Developmental changes in hypoxia-induced catecholamine release from rat carotid body, *in vitro*

D. F. Donnelly and T. P. Doyle

Department of Pediatrics, Section of Respiratory Medicine and Section of Cardiology, Yale University School of Medicine, New Haven, CT 06510, USA

1. Developmental changes in free tissue catecholamine levels were studied using Nafion-coated, carbon fibre electrodes placed in rat carotid bodies, *in vitro*. Simultaneously, single fibre chemoreceptor afferent activity was recorded from the sinus nerve. Five age groups were examined: 1, 2, 6, 10 and 20–30 days of age.

2. Using fast-scan voltammetry, similar current peaks were observed during exposure to exogenous dopamine and during superfusion with hypoxic saline. This suggests that changes in carbon fibre electrode current are due to an increase in free tissue catecholamines.

3. Baseline catecholamine levels were significantly less in the 1–6 day age groups compared to 10 day and 20–30 day rats.

4. During 1 min of hypoxia the peak concentration of tissue catecholamine was significantly less in the 1 day compared to the 2 day age groups, and these were less than in 10 day and 20–30 day rats.

5. Peak nerve response during hypoxia increased with age from $4{\cdot}5 \pm 0{\cdot}6$ Hz in the 1 day to $10{\cdot}5 \pm 1{\cdot}6$ Hz in the 6 day and to $15{\cdot}5 \pm 2{\cdot}2$ Hz in the 20–30 day rats.

6. We conclude that (1) resting free tissue catecholamine levels increase with age in the newborn period, (2) hypoxia causes enhanced catecholamine release, and (3) the magnitude of the release increases in the postnatal period as does the nerve activity.

Carotid body chemoreceptors transduce a decrease in arterial oxygen pressure into an increase in sinus nerve activity which leads, in turn, to an increase in ventilation. The mechanism for transduction is not well understood but recent attention has focused on the role of glomus cells whose presence near the afferent nerve terminal is essential for normal hypoxia sensitivity (Verna, Roumy & Leitner, 1975; Ponte & Sadler, 1989). These cells may function as oxygen sensors in that biophysical changes occur at relatively high levels of oxygen pressure (P_{O_2}), including alterations in K^+ membrane currents and intracellular calcium (Lopez-Barneo, Lopez-Lopez, Urena & Gonzalez, 1988; Urena, Lopez-Lopez, Gonzalez & Lopez-Barneo, 1989; Biscoe & Duchen, 1990; Donnelly & Kholwadwala, 1992).

The best-established putative neurotransmitter in rat glomus cells is dopamine which is secreted in response to hypoxia (Hanbauer & Hellstrom, 1978; Gonzalez, Almarez, Obeso & Rigual, 1992). Hypoxia-induced dopamine secretion was initially studied on a relatively slow time scale (minutes) using radioactive labelling of a recently synthesized catecholamine pool (Gonzalez & Fidone, 1977; Fidone, Gonzalez & Yoshizaki, 1982). Recent results from our laboratory extended these observations to a faster time scale (seconds) and to dopamine stores which are not recently synthesized (Donnelly, 1993). Using carbon fibre electrodes, we demonstrated that nerve activity and free tissue catecholamine levels change in parallel during interventions which cause variations in chemoreceptor activity, such as graded hypoxia, prolonged hypoxia and multiple exposures to hypoxia (Donnelly, 1993). Although not demonstrating causality, these parallel changes in nerve activity and catecholamine release are consistent with the postulate that catecholamine release is important for chemotransduction (Gonzalez *et al.* 1992). This is further supported by experiments on carotid bodies following catecholamine depletion with reserpine in which the hypoxia response is largely ablated (Leitner, Roumy & Verna, 1983).

The importance of catecholamine release may extend to the newborn period, at a time during which there is a developmental increase in hypoxia sensitivity. Dopamine turnover rate changes greatly in this period as does the nerve response to hypoxia (Hertzberg, Hellstrom, Lagercrantz & Pequignot, 1990; Kholwadwala & Donnelly, 1992). However, conflicting opinions exist as to the role of dopamine. As noted above, it may be argued that enhanced catecholamine secretion leads to increases in nerve activity; however, it may also be argued that dopamine acts as an inhibitory neuromodulator, and that high basal

catecholamine release inhibits chemoreceptor activity and leads to low hypoxia sensitivity in the newborn period (Hertzberg *et al.* 1990). The purpose of the present study was to begin to address the role of carotid body catecholamine during development by examining developmental changes in basal and stimulated levels of free catecholamines and afferent nerve activity.

METHODS

Carotid body isolation and nerve recording

The method of isolation and nerve recording for rat carotid bodies *in vitro* is completely described elsewhere (Kholwadwala & Donnelly, 1992). Rats of five ages (1 day old, $n = 6$; 2 days, $n = 6$; 6 days, $n = 5$; 10 days, $n = 7$, and 20–30 days, $n = 11$) were anaesthetized by placement in a closed chamber in which the atmosphere was saturated with methoxyflurane vapour. Following deep anaesthesia, indicated by repetitive slow breathing and an absence of motor movements, the rats were removed from the chamber and decapitated. Carotid bodies were harvested by removing the regions of the carotid bifurcations along with a portion of the sinus nerves and placing them in chilled, oxygenated (95 % O_2, 5 % CO_2), Ringer solution (mM: 125 NaCl, 3 KCl, 24 $NaHCO_3$, 1·25 NaH_2PO_4, 1·2 $CaCl_2$, 1·2 $MgSO_4$ and 10 glucose). The carotid body was dissected free and placed in a dilute enzymatic solution (0·02 % collagenase, 0·01 % protease, 20 min, Ringer solution, 35 °C) to aid in the preparation of the sinus nerve for recording and for removing the connective tissue around the carotid body. Following enzyme exposure, the carotid body was superfused (2·5 ml min^{-1}) with Hepes-buffered saline solution (mM: 140 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 25 glucose, 10 Hepes, pH 7·35, 34 °C) at 32–34 °C in a perfusion chamber mounted on the stage of an inverted microscope. Superfusate P_{O_2} was monitored by a platinum wire electrode placed next to the carotid body and polarized at −0·8 V. The electrode was covered with a butyl acetate membrane to present a controlled diffusion barrier to oxygen. The electrode was calibrated during superfusion with Hepes–saline equilibrated with room air ($P_{O_2} \approx 150$ mmHg) and Hepes–saline equilibrated with N_2 with the addition of glucose oxidase (10 μg ml^{-1}) which served as an oxygen scavenger ($P_{O_2} \approx 0$ mmHg).

Single-unit recordings of sinus nerve action potentials were obtained using a suction electrode applied to the cut peripheral end of the sinus nerve. The number of recorded fibres could be selected by the electrode tip size and usually single-unit activity could be well discriminated for each recording (BAK DIS-1 window discriminator, BAK Electronics, Rockville, MD, USA). The derived unit pulse triggered a storage oscilloscope sweep (Gould 2000) which includes a pre-trigger of the displayed signal. In this way, the entire action potential could be viewed for assessment of identity of height and width.

Catecholamine electrode

The catecholamine electrode was fabricated using a variation of the technique of Forni and colleagues (Forni & Nieoullon, 1984; Ganouni, Forni & Nieoullon, 1987). A single 10 μm carbon fibre (P55, Amoco Performance Products, Greenville, SC, USA) was attached to a silver wire using conductive silver paint (GC Electronics, Rockford, IL, USA). The wire and carbon fibre were placed in a glass capillary tube which was pulled to a fine tip (Sutter PC84, Sutter Instruments, Novato, CA, USA). The point of the capillary tip was broken back, allowing the carbon fibre to project about 100 μm beyond the tip. The glass was annealed to the carbon fibre by briefly exposing the tip to a small flame and was sealed by applying a layer of epoxy resin (Devcon Corp., Danvers, MA, USA). After drying, the projecting carbon fibre was cut so that the tip projected about 10 μm beyond the epoxy resin. Nafion (Aldrich Chemical Co., Milwaukee, WI, USA), dissolved in alcohol, was applied to the tip using a dip-coating procedure (Baur, Kristensen, May, Wiedeman & Wightman, 1988), and the electrode was allowed to dry. Nafion is a perfluorinated ion-exchange resin which discriminates against negatively charged compounds (e.g. the dopamine metabolite, dihydroxyphenylacetic acid) and enhances, under some conditions, the detection of positively charged compounds (e.g. dopamine) (Kristensen, Kuhr & Wightman, 1987). In most experiments the electrode current was measured in the amperometric mode with the electrode polarized to 150–160 mV (Axopatch 1D amplifier, Axon Instruments Inc., Foster City, CA, USA; CV-3 headstage, 50 GΩ feedback resistance). This is slightly above the oxidation potential for dopamine as determined using differential pulse voltammetry in our previous study (Donnelly, 1993). In some experiments, electrode current was measured during fast-scan voltammetry (300 V s^{-1}) in which the electrode voltage was scanned every 0·1 s from −500 to 1000 mV at 300 V s^{-1}. For these studies, the electrode current was measured by the headstage (Axoclamp 1D, CV-3 headstage, 500 MΩ feedback resistance), and the scanning potential was applied through the chamber ground electrode because the Axoclamp was unable to apply potentials beyond ± 200 mV (modified I–V converter, World Precision Instruments, Sarasota, FL, USA). Fifty consecutive scans were averaged and the capacitative current, obtained from the first scan and prior to stimulation, was subtracted. The scanning pulses produced artifacts on the nerve recordings; thus, nerve recordings were not obtained during scanning voltammetry.

Electrode response speeds for oxygen and catecholamine electrodes were measured for some of the electrodes by switching the placement of the pipette tips between two perfusion streams. In the case of catecholamine electrodes the perfusion streams contained Hepes–saline and Hepes–saline containing 5 μM dopamine. In the case of oxygen electrodes, the perfusion streams contained Hepes–saline equilibrated with room air and Hepes–saline bubbled with N_2 with the addition of an oxygen scavenger (10 μg ml^{-1} glucose oxidase). The electrode current was digitized (pCLAMP, Axon Instruments) and fitted to a single exponential (CLAMPFIT, Axon Instruments).

Experimental protocol

Immediately before each experimental run, the electrode was advanced into the tissue. Within 3 min the electrode current generally stabilized at a steady-state value and the carotid body was then stimulated by switching the perfusate to low P_{O_2} solution (Hepes–saline equilibrated with N_2 with the addition of 10 μg ml^{-1} glucose oxidase and generally with 20 μg ml^{-1} catalase) for 1 min. After recovery from the hypoxia stimulus, the electrode was withdrawn from the tissue but remained in the superfusate. The baseline electrode current in Hepes–saline and electrode current in Hepes–saline containing 2 μM dopamine were recorded for each trial and were used to translate the current values measured in the tissue into estimates of tissue catecholamine concentration.

Statistical analysis

The number of action potentials, bath P_{O_2} and tissue catecholamine concentration were measured or calculated each second and recorded. Plots of nerve activity and catecholamine concentration were analysed for baseline and peak values during and following the period of hypoxia. Data were analysed using ANOVA with age as a grouping variable. *Post hoc* testing was performed using Tukey's honest significant difference (HSD) test. In some cases, if the mean values were not different between age groups (e.g. 1 day *vs.* 2 day) then the age groups were combined prior to further testing.

RESULTS

Electrode response speed

The time constants for the electrode responses to changes in oxygen or catecholamine levels were less than 1 s. The time constant of the oxygen electrode during a change in P_{O_2} from 0 to 150 mmHg was 328 ± 44 ms ($n = 6$) for an increase in oxygen pressure and 396 ± 43 ms for a decrease in oxygen pressure. The catecholamine electrode time constant was 108 ± 45 ms for an increase in catecholamine concentration from 0 to 5 μM ($n = 5$) and was 330 ± 130 ms

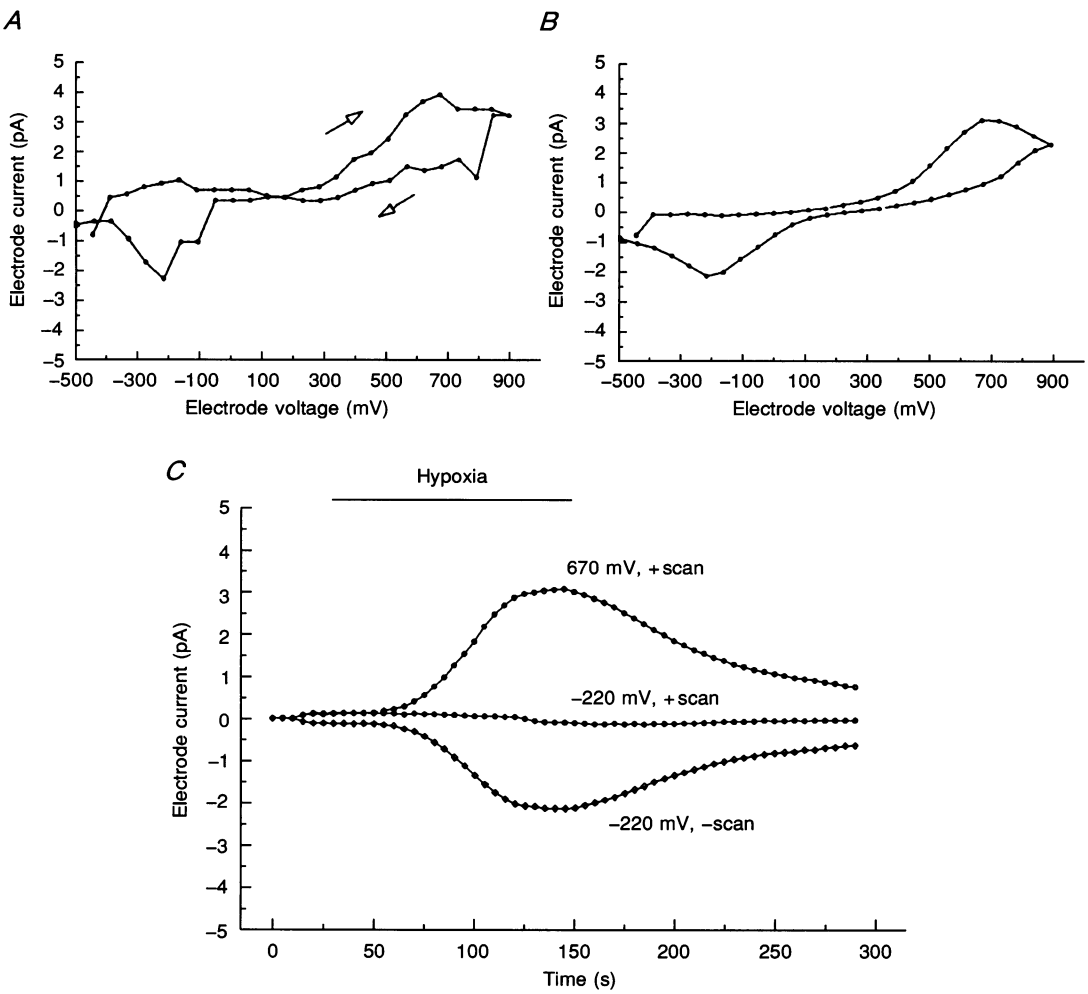

Figure 1. Fast-scan voltammetry of a carbon fibre electrode during exposure to exogenous dopamine or following placement in a carotid body

A, electrode current *vs.* voltage during exposure to exogenous dopamine (10 μM). The electrode voltage was scanned with a triangle waveform (range: −500 to 1000 mV) at high scan rate (300 V s^{-1}). Average of 20 sweeps and following background subtraction of the capacitive current. Note peak near 700 mV for the positive-going sweep and near −300 mV for the negative-going sweep. *B*, electrode current *vs.* voltage sweep for same electrode placed in a 24-day-old carotid body and during exposure to hypoxia. Note similar peaks at 700 and −300 mV. *C*, electrode current *vs.* time for the response pictured in *B*. The development of the current peaks near 700 and −300 mV occurred with the same time profile, suggesting that it was due to the same chemical species.

for a decrease in catecholamine. The longer time constant for a decrease in catecholamine may be due to a storage of catecholamine in the Nafion membrane (Baur *et al.* 1988).

Measurements using scanning voltammetry

The current–voltage response of the electrode during exposure to exogenous dopamine and following placement in the carotid body was examined in four experiments employing fast-scan voltammetry (Fig. 1). Exposure to exogenous dopamine caused an increase in electrode current near 700 mV on the positive scan and near −250 mV on the negative scan. The increase in electrode current at these potentials is probably caused by the oxidation and reduction of the 3,4-dihydroxy position of the catechol ring

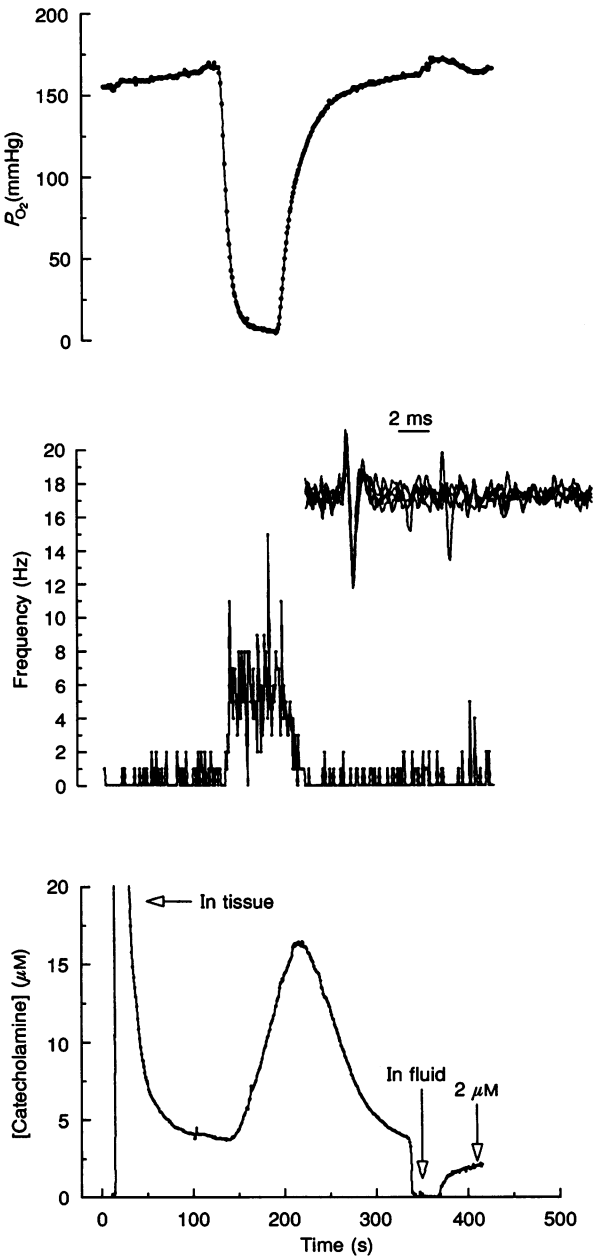

Figure 2. Nerve activity and tissue catecholamine levels in a carotid body from an adult (22 day) rat

Top, local P_{O_2} before, during and following chamber perfusion with hypoxic Hepes–saline. Middle, single-fibre unit activity before, during and following hypoxic exposure. Inset: superimposed traces of nerve recording activity triggered by the window discriminator. Scale bar is 2 ms. Bottom, carbon fibre electrode current voltage clamped at +160 mV. After reoxygenation, the electrode was removed from the tissue, but remained in the bath for calibration in Hepes–saline containing 0 and 2 μM dopamine.

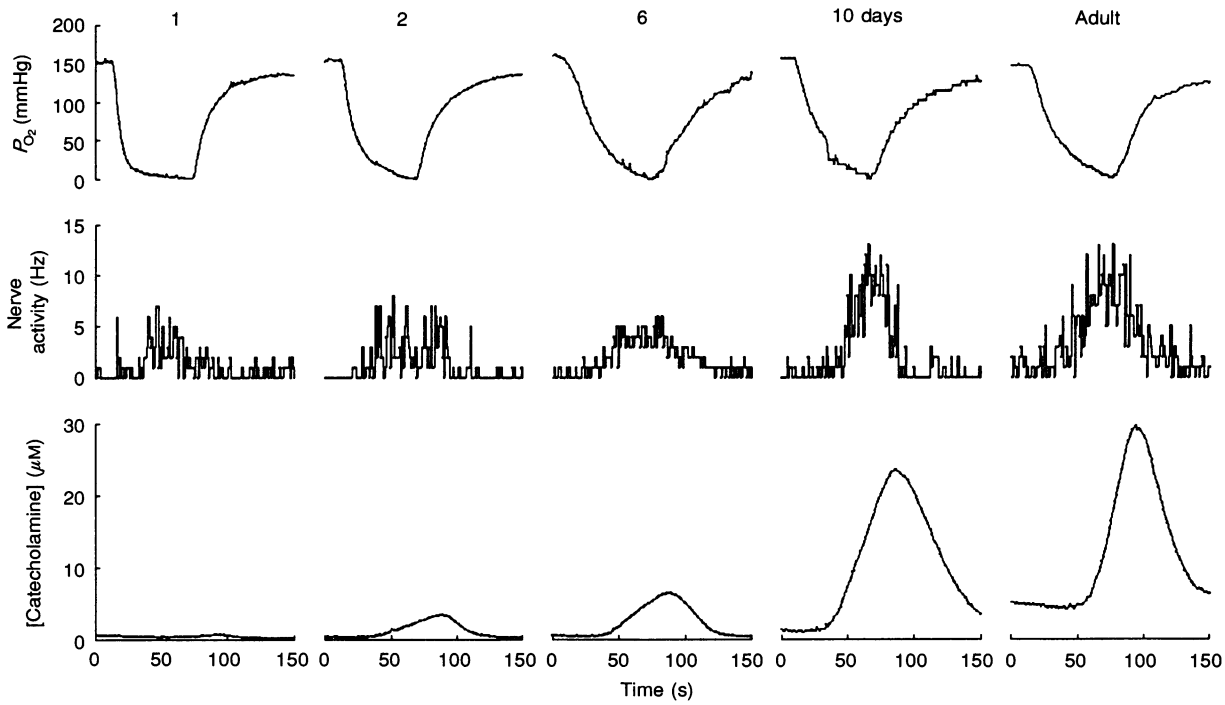

Figure 3. Excerpts of experimental records obtained as in Fig. 2 for carotid bodies of different ages
Top, P_{O_2}. Middle, single-fibre nerve activity. Bottom, carbon fibre electrode current. Note that control tissue catecholamine levels and the magnitude of the catecholamine response increased with age.

(Wightman *et al.* 1991). After placement in a carotid body and during exposure to hypoxia, an increase in electrode current at similar potentials was obtained (Fig. 1). These results are consistent with a chemical being released in the carotid body during hypoxia which can be oxidized and reduced at potentials similar to the oxidation and reduction potentials for catecholamine.

All subsequent carbon fibre electrode recordings were performed in the amperometric mode due to the inability to record action potential activity in the presence of fast scanning pulses. The oxidation potential (+160 mV) was chosen as the minimum potential above that which gives the greatest current increase during slow voltage scans in the presence of dopamine (Forni & Nieoullon, 1984;

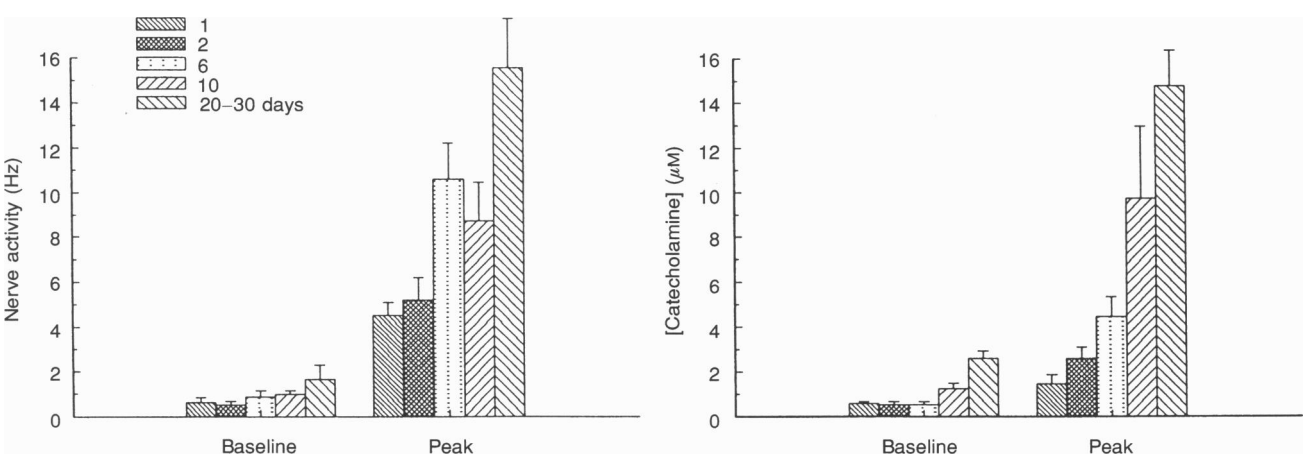

Figure 4. Baseline and peak values for tissue catecholamines and nerve activity
Mean values ± S.E.M. for carotid bodies harvested from rats aged 1 ($n = 6$), 2 ($n = 6$), 6 ($n = 5$), 10 ($n = 7$) and 20–30 days ($n = 11$).

Donnelly, 1993). However, this potential is different from that obtained in fast-scan voltammetry in which catecholamines appear as an absorbed species (Kawagoe, Jankowski & Wightman, 1991).

Response of older animals

Advancement of the carbon fibre electrode into the tissue caused a large increase in electrode current, presumably due to tissue damage from the electrode (Fig. 2). Electrode current generally fell to steady-state levels within 2–3 min and this was used to estimate the resting tissue catecholamine level which, for the 20–30 day rats, was $2{\cdot}59 \pm 0{\cdot}35\ \mu$M ($n = 11$, mean ± S.E.M.). Hypoxia caused a pronounced increase in nerve activity, and, after a short delay, an increase in catecholamine electrode current (Fig. 2). The estimated peak value of tissue catecholamines for the adult was $14{\cdot}8 \pm 1{\cdot}6\ \mu$M; however, there was considerable variability in the magnitude of increase and ranged from 10 to 25 μM. During reoxygenation, both the nerve and catecholamine levels returned to control values.

In animals of 10 days of age, the magnitude of change was similar to that noted for the adult (Figs 3 and 4). Baseline tissue catecholamine levels were $1{\cdot}26 \pm 0{\cdot}24\ \mu$M ($n = 7$) and increased to $9{\cdot}74 \pm 3{\cdot}22\ \mu$M during hypoxia

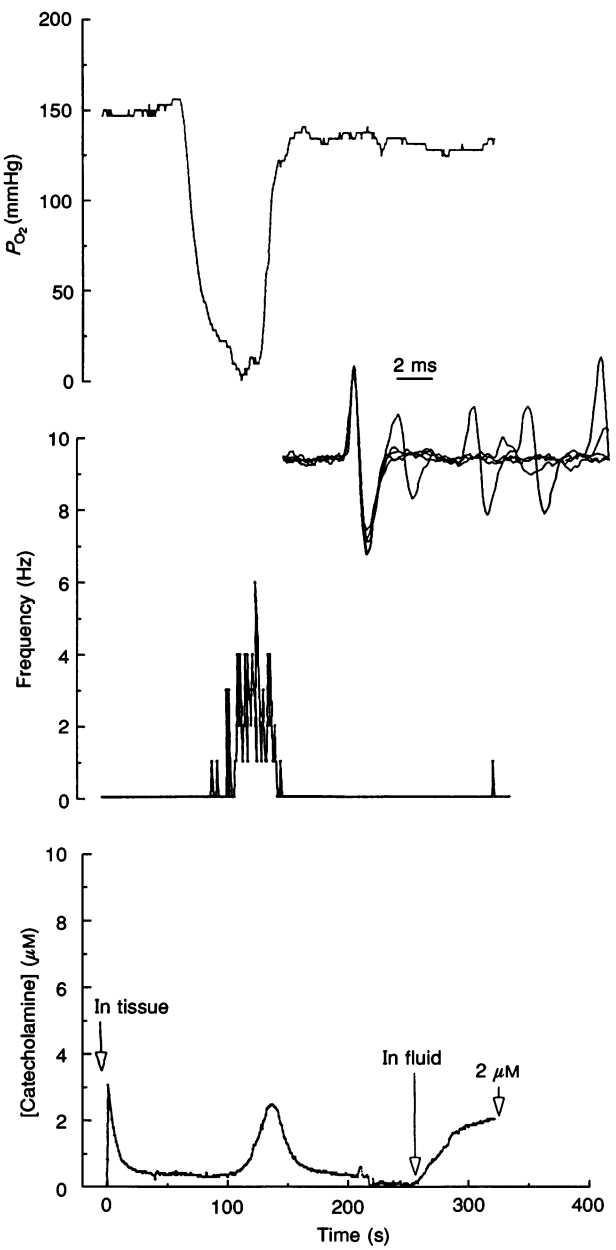

Figure 5. Nerve activity and tissue catecholamine level in a carotid body from a 2-day-old rat superfused with Ringer saline in place of Hepes–saline

Traces as in Fig. 2. The baseline catecholamine level and magnitude of increase in catecholamine was similar to that observed in Hepes–saline for 2-day-old carotid bodies (Fig. 3).

(Figs 3 and 4). Nerve activity increased from an average of $0{\cdot}9 \pm 0{\cdot}2$ to $8{\cdot}7 \pm 1{\cdot}7$ Hz. Nerve activity and catecholamine levels (baseline and peak) were not significantly different between the 10 day and 20–30 day animals.

Response of younger animals

Baseline catecholamine levels and the magnitude of increase during hypoxia were less in the younger age groups compared to older age groups ($F = 4{\cdot}1$, $P < 0{\cdot}01$; Figs 3 and 4). Resting tissue levels were $0{\cdot}58 \pm 0{\cdot}09$ ($n = 6$), $0{\cdot}53 \pm 0{\cdot}14$ ($n = 6$) and $0{\cdot}54 \pm 0{\cdot}12$ μM ($n = 5$) for carotid bodies harvested from 1, 2 and 6 day rats, respectively (Fig. 4). The values did not differ significantly among these younger aged rats, but values for each younger age group were significantly lower than that for 20–30 day animals ($P < 0{\cdot}05$, Tukey's HSD). The hypoxia-induced increase in catecholamine level peaked at $1{\cdot}47 \pm 0{\cdot}42$, $2{\cdot}60 \pm 0{\cdot}51$ and $4{\cdot}46 \pm 0{\cdot}88$ μM for 1, 2 and 6 day rats, respectively (Figs 3 and 4). This increase was significantly different across age groups ($F = 3{\cdot}6$, $P < 0{\cdot}02$); peak catecholamine levels of 20–30 day rats were significantly different than the 1 day, 2 day and 6 day age groups ($P < 0{\cdot}01$ for each, Tukey's HSD). Peak catecholamine levels were also significantly higher in the 10 day group compared to the 1 day group ($P < 0{\cdot}05$, Tukey's HSD). When the age groups were combined into one younger ($1 + 2 + 6$ day) and two older groups (10 day and 20–30 day), the magnitudes of increase in catecholamine level during hypoxia were significantly greater in both older age groups compared to the young age group ($P < 0{\cdot}01$, Tukey's HSD).

The magnitude of the nerve response followed a similar maturational course to the catecholamine response. Baseline nerve activity was not different between the three younger age groups ($0{\cdot}6 \pm 0{\cdot}2$, $0{\cdot}5 \pm 0{\cdot}2$ and $0{\cdot}8 \pm 0{\cdot}3$ Hz) but the peak activity during hypoxia was significantly greater for the 6 day ($10{\cdot}5 \pm 1{\cdot}6$ Hz) compared to the 1 day ($4{\cdot}5 \pm 0{\cdot}6$ Hz) and 2 day rats ($5{\cdot}2 \pm 0{\cdot}9$ Hz) (Figs 3 and 4). The peak activity of the 6 day rat did not differ significantly from those of 10 or 20–30 days of age.

In order to determine whether the base buffering of the superfusion medium exerted a major effect on the observed response, two experiments on 2-day-old rat carotid bodies were undertaken in Ringer saline. Under these conditions, albeit with a small sample, the magnitudes of increase in nerve activity and catecholamine level were similar to those observed in Hepes buffer (Fig. 5). Baseline values of catecholamine concentration were $0{\cdot}5$ and $0{\cdot}6$ μM and increased to $2{\cdot}2$ and $2{\cdot}5$ μM during hypoxia. This magnitude of increase was approximately that observed in the population of 2-day-old carotid bodies perfused in Hepes–saline.

DISCUSSION

These results demonstrate that developmental changes occur in the regulation of carotid body free tissue catecholamine concentration. In the very young (*ca* 1 day), baseline and hypoxia-stimulated levels for free catecholamines and nerve activity are low. By 2–6 days after birth, hypoxia-induced catecholamine secretion is much larger than in the newborn, and is about half that of the adult. By 10–20 days of age, baseline and hypoxia-stimulated catecholamine levels are considerably higher than those of the young.

Previous results from our laboratory and others have argued for a critical role for catecholamine secretion in initiating the chemoreceptor response to hypoxia. The release of radioactive dopamine following superfusion with radioactive tyrosine is established to be roughly proportional to the magnitude of the nerve activity (Gonzalez & Fidone, 1977; Fidone *et al.* 1982). Recent work in our laboratory examined, on a faster time scale, catecholamine secretion using carbon fibre electrodes similar to those used in the present study (Donnelly, 1993). Chemoreceptor afferent activity and catecholamine secretion appeared to be linked because factors which caused nerve activity to decrease (calcium-free solutions, prolonged hypoxia, multiple hypoxia exposures) also caused secretion to decrease. Although these correlated changes do not demonstrate causality, the multiple interventions causing parallel changes in nerve activity and catecholamine release indicate that the processes are closely related.

In our previous work, the time profiles for the change in nerve activity and catecholamine levels were similar to those of the present work, but the absolute magnitudes of the catecholamine values were approximately 10 % of those in the present study. We believe that this difference is directly related to the electrode size. While in the previous study electrodes were constructed from bundles of carbon fibres which projected considerably beyond the insulating glass pipette, the electrodes used in the present study were fabricated from a single carbon fibre which was cut close to the insulating pipette. Because of the difference in surface area, the magnitude of reducing current in a standard dopamine buffer was different by a factor of 100–1000 between the two types of electrodes. When placed in the tissue, it may be expected that the larger electrode would cause a larger sink for catecholamines and thereby a greater diffusion gradient within the tissue, and hence a greater underestimation of free tissue catecholamine levels. Even with the use of small electrodes, a concentration gradient exists within the tissue and this gradient is not present during calibration in a perfusion stream. Thus, the tissue values reported here are also underestimates of actual values. However, because similar electrodes were used at all developmental ages, the underestimation should be constant across ages and not affect the developmental profile of the results.

The present work extends our previous observations on the relationship between nerve activity and catecholamine release by examining this relationship over a developmental period during which the organ response to hypoxia matures (Eden & Hanson, 1987; Kholwadwala & Donnelly, 1992). The present data are generally consistent with a link

between enhanced nerve activity and enhanced catecholamine release. In the immediate newborn period, baseline and stimulated levels of nerve activity and tissue catecholamine concentrations were low, and by 10 days of age were greatly increased. However, the developmental relationship between secretion and nerve activity is less clear at intermediate ages. Although the mean peak catecholamine level during hypoxia increased smoothly with age (Fig. 4) the peak nerve activity was unchanged between 6 and 10 days of age. This apparent dissociation between catecholamine release and nerve activity may represent a physiological change in nerve sensitivity to neurotransmitter release, but more probably represents variability in the single fibre response to hypoxia. These possibilities cannot be differentiated with the size of the present sample.

The present results have important implications for one major theory of control of carotid body hypoxia sensitivity; that is, high tonic rates of dopamine secretion in the newborn period inhibit the hypoxia sensitivity (Hertzberg *et al.* 1990). This conjecture is supported by observations that basal dopamine secretion is high in the first 6 h after birth and then decreases to a steady level between 6 h and 7 days after birth (Hertzberg *et al.* 1990). Furthermore, administration of exogenous dopamine often causes inhibition of on-going activity and administration of dopamine antagonists often increase the response to hypoxia, *in vivo* (Lahiri, Nishino, Mokashi & Mulligan, 1980; Donnelly, Smith & Dutton, 1981). Although our present results do not support the contention that free tissue levels of catecholamines are particularly high in the newborn period, we have no data to address directly the early developmental period (0–6 h after birth). Our rats were generally born at night, and none of our experiments was undertaken within 6 h of birth, i.e. beyond the high turnover period identified by Hertzberg *et al.* (1990). Nevertheless, developmental changes in hypoxia sensitivity occur well beyond a period of 6 h after birth (Eden & Hanson, 1987; Kholwadwala & Donnelly, 1992).

A limitation of the present technique is the inability to differentiate between noradrenaline and dopamine. Because both chemicals have a similar net charge at pH 7·4 and both have a catechol ring, they are both equally well detected by the carbon fibre electrode. Noradrenaline and dopamine are present in the rat carotid body (Hellstrom & Koslow, 1975; Hanbauer & Hellstrom, 1978) and basal secretion rates change with maturation (Hertzberg *et al.* 1990). However, most rat carotid body noradrenaline is localized to sympathetic nerve endings (Hellstrom & Koslow, 1975) and the turnover rate is independent of hypoxia (Hellstrom, Hanbauer & Costa, 1976; Hanbauer & Hellstrom, 1978). Thus, although basal noradrenaline release may contribute to the baseline catecholamine levels measured in the present study, the major contributor to the increased catecholamine levels during hypoxia is dopamine.

One experimental variable which may have altered the magnitude of the nerve and catecholamine responses was the use of Hepes-buffered saline in place of bicarbonate-buffered saline. It was previously reported that short-duration perfusion with CO_2-free Hepes-buffered saline reduces carotid body hypoxia sensitivity (Shirahata & Fitzgerald, 1991). However, in the present study and under steady-state conditions, this did not seem to be a major factor. Using a Hepes–saline superfusate, a 5–20 times increase in nerve activity was observed during hypoxia, and, using Ringer saline superfusate, basal and stimulated catecholamine levels in two 2-day-old carotid bodies were similar to those observed in Hepes–saline.

In summary, this work demonstrates that, in rat carotid bodies, basal and hypoxia-stimulated free catecholamine levels increase with age. These observations suggest that the low hypoxia sensitivity of rat carotid bodies in the newborn period is not due to high basal tissue levels of catecholamines causing tonic inhibition of chemoreceptor activity. The results are generally consistent with the hypothesis that catecholamine secretion and afferent nerve activity change in parallel, and may be causally linked. However, because dopamine antagonists administered *in vivo* fail to block the hypoxia response (Lahiri *et al.* 1980; Donnelly *et al.* 1981), other, as yet unidentified, factors must play a dominant role.

REFERENCES

Baur, J. E., Kristensen, E. W., May, L. J., Wiedemann, D. J. & Wightman, R. M. (1988). Fast-scan voltammetry of biogenic amines. *Analytical Chemistry* **60**, 1268–1272.

Biscoe, T. J. & Duchen, M. R. (1990). Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *Journal of Physiology* **428**, 39–59.

Donnelly, D. F. (1993). Electrochemical detection of catecholamine release from rat carotid body, in vitro. *Journal of Applied Physiology* **74**, 2330–2337.

Donnelly, D. F. & Kholwadwala, D. (1992). Hypoxia decreases intracellular calcium in adult rat carotid body glomus cells. *Journal of Neurophysiology* **67**, 1543–1551.

Donnelly, D. F., Smith, E. J. & Dutton, R. E. (1981). Neural response of carotid chemoreceptors following dopamine blockade. *Journal of Applied Physiology* **50**, 172–177.

Eden, G. J. & Hanson, M. A. (1987). Maturation of the respiratory response to acute hypoxia in the newborn rat. *Journal of Physiology* **392**, 1–9.

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

Forni, C. & Nieoullon, A. (1984). Electrochemical detection of dopamine release in the striatum of freely moving hamsters. *Brain Research* **297**, 11–20.

Ganouni, S. E., Forni, C. & Nieoullon, A. (1987). In vitro and in vivo characterization of the properties of a multifiber carbon electrode allowing long-term electrochemical detection of dopamine in freely moving animals. *Brain Research* **404**, 239–256.

Gonzalez, C., Almaraz, L., Obeso, A. & Rigual, R. (1992). Oxygen and acid chemoreception in the carotid body chemoreceptors. *Trends in Neurosciences* **15**, 146–153.

Gonzalez, C. & Fidone, S. (1977). Increased release of ^{3}H-dopamine during low O_2 stimulation of rabbit carotid body *in vitro*. *Neuroscience Letters* **6**, 95–99.

HANBAUER, I. & HELLSTROM, 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.

HELLSTROM, S., HANBAUER, I. & COSTA, E. (1976). Selective decrease of dopamine content in rat carotid body during exposure to hypoxic conditions. *Brain Research* **118**, 352–355.

HELLSTROM, S. & KOSLOW, S. H. (1975). Biogenic amines in carotid body of adult and infant rats – a gas chromatographic mass spectrometric assay. *Acta Physiologica Scandinavica* **93**, 540–547.

HERTZBERG, T., HELLSTROM, S., LAGERCRANTZ, H. & PEQUIGNOT, J. M. (1990). Development of the arterial chemoreflex and turnover of carotid body catecholamines in the newborn rat. *Journal of Physiology* **425**, 211–225.

KAWAGOE, K. T., JANKOWSKI, J. A. & WIGHTMAN, R. M. (1991). Etched carbon-fiber electrodes as amperometric detectors of catecholamine secretion from isolated biological cells. *Analytical Chemistry* **63**, 1589–1594.

KHOLWADWALA, D. & DONNELLY, D. F. (1992). Maturation of carotid chemoreceptor sensitivity to hypoxia: *in vitro* studies in the newborn rat. *Journal of Physiology* **453**, 461–473.

KRISTENSEN, E. W., KUHR, W. G. & WIGHTMAN, R. M. (1987). Temporal characterization of perfluorinated ion-exchange coated microvoltammetric electrodes for *in vivo* use. *Analytical Chemistry* **59**, 1752–1757.

LAHIRI, S., NISHINO, T., MOKASHI, A. & MULLIGAN, E. (1980). Interaction of dopamine and haloperidol with O_2 and CO_2 chemoreception in carotid body. *Journal of Applied Physiology* **49**, 45–51.

LEITNER, L. M., ROUMY, M. & VERNA, A. (1983). *In vitro* recording of chemoreceptor activity in catecholamine-depleted rabbit carotid bodies. *Neuroscience* **10**, 883–891.

LOPEZ-BARNEO, J., LOPEZ-LOPEZ, J. R., URENA, J. & GONZALEZ, C. (1988). Chemotransduction in the carotid body: K current modulated by P_{O_2} in type I chemoreceptor cells. *Science* **241**, 580–582.

PONTE, J. & SADLER, C. L. (1989). Interactions between hypoxia, acetylcholine and dopamine in the carotid body of rabbit and cat. *Journal of Physiology* **410**, 395–410.

SHIRAHATA, M. & FITZGERALD, R. S. (1991). The presence of CO_2/HCO_3^- is essential for hypoxic chemotransduction in the in vivo perfused carotid body. *Brain Research* **545**, 297–300.

URENA, J., LOPEZ-LOPEZ, J., GONZALEZ, C. & LOPEZ-BARNEO, J. (1989). Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology* **93**, 979–999.

VERNA, A., ROUMY, M. & LEITNER, L. M. (1975). Loss of chemoreceptive properties of the rabbit carotid body after destruction of the glomus cells. *Brain Research* **100**, 13–23.

WIGHTMAN, R. M., JANKOWSKI, J. A., KENNEDY, R. T., KAWAGOE, K. T., SCHROEDER, T. J., LESZCZYSZYN, D. J., NEAR, J. A., DILIBERTO, E. J. JR & VIVEROS, O. H. (1991). Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. *Proceedings of the National Academy of Sciences of the USA* **88**, 10754–10758.

Acknowledgements

This work is supported by NIH grant HL46149.

Received 9 February 1993; accepted 11 August 1993.