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VENTILATORY RESPONSE TO HYPEROXIA IN NEWBORN RATS BORN IN HYPOXIA – POSSIBLE RELATIONSHIP TO CAROTID BODY DOPAMINE

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SUMMARY

1. The influence of postnatal hypoxia on regulation of breathing and turnover rate of carotid body dopamine was examined in newborn rats. The percentage change in frequency, tidal volume and ventilation elicited by transient hyperoxia was assessed by flow plethysmography in unanaesthetized pups. The alteration in ventilation was taken as an index of peripheral chemoreceptor activity.

2. The rats were born and reared in hypoxia. The inspired oxygen fraction (F_{I,O_2}) was 0·12–0·14 until 2 days after delivery when the rats were placed into room air and the ventilatory chemoreflex was tested. At 4 days of age, i.e. 2 days after termination of hypoxia, the rats were tested again. The ventilatory data were compared with those from a previous study in normoxic rats.

3. We found a smaller decrease in ventilation (8·8 ± 3·9%, mean ± S.D.) in the hypoxic rats at 2 days of age compared with normoxic rats (22·7 ± 6·4%; $P < 0·001$). In contrast, at 4 days of age there was no difference in ventilatory response between the posthypoxic rats (19·2 ± 4·6%) and normoxic pups (18·6 ± 4·9%).

4. The turnover rates of dopamine in carotid bodies were determined at 0–6, 6–12, 12–24 h and 2 days after birth in hypoxic rats and in 2-day-old posthypoxic rat pups at different time intervals after termination of hypoxia. Postnatal hypoxia sustained a high turnover rate which decreased after termination of the hypoxia.

5. We propose that the weak chemoreflex in hypoxic rat pups is brought about by a high release of carotid body dopamine.

INTRODUCTION

The arterial chemoreceptors in the carotid body have been reported to be active and responsive in the fetal lamb (Itskovitz & Rudolph, 1982; Blanco, Dawes, Hanson & McCooke, 1984; Itskovitz & Rudolph, 1987) although their sensitivity seems to be

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adjusted to the low arterial oxygen pressure (P_{a,O_2}) prevalent *in utero*. After birth, as the P_{a,O_2} rises, the chemoreceptors become silent and gradually reset their sensitivity to the postnatal blood gas levels (Blanco *et al.* 1984). The strength of the ventilatory chemoreflex increases postnatally also in the newborn cat (Hanson, Kumar & Williams, 1989), rat (Eden & Hanson, 1987*a*; Hertzberg, Hellström, Lagercrantz & Pequignot, 1990) and human (Girard, Lacaisse & Dejours, 1960; Lahiri, Brody, Motoyama & Velasquez, 1978; Hertzberg & Lagercrantz, 1987).

The P_{a,O_2} level seems to determine the sensitivity to hypoxia, since chronic hypoxaemia from birth was found to interfere with postnatal adaptation of the chemoreflex (Sørensen & Severinghaus, 1968*a*; Eden & Hanson, 1987*b*; Hanson *et al.* 1989). Furthermore, Blanco and co-workers found that increasing fetal P_{a,O_2} by ventilating the sheep fetus *in utero* increased the responsiveness of the carotid body chemoreceptors (Blanco, Hanson & McCooke, 1988).

The mechanisms underlying these changes in sensitivity are unknown. Dopamine is the most abundant catecholamine in the carotid bodies of several species (see Fidone & Gonzalez, 1986). Direct neurophysiological studies as well as reflex responses to dopamine and dopamine receptor antagonists point to an inhibitory neuromodulator role on chemoreceptor discharge in the adult (Llados & Zapata, 1978; Docherty & McQueen, 1978, 1979; Cardenas & Zapata, 1981) and also in newborn lambs (Mayock, Standaert, Guthrie & Woodrum, 1983). We have recently proposed that removal of an inhibitory dopaminergic mechanism increases the responsiveness of the chemoreceptors after birth. This was based on the observation that the emergence of the chemoreflex was preceded by a drop in carotid body dopamine turnover over the first 12 h after birth (Hertzberg *et al.* 1990).

Considering that prolonged hypoxia increases dopamine content and turnover in the carotid body of the adult rat (Hanbauer, Karoum, Hellström & Lahiri, 1981; Pequignot, Cottet-Emard, Dalmaz & Peyrin, 1987), we wanted to see if long-term hypoxia from birth would affect the carotid body turnover of dopamine and if this was associated with alterations in chemosensitivity.

METHODS

Ventilatory study

Pregnant Sprague–Dawley rats were placed in a lucid hypoxic chamber 2 days before expected delivery. The chamber was flushed with nitrogen at a flow rate of about 5 l/min. Oxygen concentration was monitored continuously by a zirconium cell analyser (Ametek, Pittsburgh, USA) to ensure an inspired oxygen fraction (F_{I,O_2}) of 0·12–0·14. The inspired carbon dioxide fraction (F_{I,CO_2}) was below 0·005 as judged by intermittent analyses. Temperature in the box was around 25 °C. The rat pups were kept in hypoxia until they were 2 days of age. At that point a normal oxygen environment was restored within the chamber following cessation of the nitrogen flow. A fan sustained adequate circulation to prevent any build-up of CO_2.

To evaluate the activity of the peripheral chemoreceptors the chemoreflex was tested by means of a hyperoxic challenge. In brief, the unanaesthetized pup was placed in a small plexiglass box. One end was open and sealed with plastic film with the rat's head emerging through a hole. Potential leaks around the neck were reduced with electrode jelly. Flow into and out of the box was measured with a pneumotachometer head. Air was passed at a rate of 1 l/min over the pup's head via a plastic hood placed over the plethysmograph. Inspired O_2 levels were continuously monitored and temperature in the plethysmograph was kept at 33–35 °C. When the pup was in a quiet state, ventilation was recorded for a 3–5 s control period after which air was substituted by pure O_2 at the same flow rate. After approximately 5 s of O_2 breathing, ventilation was measured

for another 3–5 s period and the percentage changes in tidal volumes, frequency and ventilation were calculated. Although our aim was to monitor ventilation after 5 s of hyperoxia, sometimes augmented breaths or movement artifacts necessitated a shift in the measuring period which in extreme cases started as early as 3 s or as late as 8 s after the switching of gases. The trial was repeated several times in each pup, usually three to six, and the mean response was used for further analyses. A more complete account of the method is given in a previous paper (Hertzberg *et al.* 1990).

The chemoreflex was assessed in 2-day-old pups from two litters immediately after they were taken from the hypoxic chamber into room atmosphere and again at 4 days of age, i.e. 2 days after termination of hypoxia. The results from these rats were compared to rat pups born and reared in room air and investigated according to the same protocol as in a previous study (Hertzberg *et al.* 1990).

Biochemical study

Pregnant rats were kept in an hypoxia chamber under similar conditions as in the ventilatory part of the study. Turnover rate of dopamine was determined at 0–6, 6–12, 12–24 h and 2 days of age in rats born and reared in hypoxia. Moreover, turnover rates were determined 2 days after birth in another group of pups also born under hypoxic conditions. The latter group was taken out into room air 0–6, 6–12, 12–24 h or 2 days before killing. The rationale for this was to separate changes on dopamine turnover caused by postnatal age from those induced by environmental oxygen concentration.

The turnover rate of dopamine in the carotid bodies was determined by applying steady-state kinetics after inhibition of synthesis (Brodie, Costa, Dlabac, Neff & Smookler, 1966). Rats were injected subcutaneously with α-methyl-*p*-tyrosine methyl ester (Sigma) at 250 mg/kg dissolved in 0·05 ml saline or with equal amount of saline only. This dose is sufficient to inhibit tyrosine hydroxylase, the rate limiting step in catecholamine biosynthesis. Drug-injected pups were killed by a sharp blow on the head 4 h after administration. The carotid bodies were rapidly dissected out and put into ice-cold perchloric acid (0·1 mol/l + Na_2EDTA 2·7 mmol/l) and stored at −70 °C for later analysis. High performance liquid chromatography with electrochemical detection (Eldec 102, Chromatofield, Marseille, France) was used to assay dopamine as previously described (Pequignot, Cottet-Emard, Dalmaz, De Haut & Peyrin, 1986). The sensitivity was 0·11 pmol. A semilogarithmic plot of dopamine *versus* time after injection was made and a line fitted by means of linear regression. The slope of the line multiplied with the mean dopamine contents in carotid bodies of saline injected rats was taken as the turnover rate expressed as picomoles per pair of carotid bodies per hour. Previous observations suggest that dopamine declines in an exponential fashion after α-methyl-*p*-tyrosine (Hertzberg *et al.* 1990). Thus, only two time points were used in the present study.

Statistical methods

Comparison of strength of the chemoreflex during the various experimental conditions and ages was made by one way analysis of variance. Differences in turnover rates were analysed with Dunnet's test for comparing multiple means with a control condition. $P < 0·05$ was taken as significant.

RESULTS

Ventilatory study

Hyperoxic challenge was performed on eight hypoxic pups at 2 days after birth and nine posthypoxic ones at 4 days of age. The chemoreflex in these rats was compared with our previously published data obtained from eight rats reared under normal conditions and tested at 2 and 4 days of age (Hertzberg *et al.* 1990). The percentage changes in ventilatory parameters elicited by short-term hyperoxia are shown in Fig. 1. In all groups, there was a significant decrease in ventilation ($P <$ 0·001). At 2 days of age the hypoxic rats showed a weaker response in ventilation

than the normoxic pups, $-8{\cdot}8 \pm 3{\cdot}9\%$ *vs.* $-22{\cdot}7 \pm 6{\cdot}4\%$ (mean ± S.D., $P < 0{\cdot}001$). There were also differences in the changes of frequency, $-8{\cdot}0 \pm 6{\cdot}2\%$ *vs.* $-15{\cdot}3 \pm 6{\cdot}5\%$ ($P < 0{\cdot}05$) and tidal volume, $0{\cdot}0 \pm 6{\cdot}3\%$ *vs.* $-8{\cdot}3 \pm 8{\cdot}6\%$ ($P < 0{\cdot}05$).

The posthypoxic rats decreased their ventilation more at 4 days of age ($-19{\cdot}2 \pm 4{\cdot}6\%$) compared with their hypoxic state at 2 days after delivery ($P <$

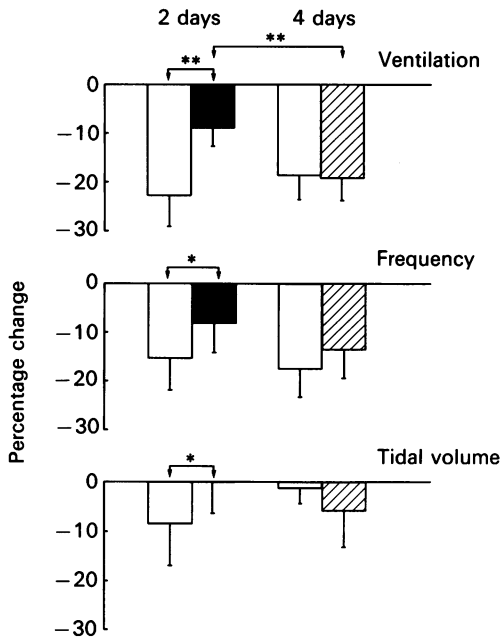

Fig. 1. Percentage changes in ventilation, frequency and tidal volume elicited by short-term hyperoxia in 2- and 4-day-old rat pups. Bars represent means ± S.D. in rats hypoxic from birth (filled bars), pups returned to room air after 2 days of postnatal hypoxia (hatched bars) and rats born and reared in normoxia (open bars). There were significant differences in relative changes of ventilation, frequency and tidal volume between 2-day-old rats hypoxic from birth compared with normoxic ones. At 4 days of age the chemoreflex was indistinguishable between posthypoxic rats and normoxic pups. $^{**}P < 0{\cdot}001$, $^{*}P < 0{\cdot}05$.

0·001) although responses in tidal volume ($-5{\cdot}7 \pm 7{\cdot}4\%$) or frequency ($-13{\cdot}5 \pm 6{\cdot}0\%$) did not change significantly. There were no differences in ventilatory parameters elicited by hyperoxia between the 4-day-old rats, whether they had been born in hypoxia or normoxia.

Dopamine contents and turnover rates

The dopamine content in untreated rats ranged from $10{\cdot}25 \pm 3{\cdot}81$ to $12{\cdot}17 \pm 1{\cdot}87$ (pmol/pair; means ± S.D.) in hypoxic rat pups and from $11{\cdot}81 \pm 1{\cdot}61$ to $17{\cdot}09 \pm 7{\cdot}21$ in the posthypoxic animals. There was no significant difference between them. Turnover rates of dopamine in carotid bodies were assessed using 128 rat pups from sixteen litters. The results from these and the turnover rates found in rat pups born in normoxia (Hertzberg *et al.* 1990) are given in Fig. 2. In the hypoxic rat pups,

dopamine turnover increased from 3·00±0·68 pmol per pair of carotid bodies per hour 0–6 h after birth to 3·60±0·45 at 12–24 h of age ($P < 0·05$) and decreased to 3·45±0·73 at 2 days of age. Two-day-old rats returned to room air after hypoxia were found to have a turnover rate of 4·59±1·18 after 0–6 h of normoxia while turnover

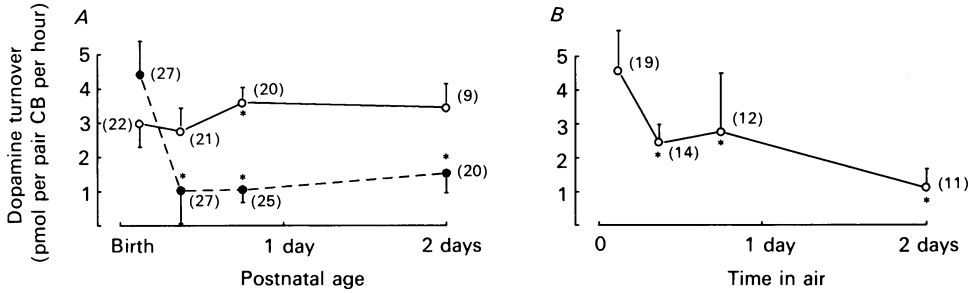

Fig. 2. *A*, turnover rates of dopamine (pmol per pair of carotid bodies (CB) per hour, means±S.D.) in chronically hypoxic rats (○). The rates found in rats born in normoxia (●) are presented for comparison (Hertzberg *et al.* 1990). Number of rats is given in parentheses. Asterisks denote significantly different turnover rate relative value at 0–6 h after birth in respective group. The turnover rate was sustained and even transiently increased by hypoxia. *B*, turnover rates of dopamine in 2-day-old rats born in hypoxia and returned to room air after various time periods. Asterisks denote significantly different turnover rate relative value at 0–6 h after termination of hypoxia.

was 2·44±0·52 6–12 h and 2·77±1·70 12–24 h after termination of hypoxia ($P < 0·01$). A further reduced turnover rate, 1·10±0·54, was observed in 2-day-old rats returned to room air soon after birth. Moreover, there was a higher turnover in 2-day-old rats 0–6 h after changing the atmosphere to room air than in 2-day-olds still in hypoxia ($P < 0·05$).

DISCUSSION

The method for measurements of relative changes in ventilation permits the repeated study of unanaesthetized rat pups. Assessing the chemoreflex by 'physiological chemodenervation' by short-term hyperoxia is well established, although in the present study the classical 'one-breath test' could not be used due to the high breathing rate of the newborn rat. Any tonic influence from the hypoxic drive is expected to decline at a sudden increase in P_{a,O_2}, thus causing a transient fall in ventilation (see Dejours, 1962). The respiratory neurons in the brainstem receive a multitude of afferents besides the chemoreceptors and ventilatory output is the net effect of all converging inputs. Therefore the changes in ventilatory parameters are only a rough index of chemoreceptor activity. The breathing pattern of the newborn rat is highly irregular with changes in tidal volume and frequency, abundant brief apnoeas and body movements (Mortola, 1984; Hertzberg *et al.* 1990). Calibration was not successful since the seal around the pups' neck was not absolutely tight and the size of leaks may have changed as the rats moved. Moreover, the integrator was modified to reset after a sudden offset of the baseline due to frequent movement artifacts, making calibration by injecting air into the chamber difficult. Thus, we

chose to rely on relative changes in ventilatory parameters rather than absolute ones. This approach is valid only if the properties of the recording apparatus are unchanged during each trial. To ensure this, any tests with gross body movements were discarded. With these considerations in mind the percentage decrease in ventilation observed during the hyperoxic challenge was taken as an estimate of chemoreceptor drive during normoxia. Advantages and limitations of the method are discussed in more detail elsewhere (Hertzberg *et al.* 1990).

We found that 2 days' exposure to mild hypoxia of the pregnant rats before delivery and 2 days postnatally was sufficient to hamper the tonic influence of the hypoxic drive in the newborn rats under normoxia. A return to room air permitted the chemoreflex to attain a strength indistinguishable from that of rats born into a normoxic environment. This increase in chemosensitivity was paralleled by a drop in the turnover rate of carotid body dopamine.

Chronic hypoxaemia from birth seems to interfere with the normal resetting of chemoreceptor sensitivity. Using acute hypoxic challenges on newborn rats Eden & Hanson (1987*b*) showed that chronic hypoxia from birth (F_{I,O_2}, 0·15) delayed the appearance of 'adult' response to further hypoxia and the 'newborn' pattern with a decrease in ventilation or a biphasic response persisted. Data from the same laboratory suggest that hypoxia also delays or abolishes maturation of chemoreceptor reflexes in the newborn kitten (Hanson *et al.* 1989). Moreover, there are observations supporting the idea that chronic postnatal hypoxia also affects ventilatory control in lambs (Grögaard, Selstam, Hascoet & Sundell, 1988) and humans (Sørensen & Severinghaus, 1968*a*; Lahiri *et al.* 1978).

Ambient oxygen affects the metabolic rate in the newborn. During the first hours of hypoxia oxygen consumption decreases in the newborn rat. After 3 days, however, there was no difference compared with normoxic controls (Piazza, Lauzon & Mortola, 1988). Moreover, 5 min of hyperoxia increased oxygen consumption in neonatal mice (Mortola & Tenney, 1986). It is therefore possible that the rat pups in the present study had a lower metabolic rate than rats born in room air. However, it is less likely that the brief exposures to pure oxygen that the animals encountered in the present study were sufficient to change oxygen consumption. Therefore, it seems reasonable to accept the percentage changes in ventilation elicited by brief hyperoxia as an indirect measure of ventilatory drive from the peripheral chemoreceptors.

Newborn animals adapt to prolonged hypoxia not only by transiently decreasing oxygen consumption but also by enhanced oxygen delivery by increasing ventilation, pulmonary surface area and haematocrit (Mortola, Morgan & Virgona, 1986; Piazza *et al.* 1988). This pattern resembles the one seen in high altitude inhabitants (Sørensen & Severinghaus, 1968*a*; Santolaya, Lahiri, Alfaro & Schoene, 1989; see also Dempsey & Forster, 1982; Weil, 1986). The strategy seems to keep energy expenditure as low as possible. In this perspective, an increased ventilatory response to hypoxaemia is not as appropriate as in non-acclimated subjects. The mechanism for this is unknown, although an altered chemoreceptor function has been suspected (see e.g. Weil, 1986). The present findings may provide the link between prolonged hypoxia and a decreased chemoreceptor sensitivity. The actual output from the carotid body chemoreceptor is difficult to estimate correctly by any indirect method. The best way would be to record directly from the sinus nerve. But to our knowledge

this has not been achieved in animals of this size. Thus, it cannot be ruled out that ventilation in the hypoxic rat is stimulated by other mechanisms, making the relative contribution from the peripheral chemoreceptors less important. Another point to consider is that hypoxia-induced changes in arterial carbon dioxide (P_{a,CO_2}) might also affect chemoreceptor sensitivity to hypoxaemia. Whether P_{a,CO_2} was different in the hypoxic compared with the normoxic rats in the present study is not known since blood gases were not measured. However, an increase in P_{a,CO_2} was found in 14- to 21-day-old rats reared in an hypoxic environment with F_{I,O_2} of 0·13–0·15 (Eden & Hanson, 1987*b*). If the same is also true of 2-day-old hypoxic rats this would increase hypoxic sensitivity and give an enhanced chemoreflex rather than the weak one observed.

The issue whether there is a critical period within which the chemoreceptor sensitivity is irreversibly determined is controversial. Sørensen & Severinghaus (1968*b*) described an irreversible blunting of chemosensitivity persisting after surgical correction of congenital cyanotic malformations of the heart while others have reported a recovery in this respect after closing the shunt (Edelman, Lahiri, Braudo, Cherniack & Fishman, 1970; Blesa, Lahiri, Rashkind & Fishman, 1977). In the present study, rats returned to room air after 2 days of hypoxia acquired a normal chemoreceptor reflex after 2 additional days, suggesting that the chemoreceptor influence on breathing can be modified by ambient oxygen levels. This indicates that the critical period within which the chemoreceptor sensitivity is set, if it exists, extends at least over the first few postnatal days in the rat. Eden & Hanson (1987*b*) reported a delayed development of hypoxic response in chronically hypoxic newborn rats suggesting that the sensitivity developed in spite of hypoxia, although at a slower rate. On the other hand, increased oxygenation in fetal lambs enhanced carotid body chemosensitivity, indicating that P_{a,O_2} has a fundamental influence (Blanco *et al.* 1988).

Dopamine is the most abundant catecholamine in the carotid body in most species studied (see Fidone & Gonzalez, 1986). Its role in chemoception is far from clear but studies point towards an inhibitory action on chemoreceptor activity in the cat (Llados & Zapata, 1978; Docherty & McQueen, 1978), goat (Kressin, Nielsen, Laravuso & Bisgard, 1986), lamb (Mayock *et al.* 1983), rat (Cardenas & Zapata, 1981) and human (Olson, Hensley & Saunders, 1982). An increase in dopamine release during hypoxia has been demonstrated *in vitro* (Fidone, Gonzalez & Yoshizaki, 1982) and hypoxia decreases carotid body levels *in vivo* (Hanbauer & Hellström, 1978) while chronic hypoxia resulted in an increase in content (Hanbauer *et al.* 1981) and turnover rate (Pequignot *et al.* 1987). Moreover, termination of prolonged hypoxia causes an acute increase in dopamine content of the carotid body in adult rats (Olson, Vidruk, McCrimmon & Dempsey, 1983), suggesting a drop in turnover rate. The approach of returning hypoxic rats to normal atmosphere was aimed at simulating the rise in P_{a,O_2} which normally occurs soon after birth and continues until 2 days of age and thus, to some extent, separating the effects of age from the effect of ambient oxygen concentrations.

In contrast to observed changes in adult rats (Hanbauer *et al.* 1981), prolonged hypoxia failed to increase carotid body dopamine significantly in newborn rats, a circumstance probably explained by the much shorter exposure to hypoxia.

Compared to rats born and reared in normoxia, the hypoxic pups demonstrated a sustained turnover rate of carotid body dopamine. Moreover, as hypoxia was terminated, there was a drop in dopamine utilization, even though the time course was slightly longer than observed in rats born in normoxia. As in a previous study, we found that the increase in the strength of the chemoreflex was preceded by a drop in the turnover rate of carotid body dopamine (Hertzberg *et al.* 1990). The turnover was sustained and even rose transiently in hypoxic rats over the first 2 postnatal days as opposed to the prompt decrease in turnover in pups born in normoxia. The posthypoxic rats had an even higher turnover rate 0–6 h after termination of hypoxia. The reason for this, as for the transient increase in turnover rate on the day after birth in the hypoxic group, is not known.

It is conceivable that local release of dopamine in the carotid body is part of a regulatory mechanism that tunes the chemoafferent input. This notion is supported by the observation that changes in the strength of arterial chemoreflexes are paralleled or preceded by changes in dopamine turnover rate. In addition, experimental support for the release of carotid body dopamine by centrifugal stimulation of the sinus nerve *in vivo* and *in vitro* have been presented (Lahiri, Smatresk, Pokorski, Barnard, Mokashi & McGregor, 1984; Almaraz & Fidone, 1986).

Postnatal changes in the afferent or central neuronal circuits may also affect chemosensitivity. Katz and co-workers have reported increased levels of tyrosine hydroxylase in the petrosal ganglion of the rat from the day before birth to the first postnatal day (see Katz, 1990). This connection is at present speculative since no studies addressing the issue on whether this can be modulated by postnatal hypoxia have been presented.

In conclusion, a weaker chemoreflex was demonstrated in rat pups born in an hypoxic environment. The strength of the chemoreflex was increased after return to normoxia. Furthermore, the postnatal drop in dopamine turnover rate in the carotid body was abolished by hypoxia while rats returned to normal atmosphere exhibited a decline in dopamine utilization. These findings support the concept of an inhibitory dopaminergic mechanism keeping sensitivity low in the immediately newborn. A lifting of this mechanism may be governed by the postnatal rise in P_{a,O_2}.

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