: glomus cells, putative neurotransmitters, and nerve endings. *American Journal of Physiology* **239**, C135–152.

EYZAGUIRRE, C. & ZAPATA, P. (1984). Perspectives in carotid body research. *Journal of Applied Physiology* **57**, 931–957.

FIDONE, S. J. & GONZALEZ, C. (1986). Initiation and control of chemoreceptor activity in the carotid body. In *Handbook of Physiology: The Respiratory System*, ed. FISHMAN, A. P., CHERNIACK, N. S. & WIDDICOMBE, J. G., pp. 247–312. American Physiological Society, Bethesda, MD, USA.

FIDONE, S., GONZALEZ, C. & YOSHIZAKI, K. (1982*a*). Effects of low oxygen on the release of dopamine from the rabbit carotid body *in vitro*. *Journal of Physiology* **333**, 93–110.

FIDONE, S., GONZALEZ, C. & YOSHIZAKI, K. (1982*b*). Effects of hypoxia on catecholamine synthesis in rabbit carotid body *in vitro*. *Journal of Physiology* **333**, 81–91.

FOLGERING, H., PONTE, J. & SADIG, T. (1982). Adrenergic mechanisms and chemoreception in the carotid body of the cat and rabbit. *Journal of Physiology* **325**, 1–21.

GIRARD, F., LACAISSE, A. & DEJOURS, P. (1960). Le stimulus O_2 ventilatoire à la période néonatale chez l'Homme. *Journal de physiologie* **52**, 108–109.

GLUCKMAN, P. D., GUNN, T. R. & JOHNSTON, B. M. (1983). The effect of cooling on breathing and shivering in unanaesthetized fetal lambs *in utero*. *Journal of Physiology* **343**, 495–506.

HANBAUER, I. & HELLSTRÖM, S. (1978). The regulation of dopamine and noradrenaline in the rat carotid body and its modification by denervation and by hypoxia. *Journal of Physiology* **282**, 21–34.

HANBAUER, I., KAROUM, F., HELLSTRÖM, S. & LAHIRI, S. (1981). Effects of hypoxia lasting up to one month on the catecholamine content in rat carotid body. *Neuroscience* **6**, 81–86.

HANSON, M. A. (1986). Peripheral chemoreceptor function before and after birth. In *Respiratory Control and Lung Development In The Fetus and Newborn* ed. JOHNSTON, B. M. & GLUCKMAN, P. D., pp. 311–330. Perinatology Press, Ithaca, NY, USA.

HANSON, M. A., KUMAR, P. & WILLIAMS, B. A. (1987). Developmental changes in the reflex respiratory response to alternations of F_{i,O_2} in the newborn kitten. *Journal of Physiology* **394**, 69*P*.

HELLSTRÖM, S., HANBAUER, I., COMMISSIONG, J., KAROUM, F. & KOSLOW, S. (1984). Role and regulation of catecholamines in carotid body. In *Dynamics of Neurotransmitter Function*, ed. HANIN, I., pp. 31–38. Raven Press, New York.

HERTZBERG, T., HELLSTRÖM, S., PEQUIGNOT, J. M. & LAGERCRANTZ, H. (1988). Carotid body catecholamines and the development of the arterial chemoreflex in the newborn rat. *IXth International Symposium on Arterial Chemoreceptors*. (abstract). Park City, Utah, USA.

HERTZBERG, T. & LAGERCRANTZ, H. (1987). Postnatal sensitivity of the peripheral chemoreceptors in newborn infants. *Archives of Disease in Childhood* **62**, 1238–1241.

HOFER, M. A. (1986). Role of carotid sinus and aortic nerves in respiratory control of infant rats. *American Journal of Physiology* **251**, R811–817.

JANSEN, A. H., IOFFE, S., RUSSEL, B. J. & CHERNICK, V. (1981). Effect of carotid chemoreceptor denervation on breathing *in utero* and after birth. *Journal of Applied Physiology* **51**, 630–633.

KRESSIN, N. A., NIELSEN, A. M., LARAVUSO, R. & BISGARD, G. E. (1986). Domperidone-induced potentiation of ventilatory responses in awake goats. *Respiration Physiology* **65**, 169–180.

LAHIRI, S., BRODY, J. S., MOTOYAMA, E. K. & VELASQUEZ, T. M. (1978). Regulation of breathing in newborns at high altitude. *Journal of Applied Physiology* **44**, 673–678.

LLADOS, F. & ZAPATA, P. (1978). Effects of dopamine analogues and antagonists on carotid body chemosensors *in situ*. *Journal of Physiology* **274**, 487–499.

MCCOOKE, H. B. & HANSON, M. A. (1985). Respiration of conscious kittens in acute hypoxia and effect of almitrine bismesylate. *Journal of Applied Physiology* **59**, 18–23.

MAYOCK, D. E., STANDAERT, T. A., GUTHRIE, R. D. & WOODRUM, D. E. (1983). Dopamine and carotid body function in the newborn lamb. *Journal of Applied Physiology* **54**, 814–820.

MILLER, H. C. & BEHRLE, F. C. (1954). The effects of hypoxia on the respiration of newborn infants. *Pediatrics* **14**, 93–103.

MIR, A. K., AL-NEAMY, K., PALLOT, D. J. & NAHORSKI, S. R. (1982). Catecholamines in the carotid body of several mammalian species: effects of surgical and chemical sympathectomy. *Brain Research* **252**, 335–342.

MODESTO, J. P. & DASILVA, K. M. C. (1982). Drift-free electronic integrator for respiratory studies. *Medical and Biological Engineering and Computing* **20**, 457–460.

MORTOLA, J. P. (1984). Breathing pattern in newborns. *Journal of Applied Physiology* **56**, 1533–1540.

PALLOT, D. J. (1987). The mammalian carotid body. *Advances in Anatomy, Embryology and Cell Biology* **102**, 1–91.

PEQUIGNOT, J. M., COTTET-EMARD, J. M., DALMAZ, Y., DE HAUT DE SIGY, M. & PEYRIN, L. (1986). Biochemical evidence for norepinephrine stores outside the sympathetic nerves in rat carotid body. *Brain Research* **367**, 238–243.

PEQUIGNOT, J. M., COTTET-EMARD, J. M., DALMAZ, Y. & PEYRIN, L. (1987). Dopamine and norepinephrine dynamics in rat carotid body during long-term hypoxia. *Journal of the Autonomic Nervous System* **21**, 9–14.

PRABHAKAR, N. R., RUNOLD, M., YAMAMOTO, Y., LAGERCRANTZ, H. & VON EULER, C. (1984). Effects of substance P antagonist on the hypoxia-induced carotid chemoreceptor activity. *Acta physiologica scandinavica* **121**, 301–303.

PURVES, M. J. (1974). Onset or respiration at birth. *Archives of Disease in Childhood* **49**, 333–343.

PURVES, M. J. (1981). Chemoreceptors and their reflexes with special reference to the fetus and newborn. *Journal of Developmental Physiology* **3**, 21–57.

RIGUAL, R., GONZALEZ, E., GONZALEZ, C. & FIDONE, S. (1986). Synthesis and release of catecholamines by the cat carotid body *in vitro*: effects of hypoxic stimulation. *Brain Research* **374**, 101–109.

SLOTKIN, T. A. (1986). Development of the sympathoadrenal axis. In *Developmental Neurobiology of the Autonomic Nervous System*, ed. GOOTMAN, P. M., pp. 69–96. Humana Press, Clifton, NJ, USA.

SMOLEN, A. J., BEASTON-WIMMER, P., WRIGHT, L. L., LINDLEY, T. & CADER, C. (1985). Neurotransmitter synthesis, storage and turnover in neonatally deafferented sympathetic neurones. *Developmental Brain Research* **23**, 211–218.

SØRENSEN, S. C. & SEVERINGHAUS, J. W. (1968). Irreversible respiratory insensitivity to acute hypoxia in man born at high altitude. *Journal of Applied Physiology* **25**, 217–220.

ZAPATA, P. (1975). Effects of dopamine on carotid chemo- and baroreceptors *in vitro*. *Journal of Physiology* **244**, 235–251.