Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells

Roger J. Thompson, Adele Jackson and Colin A. Nurse*

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

1. We investigated whether adrenomedullary chromaffin cells (AMCs) derived from neonatal (postnatal day (P) 1–P2) and juvenile (P13–P20) rats, and maintained in short-term culture (1–3 days), express O_2-chemoreceptive properties.

2. In whole-cell recordings, the majority (~70%; $n = 47$) of neonatal AMCs were sensitive to hypoxia. Under voltage clamp, acute hypoxia (P_{O_2} ~40 mmHg) suppressed voltage-dependent K^+ current by 25·1 ± 3·4% (mean ± S.E.M.; $n = 22$); under current clamp, acute hypoxia caused a membrane depolarization of 14·1 ± 1·3 mV ($n = 13$) from a resting potential of −54·8 ± 2·8 mV ($n = 13$), and this was often sufficient to trigger action potentials.

3. Exposure of neonatal AMC cultures to a moderate (P_{O_2} ~75 mmHg) or severe (P_{O_2} ~35 mmHg) hypoxia for 1 h caused a dose-dependent stimulation (~3 or 6 times normoxia, respectively) of catecholamine (CA) release, mainly adrenaline, determined by HPLC. This induced CA release was abolished by the L-type calcium channel blocker, nifedipine (10 μM).

4. In contrast to the above results in neonates, hypoxia had no significant effects on voltage-dependent K^+ current, membrane potential, or CA release in juvenile AMCs.

5. We conclude that rat adrenal chromaffin cells possess a developmentally regulated O_2-sensing mechanism, similar to carotid body type I cells.

In the perinatal period, catecholamine (CA) release from adrenomedullary chromaffin cells (AMCs) is critical for the animal's ability to survive stresses associated with delivery and the transition to extrauterine life. This release plays a vital role in the modulation of cardiovascular, respiratory and metabolic responses to stressors such as hypoxia (Lagercrantz & Slotkin, 1986; Slotkin & Seidler, 1988). In some species such as rat and man, sympathetic innervation of the adrenal medulla is immature or absent in the neonate, yet the animal can still elicit the vital catecholamine surge in response to hypoxic challenge (Seidler & Slotkin, 1985). Seidler & Slotkin (1985) showed that in the newborn rat, acute hypoxia reduces adrenal catecholamines through a 'non-neurogenic' mechanism, which disappears postnatally with a rough correlation to the maturation of the sympathetic innervation of the adrenal medulla. Furthermore, they demonstrated that sympathetic denervation of the adrenal medulla in mature animals causes a gradual re-appearance of this non-neurogenic mechanism (Seidler & Slotkin, 1986).

The mechanisms underlying the non-neurogenic response of the adrenal medulla to hypoxia are unknown. One possibility is that it is mediated indirectly, via humoral factors released into the circulation during hypoxia. Alternatively, adrenomedullary chromaffin cells might themselves possess O_2-sensing mechanisms, similar to their neural crest counterparts in the carotid body, i.e. glomus or type 1 cells, which are the prototype for O_2 chemoreceptors in mammals (Gonzalez, Almaraz, Obeso & Rigual, 1994). These cells respond to hypoxia by suppression of an outward K^+ current (López-Barneo, López-López, Urena & Gonzalez, 1988; Delpiano & Hescheler, 1989; Peers, 1990; Stea & Nurse, 1991), membrane depolarization and/or increased action potential frequency (López-Barneo *et al.* 1988; Buckler & Vaughan-Jones, 1994), leading to entry of extracellular calcium and enhanced CA release (Buckler & Vaughan-Jones, 1994; Montoro, Urena, Fernandez-Chacon, Alvarez de Toledo & López-Barneo, 1996). In this study, using whole-cell recording techniques and HPLC determination of CA release, we tested the hypothesis that newborn rat AMCs share similar O_2-sensing properties to carotid body type 1 cells, and that these properties are lost with postnatal maturation.

* To whom correspondence should be addressed.

METHODS

Pregnant or lactating Wistar rats (Charles River, Quebec, Canada) and pups were housed in our animal facility under a constant 12 h light–12 h dark cycle, according to the guidelines of the Canadian Council on Animal Care (CCAC). All procedures for animal handling and tissue removal were carried out according to CCAC guidelines. Animals were rendered unconscious by a blow to the head (1- to 14-day-old pups) or by inhalation of the anaesthetic Somnothane (Wyeth-Ayerst Canada Inc., Montreal, Quebec, Canada; 15- to 20-day-old pups). The pups were then immediately killed by decapitation or cervical dislocation and the adrenal glands removed.

Cultures

Primary cultures enriched in dissociated rat AMCs were prepared by a modification of methods previously described (Doupe, Landis & Patterson, 1985). Briefly, adrenal glands were dissected from rat pups of two age groups, i.e. neonatal (postnatal day (P) 1–P2) or juvenile (P13–P20). Most of the surrounding cortical tissue was trimmed and discarded, whereas the remaining central medulla was incubated in an enzymatic solution, containing 0·1% trypsin, 0·1% collagenase (Gibco), and 0·01% deoxyribonuclease (Millipore) for 1 h at 37 °C. Following incubation, most of the enzyme was removed with a pipette and the remainder was inactivated with growth medium consisting of F-12 nutrient medium (Gibco) supplemented with 10% fetal calf serum (Gibco), 80 U l^{-1} insulin (Sigma), 0·6% glucose, 2 mM glutamine, 1% penicillin and streptomycin (Gibco), and 0·01% dexamethasone (Sigma). In most experiments, after mechanical dissociation of the tissue with forceps and trituration with a Pasteur pipette, the resulting cell suspension was preplated for 24 h on collagen to remove most of the cortical cells. The non-adherent chromaffin cells were then replated on the central wells of modified culture dishes (Nurse, 1990), coated with Matrigel (Collaborative Research, Bedford, MA, USA). The cells were grown at 37 °C in a humidified atmosphere of 95% air–5% CO_2 for 1–3 days before they were used in the patch-clamp experiments, or for determination of CA release.

Electrophysiology

Voltage-clamp data were obtained using either conventional whole-cell or nystatin perforated-patch techniques as previously described (see Stea & Nurse, 1991). Membrane potential measurements under current clamp were obtained with the latter method. The seal resistance was in the range 2–10 GΩ and most (~75%) of the series resistance (range, 10–25 MΩ) was compensated in voltage-clamp experiments. Junction potentials (2–10 mV) were nulled at the beginning of each experiment. Voltage- and current-clamp records were obtained using an extracellular fluid with the following composition (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 10; Hepes, 10; adjusted to pH 7·4 with NaOH. The pipette solution for conventional whole-cell recording contained (mM): KCl, 135; NaCl, 5; $CaCl_2$, 0·1; EGTA, 11; Hepes, 10; Mg-ATP, 2; at pH 7·2. For perforated-patch recording the pipette solution contained (mM): potasssium gluconate, 105; KCl, 30; NaCl 5; $CaCl_2$, 0·1; Hepes, 10; at pH 7·2, plus nystatin (300 μg ml^{-1}). All recordings were obtained at room temperature (20–23 °C) with an Axopatch-1D patch-clamp amplifier equipped with a 1 GΩ head stage feedback resistor, digitized with a Digidata 1200 computer interface (Axon Instruments), and stored on disk in an IBM-compatible computer using pCLAMP software version 6.0 (Axon Instruments). Solutions were exchanged by perfusion under gravity and simultaneous removal by suction. Hypoxia was generated by bubbling 100% N_2 into the perfusion chamber, occasionally with the O_2 scavenger, 1 mM sodium dithionite, present (Peers, 1990). During stimulus application the P_{O_2} at the recording site was within 4 mmHg of that measured with an oxygen electrode placed in the perfusion chamber. The effects of hypoxia on voltage-activated outward currents were determined by comparing peak currents from four records taken at each step potential, before (control), during, and after (wash) stimulus application. Currents or current densities were compared using Student's t test and the level of significance was set at $P < 0.05$. Membrane capacitance was obtained by integration of the capacitative transient during a hyperpolarizing voltage step from −60 to −100 mV. Voltage-clamp traces shown in text are leak subtracted.

Catecholamine determination by HPLC

Catecholamines (CA) released from living cultures, were separated by HPLC (Waters, model 510) with a Spherisorb-ODS2 column (10 × 0·46 cm, 3 μm particle size; Chromatography Sciences Co., Montreal, Quebec, Canada), coupled with an electrochemical detector (Coulochem II detector, model 5200; ESA, Inc., Bedford, MA, USA). The first detector in the analytical cell was set at 0·05 V to reduce interference by contaminating electroactive compounds at the second detector which was set at −0·3 V, the potential required for electroreduction of (−)-arterenol (noradrenaline), (−)-adrenaline, 3-hydroxytyramine (dopamine) and the internal standard, di-3,4-hydroxybenzylamine hydrobromide (DHBA). The mobile phase consisted of NaH_2PO_4 (6·9 g l^{-1}; Sigma), Na_2EDTA (80 mg l^{-1}; BDH Chemicals), and heptanesulphonic acid (250 mg l^{-1}; Sigma) in water and 5% methanol; pH was adjusted to 3·5 with concentrated H_3PO_4. Chromatograms were analysed with the aid of a Waters 740 Data Module (Millipore) and were quantified by the peak area ratio method, using known external standards (25 nM) and the internal standard, DHBA.

In studies of CA release, cultures, grown on a circular area of ~8 mm diameter, were first rinsed in 1:1 Dulbecco's modified Eagle's medium/F-12 medium before a 1 h incubation at 37 °C in 100 μl bicarbonate-buffered salt solution (BBSS), under an atmosphere of 5% CO_2 plus either normoxia (P_{O_2} = 160 mmHg), moderate hypoxia (P_{O_2} ~75 mmHg), or severe hypoxia (P_{O_2} ~35 mmHg), using a Forma Scientific O_2/CO_2 incubator. The BBSS contained (mM): NaCl, 116; KCl, 5; $NaHCO_3$, 24; $CaCl_2$, 2; $MgCl_2$, 1·1; Hepes, 10; glucose, 5·5; at pH 7·4; for high-K^+ experiments 25 mM NaCl was replaced by equimolar KCl. In a few experiments the calcium channel blocker, nifedipine (10 μM; Sigma), was added to the BBSS. Each release sample was mixed with an equal volume of 0·1 M $HClO_4$ containing 2·7 mM Na_2EDTA and then stored at −80 °C prior to HPLC analysis. CA release was compared between different treatments using Student's t test with the level of significance set at $P < 0.05$. Results are presented as means ± S.E.M.

Immunofluorescence

At the end of release experiments cultures were processed for tyrosine hydroxylase (TH) immunofluorescence, to obtain an absolute count of the number of chromaffin cells present. Procedures for TH immunostaining were similar to those described previously (Nurse, 1990); the primary TH antibody (rabbit; Chemicon, El Segundo, CA, USA) was visualized with a fluorescein-conjugated goat anti-rabbit IgG secondary antibody (Cappel, Malvern, PA, USA).

RESULTS

Conventional whole-cell or nystatin perforated-patch techniques were used to study the effects of hypoxia on ionic currents or membrane potential of rat AMCs, after 1–3 days in culture. In the majority (~70%) of AMCs (33/47) derived from neonatal (P1–P2) rat pups, acute hypoxia caused either a suppression of voltage-dependent K^+ current (voltage clamp) or membrane depolarization (current clamp). In contrast, most AMC (29/30) from juvenile (P13–P20) pups were unresponsive to hypoxia during similar measurements.

Age-dependent effects of acute hypoxia on voltage-activated K^+ currents in AMCs

In voltage-clamp studies using conventional whole-cell recording, reducing P_{O_2} from 160 mmHg (normoxia) to ~40 mmHg (hypoxia) caused a suppression of the outward K^+ current in neonatal AMCs by 24·4 ± 3·1% ($n = 11$), measured during voltage steps from −60 to +20 mV. With perforated-patch recording, a similar K^+ current suppression occurred during hypoxia (26·0 ± 3·4%; $n = 11$). In many cases (e.g. Fig. 1*A*), recovery of control responses after hypoxia was complete, and generally was within 90–100% of control values. Figure 1*B* is a plot of the outward current, normalized to cell size or input capacitance (pA pF^{-1}), *versus* membrane potential before (○), and during (●) acute hypoxia (P_{O_2} ~40 mmHg) for a group of four cells with similar current densities, investigated with conventional whole-cell recording. For each of these cells recovery of control current was 95–100% complete following wash with normoxic solution, but is omitted from Fig. 1*B* for clarity. Statistical analysis revealed that during hypoxia K^+ current density (e.g. Fig. 1*B*) was significantly ($P < 0·05$) reduced at all voltage steps between −10 and +60 mV.

During conventional whole-cell recordings from rat AMCs, there was usually a characteristic 'hump' or shoulder in the *I–V* relation between +20 and +50 mV (see Neely & Lingle, 1992), suggesting the presence of a prominent Ca^{2+}-dependent K^+ current. Interestingly, for neonatal AMCs, K^+ current suppression was largest in this region (Fig. 1*B*), as previously observed in the hypoxic response of rat carotid body type 1 cells (Peers, 1990). However, with perforated-patch recording the shoulder in the *I–V* relation was less prominent, and usually absent (not shown); this may be related to differences in intracellular calcium buffering during the two recording conditions.

In contrast to the above results on neonates, the same level of hypoxia failed to elicit significant K^+ current suppression at any test potential in twenty-six out of twenty-seven AMCs derived from juvenile rats of ages P13–P20 (Fig. 1*C* and *D*). As expected (Neely & Lingle, 1992), these juvenile cells displayed a prominent hump in the *I–V* relation during conventional whole-cell recording (see Fig. 1*D*), suggesting that they too expressed a Ca^{2+}-dependent K^+ current. However, there was no significant effect of hypoxia even in the hump region of the *I–V* relation in these juvenile cells (Fig. 1*D*).

Over 1–3 days in culture, the mean input capacitance of neonatal AMCs was 8·5 ± 0·7 pF ($n = 20$), a value significantly ($P < 0·01$) larger than that for juvenile AMCs, i.e. 5·9 ± 0·2 pF ($n = 20$). It is not clear whether this difference is attributable to differences in shape changes as the cells flatten out over time in culture, since there was no significant difference between the two (7·5 ± 0·8 pF, $n = 5$, for neonatal *versus* 6·0 ± 0·6 pF, $n = 5$, for juvenile) after only 1 day in culture. The mean outward current density, calculated at voltage steps to +50 mV, was significantly ($P < 0·05$) higher in juvenile (126·0 ± 15·1 pA pF^{-1}, $n = 20$) compared with neonatal (78·9 ± 11·8 pA pF^{-1}, $n = 20$) AMCs.

Age-dependent effects of hypoxia on membrane potential

In order to test whether acute hypoxia can alter the membrane potential and/or excitability of AMCs, nystatin perforated-patch recordings were carried out in current-clamp mode. The mean resting potential in neonatal AMCs was −54·8 ± 2·8 mV ($n = 13$), a value not significantly different from that in juvenile AMCs, i.e. −54·1 ± 3·0 mV ($n = 12$). Exposure to hypoxia, however, had quantitatively different effects on cells from the two age groups. Whereas hypoxia (P_{O_2} ~40 mmHg) depolarized neonatal AMCs by 14·1 ± 1·3 mV ($n = 11$; e.g. Fig. 2*A* and *B*), it produced no detectable change in membrane potential of juvenile AMCs (Fig. 2*C* and *D*). In eight out of thirteen neonatal AMCs, the hypoxia-induced depolarization was sufficient to elicit action potentials at room temperature (e.g. Fig. 2*A*). Application of brief, constant hyperpolarizing current pulses indicated that the depolarization during hypoxia in neonatal AMCs was accompanied by a significant ($P < 0·05$) increase in input resistance (e.g. Fig. 2*B*), consistent with the closure of ion channels that were open under resting normoxic conditions. The mean input resistance in four neonatal AMCs was 2·0 ± 0·6, 2·9 ± 0·7 and 1·9 ± 0·5 GΩ before, during and after acute hypoxia, respectively. In contrast, the input resistance of juvenile AMCs was unaffected by hypoxia (Fig. 2*D*); in five juvenile cells the mean input resistance was 2·9 ± 0·7, 2·9 ± 0·7 and 2·8 ± 0·7 GΩ, before, during and after acute hypoxia, respectively.

Age-dependent effects of hypoxia on catecholamine release from AMC cultures

Do the contrasting membrane responses of neonatal *versus* juvenile AMC correlate with their secretory activities during acute hypoxia? To test this we used HPLC with electrochemical detection and compared CA secretion (Fig. 3*A*) in neonatal and juvenile AMC cultures, following 1 h exposure to moderate (P_{O_2} ~75 mmHg) or severe (P_{O_2} ~35 mmHg) hypoxia. We avoided the use of anoxia (0 mmHg) since this stimulus is known to deplete CA from adult bovine chromaffin cells (e.g. Dry, Phillips & Dart,

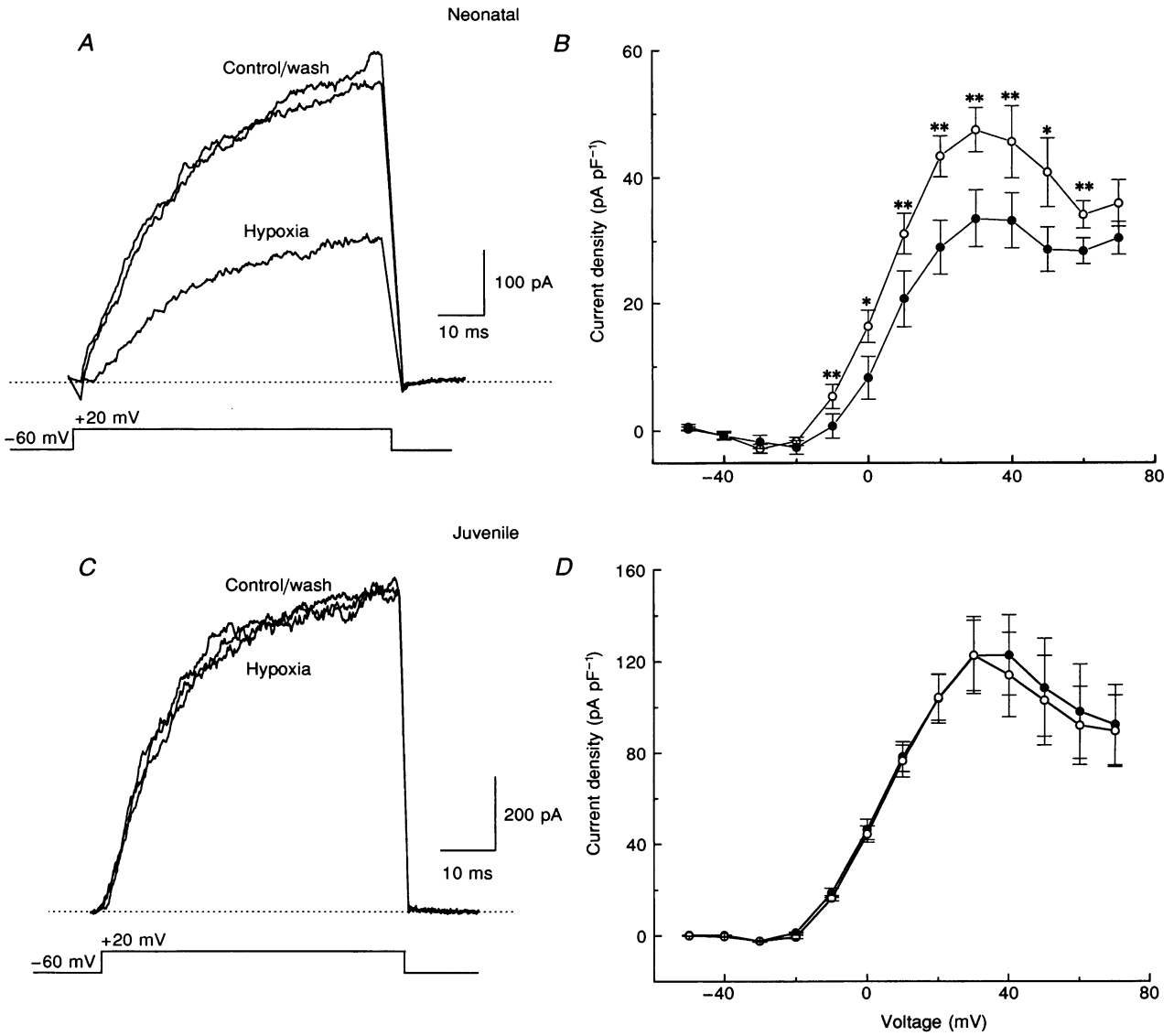

Figure 1. Contrasting effects of acute hypoxia on outward K^+ currents of neonatal *versus* juvenile rat AMCs

A and *B*, the effects of reducing P_{O_2} from 160 mmHg (control, normoxia) to ~40 mmHg (hypoxia) in neonatal (P1–P2) AMC. In *A*, the outward K^+ current, recorded with the conventional whole-cell technique, is reversibly suppressed by hypoxia; voltage step is shown below traces. Each current trace is an average of four recordings to the same step potential, and is leak subtracted. The small downward deflection at the beginning of the control current record is due to incomplete subtraction of the capacitative transience. In *B*, the *I–V* relation for the peak outward current, recorded with conventional whole-cell methods and plotted as current density (pA pF^{-1}) *vs.* membrane potential, is shown for a group ($n = 4$) of neonatal AMCs before (○) and during (●) hypoxia. After hypoxia, outward currents recovered to > 95% of control values in *B*, but are omitted for clarity. *C* and *D*, effect of reducing P_{O_2} from 160 mmHg (Control) to ~40 mmHg (Hypoxia) in juvenile (P13–P20) AMCs. Hypoxia had no effect on outward current (*C*), in contrast to neonatal cells in *A* and *B*. In *D*, the *I–V* relation is similar before and during hypoxia, and is shown for a group ($n = 5$) of juvenile cells. Recording conditions in *C* and *D* are similar to those described above for *A* and *B*. * $P < 0{\cdot}05$ and ** $P < 0{\cdot}01$, current density is significantly different from control (Student's *t* test).

1991), conceivably by a different mechanism involving calcium release from intracellular compartments, e.g. mitochondria (see Duchen & Biscoe, 1992). As shown in Fig. 3*C*, in neonatal cultures CA release per hour, normalized to 10000 AMC, was stimulated ~3 and 6 times above normoxic (P_{O_2} = 160 mmHg) basal release when cultures were exposed to a P_{O_2} of ~75 and ~35 mmHg, respectively. Counts of AMCs were obtained at the end of each release experiment following immunofluorescence staining for TH, a cytoplasmic marker (Fig. 3*B*). Though adrenaline was the major CA released (Fig. 3*C*), hypoxia also stimulated noradrenaline and dopamine release (Fig. 3*C*). However, the ratio of released adrenaline to noradrenaline was similar for normoxia and moderate hypoxia. The CA release induced by both hypoxic stimuli was abolished by the L-type calcium channel blocker, nifedipine (10 μM; Fig. 3*C*), suggesting a requirement for the entry of extracellular calcium through voltage-gated Ca^{2+} channels.

In contrast, exposure of juvenile AMC cultures to either hypoxic stimulus (P_{O_2} ~35 or 75 mmHg) had no effect on the normalized CA release relative to basal conditions (Fig. 3*D*). In addition, CA release in juvenile AMC cultures was unaffected by nifedipine at the oxygen tensions tested (Fig. 3*D*). These results indicate that the ability of AMCs to secrete CA in response to an acute hypoxic stimulus is lost by 2 weeks of postnatal life. This is probably due to a developmental loss of hypoxic chemosensitivity in AMCs, rather than a failure of the secretory machinery, since in juvenile (and neonatal) AMC cultures CA release could be stimulated by more than 2 times basal levels by a different stimulus, i.e. high extracellular K^+ (30 mM; Fig. 3*C* and *D*).

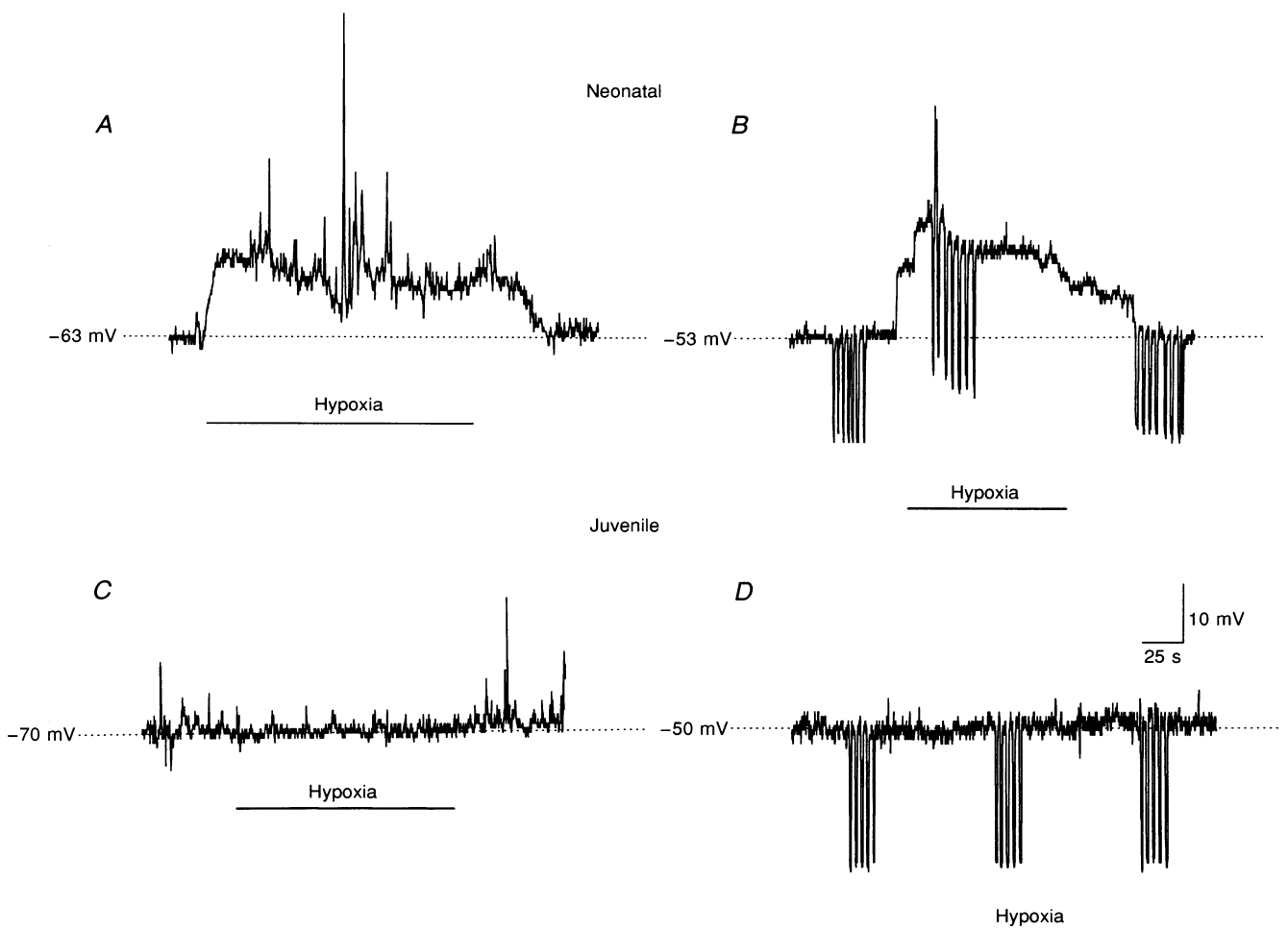

Figure 2. Contrasting effects of acute hypoxia on resting membrane potential and input resistance of neonatal *versus* juvenile AMCs

Membrane potential was recorded in current-clamp mode, using the perforated-patch, whole-cell technique. Perfusion of a hypoxic solution (P_{O_2} ~40 mmHg), during the time indicated by horizontal bar, causes ~10 mV depolarization of neonatal (P1–P2) AMCs, from a resting potential of −63 (*A*) and −53 mV (*B*). In *A*, hypoxia elicited active responses which are superimposed on the depolarization. In *B*, injection of constant hyperpolarizing current pulses (downward vertical deflections) indicates that the membrane resistance is increased during hypoxia. In contrast to its effects on neonatal cells, a similar hypoxic stimulus failed to affect either the resting membrane potential (*C* and *D*), or input resistance (*D*) in juvenile (P13–P20) AMCs. Details are as for *A* and *B*.

DISCUSSION

O_2-chemoreceptive properties are present in neonatal but not juvenile AMCs

In this study we demonstrate that acute hypoxia causes suppression of a voltage-dependent K^+ current, membrane depolarization, and stimulation of CA release in neonatal (P1–P2) AMCs. Significantly however, these properties were virtually absent in juvenile AMCs, suggesting that the mechanisms for sensing oxygen in these cells are transiently expressed in the perinatal period. In this respect, they differ from chromaffin-like (type 1) cells of the carotid body, a major O_2-chemosensory organ which, throughout postnatal life, continues to sense oxygen and regulate arterial P_{O_2} via reflex input to the respiratory centre (Gonzalez *et al.* 1994). The fact that the hypoxia-evoked CA release in neonatal AMC was abolished by the L-type calcium channel blocker, nifedipine, and that the depolarization was often sufficient to trigger action potentials, suggests that the hypoxia-sensing mechanism in these cells is similar to that described

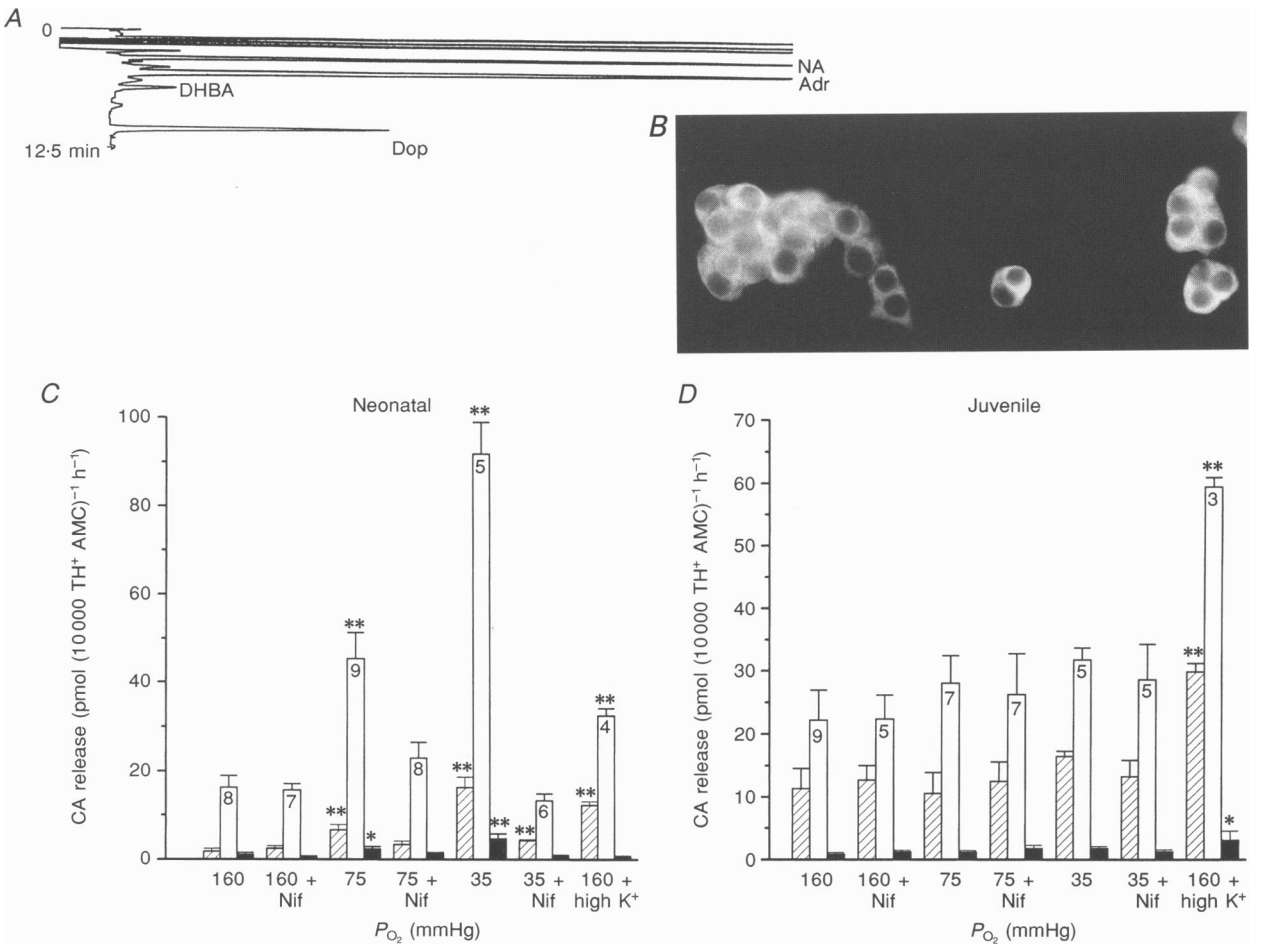

Figure 3. Comparison of normalized catecholamine release in neonatal and juvenile chromaffin cell cultures

A, HPLC record of a release sample (P_{O_2} = 160 mmHg) from a neonatal AMC culture indicating peaks corresponding to the catecholamines: noradrenaline (NA), adrenaline (Adr), dopamine (Dop), as well as to the internal standard DHBA (vertical scale readings in minutes). *B*, an example of rat chromaffin cells (diameter ~10 μm each) in a 2-day-old culture that was stained for tyrosine hydroxylase (TH) immunoreactivity, and visualized with a fluorescein-conjugated secondary antibody; cultures prepared in this way were used to obtain absolute chromaffin cell counts at the end of the release experiments, and for normalizing release to 10000 TH^+ cells in *C* and *D*. *C*, histogram illustrating the stimulatory effects of moderate (~75 mmHg) and severe (~35 mmHg) hypoxia, and high extracellular K^+ (30 mM) on catecholamine (NA, ▨; Adr, □; and Dop, ■) release in neonatal AMC cultures after 1 h exposure; basal release is represented by 160 mmHg (normoxia). Hypoxia-induced catecholamine release is abolished by the L-type calcium channel blocker, nifedipine (Nif, 10 μM). *D*, histogram illustrating the lack of effect of moderate and severe hypoxia on catecholamine release in juvenile AMC cultures; in these cultures, however, high extracellular K^+ (30 mM) significantly stimulated catecholamine release. Bars in *C* and *D* represent means ± S.E.M. for number of cultures indicated; ** $P < 0.01$ and * $P < 0.05$, release is significantly different from basal release (Student's *t* test).

in other cell types including, carotid body type 1 cells (López-Barneo *et al.* 1988; Delpiano & Hescheler, 1989; Peers, 1990; Stea & Nurse, 1991; Buckler & Vaughan-Jones, 1994; Gonzalez *et al.* 1994; Montoro *et al.* 1996), and neuroepithelial bodies (Youngson, Nurse, Yeger & Cutz, 1993).

The depolarization of neonatal AMC by hypoxia was accompanied by a decrease in membrane conductance, consistent with the closing of ion channels that were open at the resting potential. Though closing of K^+ channels is a likely explanation, these channels may well be different from the ones that were sensitive to hypoxia in our voltage-clamp studies. In the latter case, the voltage-dependent hypoxia-sensitive K^+ current was activated at potentials positive to −30 mV, well above the observed resting potentials of the order of −55 mV for chromaffin cells. Thus, the possibility that the hypoxia-induced depolarization in neonatal AMCs was due to closure of a different K^+ channel subtype cannot be excluded, and indeed, there is evidence in the carotid body that different types of K^+channels can be regulated by hypoxia (López-Barneo *et al.* 1988; Peers, 1990; Ganfornina & López-Barneo, 1992; Wyatt, Wright, Bee & Peers, 1995). Whatever the origin of the initial depolarization, the hypoxic suppression of a voltage-dependent K^+ current in this study is still likely to be physiologically important, perhaps in regulating catecholamine secretion via control of action potential frequency and/or duration. In neonatal AMC this current was activated at potentials positive to −30 mV, as occurs in other neuroendocrine cell types that sense oxygen (López-Barneo *et al.* 1988; Peers, 1990; Stea & Nurse, 1991; Youngson *et al.* 1993). The fact that with conventional whole-cell recording, hypoxic suppression was greatest in the region of the *I–V* relation where there was a characteristic 'hump', suggests the possible involvement of a Ca^{2+}-activated K^+ current as occurs in rat type 1 cells (Peers, 1990); however, further studies are required for validation of this point.

Importance of hypoxic chemosensitivity in neonatal animals

In the context of neonatal physiology, our results suggest a plausible 'non-neurogenic' mechanism for adrenal catecholamine release during hypoxic stress in the newborn rat, where sympathetic innervation of the adrenal medulla is immature or absent (Lagercrantz & Slotkin, 1986; Slotkin & Seidler, 1988; see also Cheung, 1990). This catecholamine release is crucial for survival of the neonate, producing multiple systemic effects that facilitate the transition from fetal to extrauterine life. Among these are absorption of lung fluid, secretion of surfactant (a process mediated by β_2-receptors), and regulation of cardiac function via stimulation of α-adrenergic receptors (Slotkin & Seidler, 1988). Thus, our data indicate that the increase in plasma catecholamine associated with birth could arise from the hypoxic-sensing mechanism we have uncovered in neonatal chromaffin cells, leading to membrane depolarization and increased action potential frequency, entry of extracellular calcium and catecholamine release. Interestingly, our results also account for the observation that this 'non-neurogenic' mechanism disappears in the rat during the first few weeks of postnatal life (Slotkin & Seidler, 1988). At this time adrenal catecholamine release in response to hypoxic stress is abolished by blockers of cholinergic transmission or by short-term denervation of the adrenal medulla (Slotkin & Seidler, 1988). Thus our finding that the hypoxic-sensing mechanism, present in the neonate, disappears in juvenile (P13–P20) chromaffin cells is consistent with a model where O_2 sensing in these cells is a developmentally regulated process. It remains to be determined whether preganglionic sympathetic innervation can directly modulate O_2 chemosensitivity in adrenal chromaffin cells.

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Author's email address

C. A. Nurse: nursec@mcmail.cis.mcmaster.ca

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