Induction of T-type Calcium Channel Gene Expression by Chronic Hypoxia*

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Cellular responses to hypoxia can be acute or chronic. Acute responses mainly depend on oxygen-sensitive ion channels, whereas chronic responses rely on the hypoxia-inducible transcription factors (HIFs), which up-regulate the expression of enzymes, transporters, and growth factors. It is unknown whether the expression of genes coding for ion channels is also influenced by hypoxia. We report here that the α1H gene of T-type voltage-gated calcium channels is highly induced by lowering oxygen tension in PC12 cells. Accumulation of α1H mRNA in response to hypoxia is time- and dose-dependent and paralleled by an increase in the density of T-type calcium channel current recorded in patch clamped cells. HIF appears to be involved in the response to hypoxia, since cobalt chloride, desferrioxamine, and dimethylloxalylglycine, compounds that mimic HIF-regulated gene expression, replicate the hypoxic effect. Moreover, functional inhibition of HIF-2α protein accumulation using antisense HIF-2α oligonucleotides reverses the effect of hypoxia on T-type Ca2+ channel expression. Importantly, regulation by oxygen tension is specific for T-type calcium channels, since it is not observed with the L-, N-, and P/Q-channel types. These findings show for the first time that hypoxia induces an ion channel gene via a HIF-dependent mechanism and define a new role for the T-type calcium channels as regulators of cellular excitability and calcium influx under chronic hypoxia.

Maintaining optimal oxygen homeostasis is of paramount importance for cells. Reductions of oxygen supply trigger cell adaptive responses that minimize the deleterious effects of hypoxia. Cellular responses to hypoxia can be acute, occurring over a time scale of seconds or minutes, or chronic, with time courses of hours to days (1–5). The major effectors of the acute cellular responses to hypoxia are oxygen-sensitive ion channels. These channels mediate the fast adaptive changes in cell excitability, contractility, and secretory activity that occur in response to low ambient oxygen tension (PO2) (1, 5). On the other hand, chronic cellular responses to hypoxia, studied in great detail in the past few years, are mediated by ubiquitously expressed hypoxia-inducible transcription factors (HIF-1α and isoforms). Stabilization and transcriptional activity of HIF depend on oxygen-regulated hydroxylases (6–8). Hypoxia-inducible factors regulate the expression of a wide repertoire of oxygen-sensitive genes with roles in diverse cellular functions such as angiogenesis, red blood cell production, glucose and energy metabolism, apoptosis, and cell proliferation (1–5).

Despite progress in the understanding of the role of ion channels and gene expression in the cellular responses to hypoxia, long-term regulation of ion channel expression by maintained low PO2 is poorly known. There are previous reports showing that prolonged hypoxia down-regulates various voltage-gated K+ (Kv) channel genes in pulmonary artery smooth muscle cells (9), and the opposite has been observed with the Kv1.2 gene in PC12 cells (10). Nevertheless, the effect of chronic hypoxia on voltage-gated ion channel genes has not been systematically addressed, and key questions remain regarding the involvement of the ubiquitous HIF-1α-mediated pathway in the modulation by hypoxia of ion channel gene expression.

In this work, we have focused on the regulation by protracted hypoxia of voltage-gated Ca2+ channel genes. These channels are the major pathway for regulated influx of Ca2+ into the cells and play critical roles in diverse cellular processes such as electrical excitability and contraction, hormone secretion, enzyme activity, and gene expression. Importantly, Ca2+ entry through voltage-gated Ca2+ channels is necessary for the acute response to hypoxia of neurosecretory cells (1). There are two major classes of voltage-dependent Ca2+ channels: low voltage-activated or T-type channels and high voltage-activated (HVA) channels, which include the L-, N-, P/Q-, and R-subtypes (11, 12). T-type channels regulate cellular functions susceptible to modulation by low oxygen concentration, such as cellular excitability, differentiation, growth, and proliferation (12). They are predominantly expressed in the G0/G1 transition stage of the cell cycle (13) as well as in the early stages of differentiation of many embryonic and neonatal tissues (14, 15). In addition, T-type channels are up-regulated by a variety of mitogens and are expressed in various tissues under proliferative conditions where oxygen supply decreases, such as cardiac cells during heart hypertrophy or cardiomyopathy (16, 17), smooth muscle cells following vascular injury (18), and prostatic tumor cells (19). Based on these precedents, we hypothesized that T-type Ca2+ channels could be a target of the gene expression program developed under hypoxia. We have used for our study the oxygen-sensitive pheochromocytoma-derived PC12 cell line as a model system. These are excitable cells that respond to acute

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1 The abbreviations used are: HIF, hypoxia-inducible transcription factor; Kv, voltage-gated K+; HVA, high voltage-activated; pF, picofarads.
hypoxia with membrane depolarization, increase of extracellular Ca\textsuperscript{2+} influx, and catecholamine secretion (20–22). Chronic hypoxia induces in these cells tyrosine hydroxylase and other genes (23, 24). We show here, both at the molecular and electrophysiological levels, that PC12 cells contain T-type Ca\textsuperscript{2+} channels and that proliferated hypoxia markedly up-regulates their expression. We also present evidence indicating that HIF is involved in this process. Importantly, the low PO\textsubscript{2}-dependent up-regulation is specific for the T-type Ca\textsuperscript{2+} channel, since it is barely affected by other Ca\textsuperscript{2+} channels expressed in PC12 cells. These findings define a new role for the T-type Ca\textsuperscript{2+} channels as regulators of cell excitability and Ca\textsuperscript{2+} influx during cellular adaptive responses to prolonged hypoxia.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Treatments**—PC12 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum, 10% horse serum, 100 units/ml penicillin, and 100 \mu g/ml streptomycin. Cells were routinely cultured in 90% air, 10% CO\textsubscript{2} (normoxic conditions) at 37 °C and subcultured every 5 days at 1:5 dilution. For hypoxic treatment, the modified Eagle’s medium was replaced with 0.11 mg/ml sodium pyruvate and 4 \mu g/ml-glutamine to match the composition of the control medium. Cobalt chloride and desferrioxamine mesylate were obtained from Sigma. Dymetoxylglycine was synthesized in facilities of the University of Seville. For electrophysiological recordings, cells were plated on poly-L-lysine-coated glass coverslips prior to the hypoxic treatments.

**Reverse Transcription and Polymerase Chain Reaction**—Total RNA was isolated with Nucleospin RNA II (Macherey-Nagel) following the manufacturer’s instructions with the addition of an extra acid phenol/ chloroform extraction followed by RNA precipitation. First strand cDNA was synthesized from 2–4 \mu g of total RNA using the SuperScript\textsuperscript{TM} first strand synthesis system for reverse transcriptase-PCR (Invitrogen) with random primers according to the manufacturer’s directions. To analyze the expression of T-type channels in PC12 cells, the PCRs were usually carried out for 30 cycles of 96 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min with 2 \mu l of cDNA. The primers used were as follows: rat \alpha\textsubscript{T1} forward (5'-GGGCTGTTGGGTAGGAACTT-3') and reverse (5'-GATGATGGTGGGATTGAT-3') (GenBank\textsuperscript{TM} accession number AF290212); rat \alpha\textsubscript{1H} forward (5'-GGTATTAGACA-CCTGCTCCAGAAAGA-3') and reverse (5'-GGCGTGGTGGTGGAGAACTT-3') (GenBank\textsuperscript{TM} accession number AF290213); and rat \alpha\textsubscript{1A} forward (5'-GAGTTAGACATATTCCAAGCTGCCCAGACTGCCCAGCTCCTG-3'). The PCR products were subcloned in pGem\textsuperscript{TM}T or pBluescript vectors, and their identities were confirmed by complete DNA sequencing.

**Northern Blot Analysis**—mRNA was isolated from total RNA with NucleoTrap mRNA minikit (Macherey-Nagel) following manufacturer’s instructions. Equal amounts (5 \mu g) of mRNA were analyzed by Northern blots according to standard procedures for separation using 1% agarose gel containing formaldehyde (25). RNA from gels was trans- ferred to nylon membranes (Am- bion) and UV-cross-linked prior to hybridization. Labeling of radioac- tive DNA probes was performed using [\text{\text{\textsuperscript{32}P}}]dCTP and Rediprime\textsuperscript{TM} II random prime labeling system (Amersham Biosciences). The probes were subsequently purified with MicroSpin\textsuperscript{TM} S-400 HR columns (Am- ersham Biosciences). Hybridization was carried out overnight at 42 °C with Ultrahyb hybridization buffer (Ambion), after which the mem- branes were washed twice to a stringency of 2\times SSC, 0.1% SDS at 55 °C for 15 min followed by three 5 min washes with 0.5\times SSC, 0.1% SDS at 55 °C. RNA loading on gels was monitored by etidium bromide stain- ing and by probing with cyclophilin as a control. Autoradiography was carried out at ~80 °C with intensifying screens. Rat cDNA fragments containing the \alpha\textsubscript{1A} subunit and \alpha\textsubscript{1H} subunit were used to make the radioactive probes. RNA bands on the autoradiographs were quantified using a CanoScan N500U scanner and National Institutes of Health software.

**Real Time PCR**—Total RNA was isolated from cells after the different treatments, and 4 \mu g were subsequently used for cDNA synthesis as described above. Real time PCR was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using SYBR Green PCR Master mix (Applied Biosystems) and the thermocycler conditions recommended by the manufacturer. PCRs were performed in duplicates in a total volume of 30 \mu l containing 1 or 2 \mu l of the reverse trans- scriptase reaction. Each sample was analyzed for \beta-actin to normalize for RNA input amounts and to perform relative quantifications. Prim- ers were designed using the computer program Primer Express (Ap- plied Biosystems). Primers (forward 5'-ACTTGGCAGCTGCTCCTCTT- A'-3' and reverse 5'-GGGGCGTCTACCTGCAATCTC-3') were generated to the rat \alpha\textsubscript{T1} subunit of T-type Ca\textsuperscript{2+} channels and used to amplify a 64-base pair fragment. For the \alpha\textsubscript{1H} subunits of the HVA Ca\textsuperscript{2+} channels, primers were as follows: forward (5'-TCTTCTCGACCCAAACAAC- GAGCTCCTG-3') and reverse (5'-TTGGAATGGCTTTGGAGAC-GAGGG-3'). For the rat \alpha\textsubscript{1AI} subunit (GenBank\textsuperscript{TM} accession number M67516); forward 5'-AATGC- CCTGTCACAGAGAAGA-3' and reverse 5'-CAGAGCTGCCCGTTCAGGGA- GG' for the \alpha\textsubscript{1H} subunit (GenBank\textsuperscript{TM} accession number M929905); and forward 5'-GATGGCTCAAAGAACAGCAT-3' and reverse 5'-G- GCCGAGCTCAGGATGCGTC-3' for the \alpha\textsubscript{1A} subunit (GenBank\textsuperscript{TM} accession number M64373). Melting curve analysis showed a single sharp peak with the expected T\textsubscript{m} for all samples.

**Western Blotting**—Cells were homogenized in lysis buffer containing 50 mM Heps-KOH, pH 7.3, 250 mM NaCl, 5 mM EDTA, 0.2% Nonid- et N-40, 5 mM dithiothreitol, and protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 1 mM Na\textsubscript{3}VO\textsubscript{4}, 1 \mu g/ml aprotinin, 10 \mu g/ml pep- tstatin, 1 \mu g/ml leupeptin) for 10 min at 4 °C. After centrifugation at 8,000 \times g for 5 min, the supernatants were recovered, and protein concentrations were determined with Bradford protein assay reagent. Lysates (20 \mu g) were loaded and resolved on 6% SDS-polyacrylamide gel electrophoresis followed by transfer to Immob-Blot polyvinylidene difluoride membrane (Bio-Rad). Membranes were probed with 1:500 anti-HIF 2 \alpha (Novus-Bio- logicals) or 1:1000 anti-\alpha-tubulin antibodies (Sigma) and developed with the enhanced chemiluminescence plus Western blotting detection system (Amersham Biosciences).

**Antisense Oligonucleotide Treatment**—Phosphorothioate oligonu- cleotides for HIF-2 \alpha depletion experiments were as follows: antisense, 5'-GCGAGCTCGCTGAGCTCGTC-3'; sense, 5'-GAGCGACTGCTGACTGCT- GAGG-3'. The 5\textsuperscript{'}-C subunit of the HVA Ca\textsuperscript{2+} channel was chosen to serve as a control for antisense oligonucleotide transfer re- agent according to the manufacturer’s protocol (Invitrogen). 72 h after transfection, treated cells were exposed to hypoxia or normoxia for 12 h, and afterward, cells were lysed, and total RNA and protein were ex- tracted. \alpha\textsubscript{T1} and HIF 2 \alpha mRNA levels were quantified by real time PCR as described above. The primers used for HIF-2 \alpha mRNA quantification were as follows: 5'-CGCATGAGATACTGGTACTGGAAGGG-3' and 5'- CTGACAGAAGGATCATATCAGCTGCTT-3'.

**5'-Flanking Sequences Analysis**—The 5\textsuperscript{'}-upstream sequences of the rat, mouse, and human \alpha\textsubscript{1H} genes were obtained from the Ensembl software system (available on the World Wide Web at www.ensembl.org). Primers (forward 5'-GCTTCCCAATCCCTCAAAGAGC-3' and reverse 5'-GCTTTTCCCCTATCCGCTG-3') were loaded and resolved on 6% SDS-polyacrylamide gel electrophoresis followed by transfer to Immob-Blot polyvinylidene difluoride membrane (Bio-Rad). Voltage clamp recordings were obtained with an EPC-8 patch clamp amplifier (Heka Elektronik) using standard voltage clamp protocols designed with Pulse software (Heka Elektronik). Unless otherwise noted, holding potential was ~80 mV. Data were filtered at 10 kHz, digitized at a sampling interval of 20 \mu s with an ITC-16 A-D converter (Instrutech), and stored on a Macintosh computer. Off-line analysis of the data was performed using P-Clamp software and Pulse Fit (Heka Elektronik). All experiments were conducted at room temperature, 22–24 °C. For whole cell patch recordings, the internal solution contained 110 mM CsCl, 30 mM Cs\textsuperscript{2+}, 10 mM EGTA, 10 mM HEPES, and 4 mM Mg\textsuperscript{2+}. pH was adjusted with CsOH to 7.2, and osmolality was 285 mOsm/kg. The standard bath solution contained 140 mM Na\textsuperscript{2+}, 4 mM n-methyl-D-glucamine, 9 mM Ba\textsuperscript{2+}, 1 mM Ca\textsuperscript{2+}, 1 0 mM HEPES, and 10 mM glucose. pH was adjusted with HCl to 7.4, and osmolality was 300 mOsm/kg.

**Statistical Analysis**—Data were analyzed using Student’s t test for unpaired observations with the SigmaPlot program (Jandel). Values
amplified from rat brain RNA. The specificity of the amplification products was further confirmed by complete DNA sequencing. The PCR product for the α₁H gene was consistently more abundant than for the α₁G gene, suggesting the existence of different levels of expression of the two channel isoforms. Northern blot analyses were then performed to confirm the above results. As shown in Fig. 1B, a probe specific for the α₁H subunit hybridized to a single ~8.5-kb transcript in PC12 cells, which was also detected in rat brain as previously reported (30). A second less abundant band of ~10 kb, detected in rat brain, was not present in PC12 cells. In contrast to the α₁H subunit, no transcript was detected when a probe specific for the α₁G gene was used, although, as shown in previous reports (31), two mRNAs of ~8.5 and ~10 kb were observed in rat brain. The absence of signal of α₁G mRNA in Northern blots and the PCR data suggest the existence of a very low level of expression of the α₁G as compared with the α₁H subunit. Altogether, these results reveal that PC12 cells express genes that encode for the α₁H and α₁G subunits of T-type Ca^{2+} channels, α₁H mRNA being the most abundant.

The presence of functional T-type Ca^{2+} channels in PC12 cells was confirmed by analysis of the total Ca^{2+} currents recorded with the whole cell configuration of the patch clamp technique. Fig. 1C shows a representative example of Ca^{2+} current recorded in a PC12 cell during a depolarizing pulse to +20 mV followed by repolarization to −70 mV. Consistent with other analyses on Ca^{2+} currents in PC12 cells (28, 29), the recorded current appeared to result predominantly from the expression of HVA and fast deactivating channels, as evidenced by the fast tail current generated when the membrane was repolarized to −70 mV. The apparent activation threshold of the whole cell Ca^{2+} current in PC12 cells was observed at about −50 mV, and maximal current amplitude was observed at +20 mV (see below). However, a slow component in the deactivation, or closing, tail current (indicated by the small arrowheads in Fig. 1, C and D) was consistently observed, suggesting the presence of a population of T-type Ca^{2+} channels, which are known to close about 10–20 times more slowly than HVA channels (12, 27, 32–34). The calcium tail currents evoked upon repolarization were analyzed in more detail (Fig. 1D). To quantify the closing time constant (τ), we fitted single and double exponential functions to the deactivating segment of the current (top and bottom panels in Fig. 1D). Analysis of the data revealed that the deactivating current is well fitted by a double but not by a single exponential function; therefore, two time constants (τ_{fast} and τ_{slow}) were estimated (27, 32, 33). At −70 mV, average τ_{fast} and τ_{slow} values were 0.11 ± 0.005 and 1.34 ± 0.09 ms, respectively (n = 45 cells). These data are compatible with the existence of two populations of Ca^{2+} channels in PC12 cells, HVA channels that deactivate rapidly, and T-type channels that deactivate slowly. Significantly, our data are in close agreement with the reported electrophysiological parameters for the recombinant rat α₁H gene (30). The presence of T-type Ca^{2+} currents in PC12 cells was further confirmed by their rapid inactivation, another distinct feature of T-type Ca^{2+} channels (27, 32, 35, 36). Fig. 1E shows representative examples of the deactivating currents recorded in PC12 cells upon repolarization of the cell to −70 mV after a depolarizing pulse to +20 mV lasting either 10 ms (short pulse) or 50 ms (long pulse). The experiments clearly indicated that the slow component of the tail current present at the end of 10-ms pulses disappeared almost completely at the end of the 50-ms pulses, suggesting inactivation of the T-type channels during the maintained depolarization. Therefore, Ca^{2+} currents in PC12 cells possess a slowly deactivating and fast inactivating component typical of T-type Ca^{2+} channels. The molecular and the
electrophysiological data indicate that α_{1H} is the major T-type Ca^{2+} channel subunit functionally expressed in PC12 cells.

Selective Induction of T-type Ca^{2+} Channel Gene Expression by Hypoxia—Northern blot and quantitative real-time PCR analyses were performed to determine whether the α_{1H} gene expression is regulated by hypoxia. Fig. 2A shows Northern blot data obtained on PC12 cells after exposure to reduced PO_{2} (3% oxygen) for 1, 6, 12, and 24 h. In all our experiments, sensitivity of PC12 cells to hypoxia was confirmed by the induction of tyrosine hydroxylase, a well characterized hypoxia-sensitivity of PC12 cells to hypoxia was confirmed by the in-electrophysiological data indicate that α_{1H} is the major T-type Ca^{2+} channel subunit functionally expressed in PC12 cells.

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suggested a high presence of T-type Ca\(^{2+}\) channels in the cells. As shown above for control cells (see Fig. 1E), the slow component of the tail currents, reflecting the closure of the T-type channel population, was also markedly reduced after long lasting pulses in hypoxia-treated cells (Fig. 5, A and B). Shown in Fig. 5, C and D, are frequency histograms of the amplitude of the slowly deactivating current component expressed as percentage of the total tail current. These data show that exposure to hypoxia increased the population of cells with a large component of slowly deactivating tail current; in some cells, the amplitude of the slowly deactivating current was more than half of the total tail current. Although chronic hypoxia increased the expression of T-type channels, the values of the fast and slow closing time constants of the current measured at −70 mV remained unchanged (\(\tau_{fast} = 0.12 \pm 0.01\) and \(\tau_{slow} = 1.37 \pm 0.06\) ms, \(n = 42\) cells; \(p > 0.05\) in the two cases when compared with the respective values in control cells). The slowly deactivating component of the tail currents induced by hypoxia was selectively reduced in amplitude by application of 50 \(\mu\)M nickel to the external solution (Fig. 5, E and F), thus further indicating that this component of the Ca\(^{2+}\) current represented the activity of T-type Ca\(^{2+}\) channels (37).

Induction of T-type Ca\(^{2+}\) channel expression by chronic hypoxia is further illustrated in Fig. 6. The average current density due to T-type Ca\(^{2+}\) channels measured during repolarizations from +20 to −70 mV (3.2 ± 0.47 pA/pF, \(n = 45\) cells) increased almost 2.5 times upon exposure of the cells to hypoxia (7.4 ± 1.4 pA/pF, \(n = 42\) cells) (Fig. 6A). In contrast, the same treatment produced no effect on, or even a slight reduction in, the current density mediated by the fast deactivating Ca\(^{2+}\) channels (Fig. 6B). Accordingly, the ratio of slow/fast deactivating current densities measured in each of the cells was markedly increased in hypoxia (Fig. 6C). These results indicate that, in accord with the up-regulation of \(\alpha_{1H}\) mRNA expression detected by molecular biology, protracted hypoxia induces a significant increase in the number of functional T-type Ca\(^{2+}\) channels in PC12 cells.

**T-type Ca\(^{2+}\) Channel Induction Depends on HIF Activation**—It is well known that the effects of hypoxia on gene expression are mimicked by agents such as cobalt chloride and the iron chelator desferrioxamine, which act as nonspecific...
To determine whether HIF is indeed functionally involved in the hypoxic up-regulation of T-type Ca$^{2+}$ channel expression, we exposed cells to hypoxia once they had been incubated with antisense HIF oligonucleotides (41, 42). In these experiments, we used antisense HIF-2α oligonucleotides, since this appears to be the HIF isoform most actively induced by hypoxia in PC-12 cells (43, 44). As described before (45), HIF-2α is strongly induced by hypoxia at the level of protein but only slightly at the level of mRNA (Fig. 8A). However, incubation with antisense HIF-2α oligonucleotides resulted in a marked decrease in the HIF-2α mRNA and protein induced by hypoxia in these cells (Fig. 8A). In fair agreement with these data, antisense inhibition of HIF-2α strongly decreased the hypoxic induction of the α1H subunit, reducing the mRNA levels to values close to those seen in normoxia. Antisense HIF-2α oligonucleotides had no effect on the levels of the T-type channel mRNA in basal conditions (Fig. 8B). In control experiments, sense HIF-2α oligonucleotides showed no effect on the hypoxic induction of α1H mRNA. The involvement of HIF in the hypoxic up-regulation of the α1H Ca$^{2+}$ channel suggested by these experiments is further supported by the presence of hypoxia-responsive elements in the 5′-flanking region of the α1H gene. We identified numerous sequences compatible with hypoxia-responsive elements (44, 46–49) in a region of ~1300 bp upstream of the coding sequence of the rat α1H gene. This region is highly conserved among mammals, with more than 71% similarity between rodents and humans and 93% similarity between rats and mice. In Fig. 8C, we represent selected fragments of the aligned rat, mouse, and human sequences, showing six putative HIF consensus DNA binding sites. The core motifs are in boldface type and overlined by arrows to indicate the plus (right arrow) or minus (left arrow) DNA strand location.

**Dependence on Ca$^{2+}$ of the Regulation of T-type Ca$^{2+}$ Channel Gene Expression by Hypoxia**—One of the earliest known responses to hypoxia in PC12 cells is membrane depolarization and increase of intracellular Ca$^{2+}$ levels (20). Previous reports have shown that increase of intracellular free Ca$^{2+}$ is required...
induction of hypoxia-inducible genes, including tyrosine hydroxylase, in PC12 cells (24, 50). To test whether the induction of $\alpha_{1H}$ expression by hypoxia is $Ca^{2+}$-dependent, PC12 cells were incubated in $Ca^{2+}$-free medium supplemented with 1 mM EGTA and exposed to normoxia or hypoxia (3% oxygen) for 12 h. Fig. 9 (A and B) shows that the accumulation of $\alpha_{1H}$ mRNA induced by hypoxia is maintained in the absence of extracellular $Ca^{2+}$. Thus, an increase in intracellular free $Ca^{2+}$ does not seem to be required for induction of $\alpha_{1H}$ gene expression by hypoxia in PC12 cells. In contrast, it seems that maintained $Ca^{2+}$ influx might even down-regulate $\alpha_{1H}$ expression.$^2$

**DISCUSSION**

For the last decade, research on cellular oxygen homeostasis has rapidly progressed, due, on one hand, to the characterization of the HIF-dependent pathway that regulates the expression of enzymes and growth factors in response to chronic deficiency of $O_2$ and, on the other, to the identification of $O_2$-sensitive ion channels as mediators of the acute cardiovascular and respiratory reflexes evoked by hypoxia (1, 2, 5, 51). There is, however, almost no knowledge of the long lasting adaptive changes of ion channel gene expression in prolonged hypoxia. Among all voltage-sensitive ion channels, the $T$-type $Ca^{2+}$ channels are of special interest, because they regulate cellular excitability, which changes during acute and protracted hypoxia, and appear to be also involved in cellular proliferation, which is tightly constrained by the need of precise oxygen homeostasis. We report here that $T$-type $Ca^{2+}$ channels are expressed in PC12 cells. More importantly, we show both at the molecular and the functional levels that hypoxia greatly induces the expression of the $\alpha_{1H}$ subunit of $T$-type $Ca^{2+}$ channels through activation of the HIF-dependent pathway.

Among the three $\alpha$-subunits ($\alpha_{1H}$, $\alpha_{1G}$, and $\alpha_{1I}$) of T-type channels already cloned (30, 31, 52, 53), $\alpha_{1H}$ (Cav3.2 gene) is the most abundantly expressed in PC12 cells. The $\alpha_{1G}$ mRNA is detectable only by PCR, and the $\alpha_{1I}$ is undetectable. Accordingly, the electrophysiological data reveal that besides a large population of fast deactivating, or closing, HVA $Ca^{2+}$ channels, PC12 cells also express a smaller population of slowly deactivating and fast inactivating T-type $Ca^{2+}$ channels. Significantly, the estimated deactivation time constant of the native T-type $Ca^{2+}$ channels in PC12 cells is similar to the reported value for the recombinant rat $\alpha_{1H}$ subunit, which has been shown to display faster closing kinetics than the $\alpha_{1G}$ and $\alpha_{1I}$ subunits (30). To our knowledge, T-type $Ca^{2+}$ channels had been documented in PC12 cells that have undergone neuroendocrine differentiation (54) but not in undifferentiated PC12 cells. The very scant level of expression and the relatively fast deactivation kinetics of the $\alpha_{1I}$ subunit have probably hampered the separation of the T-type $Ca^{2+}$ channels from the fast deactivating HVA channels in previous electrophysiological studies on PC12 cells. Likewise, T-type $Ca^{2+}$ channels have not been detected in chromaffin cells until recently by using molecular approaches and analysis on different stages of cellular development. Remarkably, the T-type channels expressed in rat chromaffin cells share properties with the $\alpha_{1H}$ subunit (55). These data are in good agreement with studies showing that the $\alpha_{1H}$ subunit is mostly expressed in peripheral tissues (53), in contrast with $\alpha_{1G}$ and $\alpha_{1I}$ subunits that are more abundant in the brain (52, 56).

Our results clearly show that the $\alpha_{1H}$ T-type $Ca^{2+}$ channel is overexpressed in chronic hypoxia. This was demonstrated by accumulation of the specific mRNA and by an increase in the membrane current density with kinetic and pharmacological (nickel inhibition) features characteristic of this class of T-type channels.

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$^2$ R. Del Toro, K. L. Levitsky, J. López-Barneo, and M. D. Chiara, unpublished results.
channels (12, 27, 32–34, 37). Induction of the α1H subunit by hypoxia was time- and dose-dependent, reaching maximal levels (about 5–8-fold) after 12–24 h of exposure to 3% oxygen. The same hypoxic treatment produced only a 2.5-fold increase in the amplitude of T-type channel current, possibly because these channels are also subjected to posttranscriptional regulation by hypoxia and other signaling variables (57). Interestingly, hypoxia has not a marked effect on any of the HVA Ca2+ channel genes expressed in PC12 cells. Neither significant accumulation of the α1L (L-type), α1N (N-type), and α1S (P/Q-type) mRNAs nor significant increase of the fast deactivating Ca2+ mean current density is observed in PC12 cells exposed to hypoxia. On the contrary, a small but significant decrease in the HVA channel mean current density was observed upon hypoxia exposure of the PC12 cells. Although our molecular, electrophysiological, and pharmacological data suggest that the effect of hypoxia is rather selective for a subtype of T-type Ca2+ channels, there is a previous report showing that chronic hypoxia increases the amplitude of Ca2+ currents in PC12 cells (58). In these last experiments, however, the hypoxia challenge was very mild (10% oxygen), and T-type channels were not studied with electrophysiological or molecular techniques. Induction of T-type Ca2+ channels genes by hypoxia is not a singular property of our PC12 cells but a rather general phenomenon, since it has also been observed in adrenal chromaffin cells as well as in primary cultures and clonal cell lines of aortic smooth muscle.3

Induction of T-type Ca2+ channel expression by low PO2 has the same general features (O2 levels, time course, etc.) of the classical hypoxia-inducible genes such as erythropoietin or vascular endothelial growth factor (1–5). Although there exists the possibility that hypoxia-induced up-regulation of the α1H Ca2+ channel subunit depends on general changes in the cells (i.e. cytoplasmic acidification and subsequent activation of immediate early response genes), our experiments strongly suggest that specific activation of the HIF pathway mediates this effect. Induction of T-type Ca2+ channel gene expression by low PO2 was replicated by cobalt chloride, the iron quelant desferrioxamine, and dimethylxaloylglycine (4, 5, 39, 40). These compounds have been shown to mimic hypoxia by inhibiting an oxygen-, Fe2+, and exoglutamate-dependent dioxygenase that under normoxic conditions hydroxylates specific proline and asparagine residues in HIF prior its degradation (6–8). Moreover, we have shown using antisense HIF-2α oligonucleotides that functional inhibition of HIF-2α protein accumulation reverses the effect of hypoxia on T-type Ca2+ channel expression. This experimental observation is in fair agreement with the existence in the 5′-flanking region of the α1H gene of several putative HIF consensus DNA binding sites (46–49). We have also investigated whether Ca2+ is critical for the hypoxia-induced regulation of α1H Ca2+ channel expression. Interestingly, the increase in α1H mRNA induced by hypoxia was completely unaffected by the removal of extracellular Ca2+. This finding contrasts with the observed Ca2+ dependence of the hypoxic induction of some genes in PC12 cells (24, 50). However, recent data demonstrate that intracellular Ca2+ operates through a HIF-1α-independent signaling pathway to activate transcription of hypoxia-inducible genes (59).

The information available on the induction of ion channel coding genes by hypoxia is very scant, and to our knowledge the participation of HIF-dependent mechanisms in the regulation of these genes has not been previously studied. Chronic hypoxia has been reported to decrease the mRNA and protein levels of the K+ channels Kv1.1, Kv1.5, Kv2.1, Kv4.3, and Kv9.3 in pulmonary arterial myocytes but not in the mesenteric artery (9, 60). Although the role of HIF in the regulation of these Kv genes was not documented, the facts that the down-regulation is constrained to specific arteries and occurs after very prolonged periods of hypoxia (60–72 h) suggest a HIF-independent mechanism. In addition, an opposite effect of hypoxia on selective increase of the Kv1.2 gene expression, has been described in PC12 cells (10). Again, the possible participation of HIF in the hypoxic regulation was not addressed in the latter report.

We focused on voltage-dependent T-type Ca2+ channels, because Ca2+ influx through these channels might have a major role in the adaptive cellular responses to prolonged hypoxia. The increase of cell excitability resulting from T-type Ca2+ channel overexpression (30) may be of importance in the control of secretion in chronically hypoxic cells. Accordingly, it has been reported that chronic hypoxia increases free intracellular Ca2+ and enhances the secretory response of PC12 cells to acute hypoxia (21). Chronic intermittent exposures to low PO2 have been shown to increase the excitability of carotid body and sympathetic tissues (61). Besides the possible role of T-type channels in the modulation of cell excitability, they have been suspected of participating in cell cycle progression and proliferation, although the evidence is still being debated (62). It is therefore possible that Ca2+ entry through T-type Ca2+ channels is implicated in the cellular proliferation that usually occurs after acute or chronic hypoxia damage and in the hypoxic environment of proliferating tumor cells. In fact, a recent report has revealed that the α1H T-type Ca2+ channels are overexpressed in human prostate cancer cells in their more aggressive and invasive stages (19). Extreme hypoxia is a hallmark of solid tumors that leads to phenotypic alterations promoting tumor growth and progression (63). A potential role of T-type Ca2+ channels in malignant cellular proliferation would offer a new molecular target that could be exploited therapeutically. Therefore, it will be of particular interest to assess the role of T-type Ca2+ channels in cell growth and tumor biology.

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REFERENCES

1. López-Barneo, J., Pardal, R., and Ortega-Sáenz, P. (2001) Annu. Rev. Physiol. 63, 259–287

2. Semenza, G. L. (1999) Annu. Rev. Cell Dev. Biol. 15, 551–578

3. Wenger, R. H. (2002) FASEB J. 16, 1151–1162

4. Ratcliffe, P. J., Gleadle, J. M., Maxwell, P. H., O’Rourke, J. F., Pugh, C. W., and Wood, S. M. (1998) in Oxygen Regulation of Ion Channels and Gene Expression (López-Barneo, J., and Weir, E. K., eds) pp. 67–85, Futura Publishing Co., Armonk, NY

5. Bunn, H. F., and Poyton, R. O. (1996) Physiol. Rev. 76, 839–885

6. Jaakola, P., Mole, D. R., Tian, Y.-M., Wilson, M. I., Giebing, J., Gaskell, S. J., Von Kriesche, A., Hobesbest, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W., and Ratcliffe, P. (2001) Science 292, 468–472

7. Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salie, A., Asara, J. M., Lane, W. S., and Tavan, W. G. (2001) Science 292, 464–468

8. Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J., and Whitelaw, M. L. (2002) Science 295, 858–861

9. Wang, J., Jushavkova, M., Rublin, L. J., and Yuan, X.-J. (1997) J. Clin. Invest. 100, 2347–2355

10. Conforti, L., and Millhorn, D. E. (1997) J. Physiol. 502, 293–305

11. Catterall, W. A. (1995) Annu. Rev. Biochem. 64, 493–531

12. Huguenard, J. R. (1996) Annu. Rev. Physiol. 58, 329–348

13. Kuga, T., Kobayashi, S., Hirakawa, Y., Kanaide, H., and Takeshita, A. (1996) Circ. Res. 79, 14–19

14. McCobb, D. P., Best, P. M., and Beam, K. G. (1989) Neuron 2, 1633–1643

15. Bijlenga, P., Liu, J.-H., Espinos, E., Haenggeli, C.-A., Fisher-Louheed, J., Bader, C. R., and Bernheim, L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7627–7632

16. Nuss, H. B., and Houseer, S. R. (1993) Circ. Res. 73, 777–782

17. Sen, L., and Smith, T. W. (1994) Circ. Res. 75, 149–155

18. Schmitt, R., Clozel, J. P., Iberg, N., and Buhler, P. R. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1161–1165

19. Mariot, P., Vanoverberghe, K., Lalevee, N., Rossier, M. F., and Prevorskaya, N. (2002) J. Biol. Chem. 277, 10824–10833

---

2 J. Navarro-Antolín, K. L. Levitsky, and J. López-Barneo, unpublished results.
23. Czyzyk-Krzeska, M. F., Furnari, B. A., Lawson, E., and Millhorn, D. E. (1994) *J. Biol. Chem.* 269, 760–764

24. Millhorn, D. E., Beitner-Johnson, D., Conforti, L., Conrad, P. W., Kobayashi, S., Yuan, Y., and Rust, E. (2000) *Adv. Exp. Med. Biol.* 475, 131–142

25. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, pp. 7.43–7.45, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

26. Hamill, O., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. (1981) *J. Physiol.* 306, 340–361

27. Castellano, A., and López-Barneo, J. (1991) *J. Gen. Physiol.* 97, 303–320

28. Liu, H., Felix, R., Gurnett, C. A., De Waard, M., Witcher, D. R., and Campbell, K. P. (1990) *J. Neurosci.* 10, 557–576

29. Usowicz, M. M., Porzig, H., Becker, C., and Reuter, H. (2001) *J. Biol. Chem.* 276, 3999–4011

30. Perez-Reyes, E., Cribbs, L. L., Daud, A., Lacerda, A. E., Barclay, J., Williamson, M. P., Fox, M., Rees, M., and Lee, J.-H. (1998) *Nature* 391, 896–900

31. Matteson, D. R., and Armstrong, C. M. (1986) *J. Gen. Physiol.* 88, 161–182

32. Swandulla, D., and Armstrong, C. M. (1988) *J. Gen. Physiol.* 91, 197–218

33. Williams, M. E., Maraho, I. M., Deal, C. R., Hans, M., Brust, P. F., Phililson, L. H., Miller, R. J., Johnson, E. C., Harpold, M. M., and Ellis, S. B. (1994) *J. Biol. Chem.* 269, 22347–22357

34. Llinás, R., and Yarom, Y. (1981) *J. Physiol.* 315, 549–567

35. Carbone, E., and Lux, H. D. (1984) *Nature* 310, 501–502

36. Lee, J. H., Moseley, J. C., Cribbs, L. L., and Perez-Reyes, E. (1999) *Biophys. J.* 77, 3034–3042

37. Goldberg, M. A., Dunning, S. P., and Bunn, H. F. (1988) *Science* 242, 1412–1415

38. Wang, G. L., and Semenza, G. L. (1993) *Blood* 82, 3610–3615

39. Minchenko, A., Leshchinsky, I., Opentanova, I., Sang, N., Sririvas, V., Armstead, V., and Caro, J. (2002) *J. Biol. Chem.* 277, 6183–6187

40. Brussaard, A. L. (1997) *J. Neurosci. Methods* 71, 55–64

41. Caniggia, I., Mustachhi, H., Winter, J., Gassmann, M., Lye, S. J., Kuliszewski, M., and Poust, M. (2000) *J. Clin. Invest.* 105, 577–587

42. Conrad, P. W., Freeman, T. L., Beitner-Johnson, D., and Millhorn, D. E. (1999) *J. Biol. Chem.* 274, 33709–33713

43. Conrad, P. W., Conforti, L., Kobayashi, S., Beitner-Johnson, D., Rust, R. T., Yuan, Y., Kim, H. W., Kim, R. H., Seta, K., and Millhorn, D. E. (2001) *Comp. Biochem. Physiol.* 128, 187–204

44. Wiesener, M. S., Turley, H., Allen, W. E., William, C., Eckardt, K. U., Talks, K. L., Wood, S. M., Gatter, K. C., Harris, A. L., Pugh, C. W., Ratcliffe, P. J., and Maxwell, P. H. (1998) *Blood* 92, 2260–2268

45. Firth, J. D., Ebert, B. L., and Ratcliffe, P. J. (1995) *J. Biol. Chem.* 270, 21021–21027

46. Semenza, G. L., Jiang, B. H., Leung, S. W., Passantino, R., Concordet, J. P., Maire, P., and Giallongo, A. (1996) *J. Biol. Chem.* 271, 32529–32537

47. Lee, P. J., Jiang, B. H., Chiu, B. Y., Iyer, N. V., Alam, J., Semenza, G. L., and Choi, A. M. K. (1997) *J. Biol. Chem.* 272, 5375–5381

48. Wiesener, M. S., Turley, H., Allen, W. E., Weir, E. K. (1998) *Oxygen Regulation of Ion Channels and Gene Expression*, Futura Publishing Co., Armonk, NY

49. Lee, J.-H., Daud, A. N., Cribbs, L. L., Lacerda, A. E., Pereverzev, A., Klockner, U., Schneider, T., and Perez-Reyes, E. (1999) *J. Neurosci.* 19, 1912–1921

50. Cribbs, L. L., Lee, J. H., Yang, Y., Daud, A., Barclay, J., Williamson, M. P., Fox, M., Rees, M., and Perez-Reyes, E. (1998) *Circ. Res.* 83, 103–109

51. Garber, S. S., Hoshi, T., and Aldrich, R. W. (1989) *J. Neurosci.* 9, 3976–3987

52. Bournazou, R., Hidalgo, J., Yu, H., Jaimovich, E., and Shimahara, T. (2001) *J. Biol. Chem.* 276, 35–44

53. Monteil, A., Chemin, J., Bourinet, E., Mennessier, G., Lory, P., and Nargeot, J. (2000) *J. Biol. Chem.* 275, 6690–6610

54. Fearn, I. M., Randall, A. D., Perez-Reyes, E., and Peers, C. (2000) *Pflügers Arch.* 441, 181–188

55. Green, K. N., Boyle, J. P., and Peers, C. J. (2002) *J. Physiol.* 541, 1013–1023

56. Saluzokow, K., Kius, T., Cotten, M., Hynning, D., Demidenko, Z. N., Xie, K., and Blagosklonny, M. V. (2002) *J. Biol. Chem.* 277, 1734–1741

57. Platschyn, O., Yu, Y., Grelka, V. A., McDaniel, S. S., Krick, S., Li, L., Wang, J.-Y., Rubin, L. J., and Yuan, J. J.-Y. (2001) *Am. J. Physiol.* 280, L801–L812

58. Prabhakar, N. R., Fields, R. D., Baker, T., and Fletcher, E. C. (2001) *Am. J. Physiol.* 281, L524–L528

59. Chemin, J., Monteil, A., Briquaire, C., Richard, S., Perez-Reyes, E., Nargeot, J., and Lory, P. (2000) *FERS Lett.* 478, 166–172

60. Harris, A. L. (2001) *Nat. Rev.* 2, 38–47
Induction of T-type Calcium Channel Gene Expression by Chronic Hypoxia
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