Ca$^{2+}$/Calmodulin Kinase-dependent Activation of Hypoxia Inducible Factor 1 Transcriptional Activity in Cells Subjected to Intermittent Hypoxia*

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Intermittent hypoxia (IH) occurs in many pathological conditions. However, very little is known about the molecular mechanisms associated with IH. Hypoxia-inducible factor 1 (HIF-1) mediates transcriptional responses to continuous hypoxia. In the present study, we investigated whether IH activates HIF-1 and, if so, which signaling pathways are involved. PC12 cells were exposed to either 2% O$_2$ (non-hypoxic control) or to 60 cycles consisting of 30 s at 1.5% O$_2$ followed by 4 min at 20% O$_2$ (IH). Western blot analysis revealed significant increases in HIF-1α protein in nuclear extracts of cells subjected to IH. Expression of a HIF-1-dependent reporter gene was increased 3-fold in cells subjected to IH. Although IH induced the activation of ERK1, ERK2, JNK, PKC-α, and PKC-γ, inhibitors of these kinases and of phosphatidylinositol 3-kinase did not block HIF-1-mediated reporter gene expression induced by IH, indicating that signaling via these kinases was not required. In contrast, addition of the intracellular Ca$^{2+}$/chelator BAPTA-AM or the Ca$^{2+}$/calmodulin-dependent (CaM) kinase inhibitor KN93 blocked reporter gene activation in response to IH. CaM kinase activity was increased 5-fold in cells subjected to IH. KN93 prevented IH-induced transactivation mediated by HIF-1α, and its coactivator p300, which was phosphorylated by CaM kinase II in vitro. Expression of the HIF-1-regulated gene encoding tyrosine hydroxylase was induced by IH and this effect was blocked by KN93. These observations suggest that IH induces HIF-1 transcriptional activity via a novel signaling pathway involving CaM kinase.

Sleep-disordered breathing with recurrent apnea is a major cause of morbidity and mortality in the United States population, affecting an estimated 18 million people (1). In this condition, transient repetitive apnea (cessation of breathing) results in periodic hypoxemia (decreased arterial PO$_2$). In severely affected patients, the frequency of apneas may exceed 60 episodes per hour and blood hemoglobin saturation of O$_2$ can be reduced to as low as 50%. Patients with chronic intermittent hypoxia (IH) caused by sleep apnea have a greatly increased risk for the development of systemic hypertension (2, 3). Rats exposed to chronic IH also develop systemic hypertension (4). However, rats in which the carotid bodies have been denervated show no increase in blood pressure in response to IH (5). Studies in humans and rodents suggest that the carotid body, which is the primary chemoreceptor for detecting changes in arterial PO$_2$, mediates reflex increases in the activity of the sympathetic nervous system that result in elevated blood pressure (6). Chronic IH induces long lasting sensory excitation of the carotid body (7), which in turn may contribute to increased sympathetic tone and systemic hypertension.

The transcriptional activator hypoxia inducible factor 1 (HIF-1) plays an essential role in O$_2$ sensing by the carotid body (8). HIF-1 is a global regulator of oxygen homeostasis that controls multiple key developmental and physiological processes (9, 10). Over 60 HIF-1 target genes have been identified, including those encoding erythropoietin (EPO) and vascular endothelial growth factor (VEGF). HIF-1 is a heterodimeric protein that is composed of a constitutively expressed HIF-1β subunit and an O$_2$-regulated HIF-1α subunit. Analysis of Hif1α$^{-/-}$ mice that were heterozygous for a knock-out allele at the locus encoding HIF-1α revealed that their carotid bodies were markedly impaired in the ability to sense and/or respond to hypoxia (8). Carotid body histology was normal, including the presence of glomus cells, which perform the O$_2$-sensing function of the carotid body, and Hif1α$^{-/-}$ carotid bodies responded normally to cyanide, indicating a specific defect in O$_2$ sensing (8).

HIF-1 activity is induced under conditions of continuous hypoxia as a result of a decreased rate of O$_2$-dependent proline hydroxylation, ubiquitination, and proteasomal degradation of the HIF-1α subunit (11–14). HIF-1α transcriptional activity is also regulated via O$_2$-dependent arginine hydroxylation that blocks coactivator recruitment (15). However, less is known about the effect of IH on HIF-1 activity. Recent studies suggest that HIF-1-regulated gene expression is activated by repeated cycles of hypoxia and reoxygenation. Thus, exposure of mice to

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1 The abbreviations used are: IH, intermittent hypoxia; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; CaM, calmodulin; CaMK, CaM kinase; HIF, hypoxia inducible factor; PMSF, phenylmethylsulfonyl fluoride; GST, glutathione S-transferase; PKR, phosphatidylinositol kinase; TH, tyrosine hydroxylase; EPO, erythropoietin; VEGF, vascular endothelial growth factor; DMOG, dimethyl-oxalylglcine; MAP, mitogen-activated protein; HRE, hypoxia response element; β-gal, β-galactosidase.
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an IH protocol (6% O2 for 6 min, followed by 21% O2 for 6 min, repeated for a total of 5 cycles) was shown to induce expression of erythropoietin (EPO), which mediated cardiac protection against ischemia-reperfusion injury (16). EPO expression immediately following IH and cardiac protection 24 h later were not induced in HiF1a−/− mice, demonstrating critical involvement of HIF-1 in these responses. Clinical studies indicate that recurrent sleep apnea is associated with increased serum levels of EPO and VEGF (17–20).

To further evaluate the effect of IH on HIF-1 activity, we have utilized PC12 rat pheochromocytoma cells. PC12 cells share many properties with glomus cells of the carotid body including O2-regulated neurotransmitter release (21) and expression of tyrosine hydroxylase, the rate-limiting enzyme for catecholamine production (22). In this study, we demonstrate that HIF-1α protein expression and HIF-1 transcriptional activity are induced in PC12 cells subjected to IH via signal transduction pathways that are distinct from those involved in the response to continuous hypoxia.

EXPERIMENTAL PROCEDURES

Cell Culture—PC12 cells (original clone from Dr. L. Green) were cultured in Dulbecco’s Modified Eagle’s medium supplemented with 10% horse serum, 5% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) under 90% air and 10% CO2 at 37 °C. Prior to all experiments, the cells were placed in antibiotic-free medium and serum-starved for 16 h to avoid any confounding effects of serum on HIF-1 activity. In the experiments involving treatment with drugs, cells were preincubated for 30 min with either drug or vehicle.

Exposure to Intermittent Hypoxia—Cells were exposed to alternating cycles consisting of 1.5% O2 for 30 s followed by 20% O2 for 4 min at 37 °C in a 12 × 12 × 7 inch Lucite chamber. The chamber was equilibrated with gases at a flow rate of 2.4 liter/min by timer-controlled solenoid valves. O2 levels were monitored by an electrode (LiquiTech, Northridge, CA) in a Lucite cylinder by using a Beckman LB2 placed in the chamber. 45 s was required for ambient O2 to reach 1.5% O2 during de-oxygenation and 50 s for return to 20% O2 during re-oxygenation.

Chemicals—All chemicals and reagents were of analytical grade and obtained from Sigma unless otherwise mentioned. Dimethylsulfoxide (DMSO) was a gift from Dr. F. Ratcliffe (University of Oxford). Plasmids—The following plasmids used in the present study have been described previously: p21 (23), pGalAl (24), pGE51bLac, pCaMKII-WT, and pCaMKII-290 (25). pRSV-LacZ was from the American Type Culture Collection.

Preparation of Nuclear Extracts—Cells were lysed in hypotonic solution (10 mM KCl, 10 mM HEPES, 1 mM PMSF, pH 7.8) in the presence of aprotinin (100 μg/ml) and a complete protease inhibitor mixture (Roche Applied Science) and centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was mixed with a 50% solution of 5% Nonidet P-40, 25% deionized formamide, 1% SDS, and 5 mM EDTA at 4 °C for 20 min to remove debris. 20 μl of cell lysate was mixed with 100 μl of buffer containing luciferin. Relative luminescent light units were recorded in a Berthold luminometer. For measurement of β-galactosidase activity, 10 μl of cell lysate was incubated with 100 μl of the reaction mixture containing β-galactamase (Tropix) as substrate for 30 min at room temperature. Following incubation, 150 μl of luminescence enhancement buffer (Bio-Rad) was added to the reaction mixture and the resulting luminescence was measured. Protein analysis was performed using a protein assay kit (Bio-Rad). We verified that all reporter gene assays were in the linear range.

Probe Preparation—DNA oligonucleotides were synthesized complementary to nucleotides 902–959 and 1435–1489 of rat tyrosine hydroxylase (TH) mRNA (30), end-labeled using T4 polynucleotide kinase in the presence of γ-32P-ATP, purified using QuickSpin Sephadex G25 columns (Roche Applied Science), and quantitated by liquid scintillation counting.

Northern Blot Hybridization—Total RNA was isolated from PC-12 cells using the RNAeasy kit (Qiagen), and aliquots (10 μg) were purified by formaldehyde-agarose-ethidium gel electrophoresis. RNA was transferred to a nitrocellulose membrane (Gene Screen Plus, Schleicher and Schuell) and UV-cross-linked (model FB UX-1100, Fisher Scientific). Following prehybridization at 42 °C for 4 h, 5 × 10^6 cpm of 32P-labeled oligonucleotide probe was added with fresh hybridization buffer (containing a final concentration of 5 SSPE, 5 × Denhardt’s solution, 25% deionized formamide, 1% SDS, and 5 μg/ml salmon sperm DNA) and incubated with rotation at 42 °C for 16 h. The membrane was washed twice with 2 × SSC solution containing 0.1% SDS and then washed twice in 1× SSC containing 0.1% SDS at 50 °C for 15 min. The membrane was then exposed to x-ray film for autoradiography. The probe was stripped off the membrane by boiling in 0.1× SSC containing 1% SDS for 30 min with shaking. The stripped membrane was re-probed with 32P-labeled rat β-actin cDNA. The relative amounts of β-actin mRNA were determined by optical density measurements and were used to normalize the expression levels of TH mRNA.

GST Fusion Protein Expression and Purification—Plasmid pGEXP300TD (generously provided by J. Caro, Jefferson Medical College, Philadelphia) was transformed into BL21-competent cells (Invitrogen), which were cultured in LB medium supplemented with 50 μg/ml of ampicillin. Expression of the fusion protein was induced by the addition of 0.1 mM isopropyl β-thiogalactoside (Promega) and puriﬁcation was essentially the same as described previously (31). Brieﬂy, cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 1% Triton X-100, 5 mM EDTA, 50 mM NaF, 0.1 mM Na3VO4, 1 mM PMSF, protease inhibitor mix). The cellular fraction was centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was mixed with a 50% saturation of ammonium sulfate and was left overnight on ice without the lysis buffer. The fusion protein was eluted with 50 mM Tris-HCl (pH 8.0) containing 5 mM reduced glutathione at 4 °C for 20 min. After centrifugation, the supernatant were collected, and the protein concentration was determined using a protein assay kit (Bio-Rad).

In Vitro Kinase Assay—CaM kinase II (CaMK II) activity was measured as described previously (32). Brieﬂy, cells (5 × 10^6) were washed in buffer containing 25 mM HEPES, pH 7.4, 150 mM NaCl, 2.5 mM MgCl2,
PC12 cells were exposed to IH consisting of alternating cycles of hypoxia (30 s at 1.5% O2) and re-oxygenation (20% O2) for 4 min. Under these conditions the expression of HIF-1α protein increased in a dose-dependent manner as the duration of IH was increased from 10 to 60 cycles (Fig. 1A). HIF-1α protein levels were unaffected by exposing cells to alternating cycles of normoxia instead of hypoxia (data not shown). To demonstrate an increase in HIF-1 transcriptional activity, PC12 cells were transfected with reporter gene p2.1, in which the expression of firefly luciferase was driven by a HIF-1-dependent hypoxia response element (HRE) upstream of an SV40 promoter (23). HRE-dependent transcriptional activity was induced by IH although, relative to the induction of HIF-1α protein expression, the response was not as dramatic and required an increased number of cycles (Fig. 1B). Transcription of a co-transfected reporter gene in which β-gal expression was driven by a Rous sarcoma virus (RSV) promoter was not induced by IH. 120 cycles of IH, which represents a total of 60 min of hypoxic exposure, induced HRE-dependent transcription, whereas 60 min of continuous hypoxia did not (Fig. 1C).

**MAP kinase, PKC, and PI 3-kinases Are Not Required for IH-induced HIF-1 Activation—**Exposure of PC12 cells to continuous hypoxia induces membrane depolarization, increased intracellular Ca2+ levels, and increased activity of phosphatidylinositol 3-kinase (PI3K), p42/44ERK1 and p44/42ERK2 (33). We therefore investigated the involvement of major intracellular signaling pathways in the induction of HRE-dependent transcription by IH. Immunoblot analysis revealed that IH induced increased phosphorylation of ERK1, ERK2, and Jun N-terminal kinase (JNK) (Fig. 2, A and B, top panel) but not p38 (data not shown). However, pretreatment of cells with increasing doses of PD98059, a selective inhibitor of MAP kinase/ERK kinase (MEK), or SP600125, an inhibitor of JNK, did not inhibit HRE-dependent transcription induced by IH (Fig. 2, A and B, bottom panel). We then examined the effects of IH (60 cycles) on the translocation of PKC isofoms to the plasma membrane as an index of activation. Of the several PKC isofoms (α, β, γ, δ, λ, ω), IH increased PKC-α and PKC-γ levels in the membrane fraction. Similar increases in PKC-α and PKC-γ were observed after treatment with TPA (100 nM), a potent activator of PKC that served as a positive control. We examined the effects of bisindolylmaleimide 1 (Bis), which inhibits activity of the PKC α, β1, β11, γ, δ, and ε isofoms, on IH-induced HRE-activation. As shown in Fig. 2C, HRE activation was unaffected by 3 μM Bis, whereas increasing the concentration to 10 μM resulted in a modest but significant inhibition of IH-induced HRE activation (Fig. 2C). Because hypoxia activates PI3K in PC12 cells (33), we also tested the effects of the inhibitors LY294002 and wortmannin. Neither LY294002 nor wortmannin blocked IH-induced transcriptional activity (data not shown).

Ca2+ Signaling Pathways Involving CaMK II Are Required for IH-induced HIF-1 Activity—**Pretreatment of cells with the intracellular Ca2+ chelator BAPTA-AM resulted in potent inhibition of HRE-dependent transcription induced by IH (Fig. 3A). These observations indicate that Ca2+ signaling pathways...
are involved in IH-induced HIF-1 transcriptional activity. CaMKs are downstream signaling molecules that participate in Ca\(^{2+}\)/H11545-mediated gene regulation. Our previous studies have shown that PC12 cells express CaMK II but not CaMK IV and that continuous hypoxia transiently increases CaMK II activity (32). We therefore examined whether CaMK II participates in IH-induced HIF-1-mediated transcription. As shown in Fig. 3B, IH-induced reporter gene expression was inhibited in a dose-dependent manner by pretreatment with the selective CaMK inhibitor KN93 (Fig. 3B) as well as with W-7, a potent inhibitor of calmodulin (Fig. 3C). The inhibitory effect of these agents was specific for IH-induced HRE-dependent transcription since in all cases as the expression was normalized to that of the co-transfected RSV-LacZ reporter. Further analysis of PC12 cell lysates revealed an exponential increase in CaMK II activity in response to increasing duration of IH (Fig. 4A) and an associated increase in the phosphorylation of CaMK II protein (Fig. 4B).

CaMK II Is Required for IH-induced HIF-1α Transactivation Function but Not for HIF-1α Protein Expression—We next examined whether CaMK II activity mediates changes in HIF-1α protein expression or transactivation function in cells exposed to IH. Remarkably, ectopic expression of constitutively active form CaMK II alone or in combination with HIF-1α had no significant effect on HIF-1α protein expression (Fig. 5A). Furthermore, KN93 did not block the induction of HIF-1α protein expression induced by IH (Fig. 5B). However, consistent with...
the inhibition of IH-induced transcription by CaMK II inhibitors (see Fig. 3B), co-transfection of CaMK II-290, encoding a constitutively active form of CaMK II, dramatically induced HRE-dependent transcription under non-hypoxic conditions, and this induction was inhibited by KN93 (Fig. 5C).

To investigate the role of CaMK II in the regulation of HIF-1-dependent transcription in a greater detail, PC12 cells were cotransfected with pG5E1bLuc, a reporter plasmid in which luciferase coding sequences are present downstream of five GAL4 binding sites, and pGAL4/HIF-1α-(531–826), which encodes the DNA-binding domain of the yeast GAL4 protein fused to the transactivation domains (TADs) of HIF-1α. Increased transactivation of pG5E1bLuc by GAL4/HIF-1α-(531–826) was induced in cells exposed to continuous hypoxia (24). Transactivation mediated by GAL4/HIF-1α-(531–826) was induced in PC12 cells exposed to IH (Fig. 6A) or co-transfected with CaMK II-290 (Fig. 6B) and, in both cases, the induction was inhibited by KN93 treatment. In contrast, KN93 had no effect on reporter gene transactivation induced by 6 h of continuous hypoxia (Fig. 6A).

**IH and CaMK II Stimulate HIF-1α Transactivation by a Mechanism That Is Independent of Asparaginyl Hydroxylation**—GAL4/HIF-1α-(531–826) contains the TAD-N (N-terminal TAD; amino acids 531–575) and the TAD-C (C-terminal TAD; amino acids 786–826), which are separated by the inhibitory domain (24). FIH-1 (factor inhibiting HIF-1) binds to the inhibitory domain (34) and mediates the O2-dependent hydroxylation of Asn-803, which prevents binding of the coactivators p300 and CBP to TAD-C (15). As a result, transactivation mediated by GAL4/HIF-1α-(531–826) is induced by continuous hypoxia, whereas GAL4/HIF-1α-(786–826), which lacks the FIH-1 binding site, is constitutively active. As shown in Fig. 6C, the activity of GAL4/HIF-1α-(786–826) was increased in response to IH. Co-transfection of pGAL4/HIF-1α-(786–826) with CaMK II-290 mimicked the effects of IH under non-hypoxic conditions, and KN-93 blocked the effect of IH or CaMKII-290 on GAL4/HIF-1α-(786–826) (Fig. 6D). However, KN-93 had no significant effect on baseline levels of transactivation mediated by either GAL4/HIF-1α-(531–826) or GAL4/HIF-1α-(786–826) under normoxia (Fig. 6, B and D). These results indicate that CaMK II stimulates TAD function via a mechanism that is independent of asparaginyl hydroxylation.

**CaMK II Signaling Promotes Transactivation Mediated by p300**—Several lines of evidence suggest that p300/CBP are the major coactivators for HIF-1 activation (31, 35–38). To investigate whether p300 is involved in IH- and CaMK II-mediated activation of HIF-1 transcriptional activity, PC12 cells were co-transfected with pGAL4p300 (encoding a GAL4 fusion protein containing amino acids 1–2414 of p300) and pG5E1bLuc, and exposed to 120 cycles of IH. As shown in Fig. 7A, IH increased p300 transcriptional activity. Co-transfection of CaMK II-290 and pGAL4p300 mimicked the effects of IH and KN-93 prevented activation of p300 by IH or CaMK II-290 (Fig. 7A). We next tested whether p300 was a direct substrate of CaMK II. We expressed and purified amino acids 1572–2370 of p300 as a GST fusion protein and performed an in vitro CaMK II assay. As can be seen from Fig. 7B, p300 was specifically phosphorylated by CaMK II in vitro.
**FIG. 6.** Induction of HIF-1α transactivation domain function by IH or constitutively active CaMK II. 

A, PC12 cells were transfected with pRSV-LacZ, pGAL4/HIF-1α-(531–826) encoding a fusion protein consisting of the GAL4 DNA binding domain fused to the HIF-1α transactivation domain, and pG5E1bLuc, which contains 5 GAL4 DNA binding sites upstream of E1b promoter and luciferase coding sequences. Transfected cells were exposed to 20% O2 (N) or 120 cycles of IH in the absence or presence of KN93. 

B, cells were co-transfected with GAL4-HIF-1α-(531–826) along with pCMV, or pCaMK II-WT or pCaMK II-290 in the absence or presence of KN93 and exposed to normoxia. 

C, cells were transfected with pGAL4-HIF-1α-(786–826) and pG5E1bLuc and exposed to normoxia (N) or 120 cycles of IH in presence and absence of KN93 or were untreated (C). 

D, cells were co-transfected with pGAL4/HIF-1α-(786–826) along with pCMV, pCaMK II-WT or pCaMK II-290 in the absence or presence of KN93 or were untreated (C). 

E, cells were transfected with pRSV-LacZ, pGAL4/HIF-1α-(531–826) and exposed to 20% O2 (N) or to continuous hypoxia (1% O2 for 6 h) in presence or absence of KN93. Data presented are percent change from control (mean ± S.D. from three independent experiments). **, p < 0.01 compared with control; N.S., no significant difference.

**FIG. 7.** Effects of IH and CaMK II on p300. 

A, IH and CaMK II induce transcriptional activation mediated by a GAL4/p300 fusion protein. PC12 cells were co-transfected with pRSV-LacZ, pGAL4/p300, and pG5E1bLuc with or without pCaMK II-290. Transfected cells were exposed to 20% O2 (N) or 120 cycles of IH in the absence or presence of KN93. Data presented are mean ± S.D. (n = 3). **, p < 0.01 compared with control (N). 

B, direct phosphorylation of p300 in vitro by CaMK II. GST/p300TD, a fusion protein consisting of glutathione S-transferase and p300, was expressed in and purified from BL21 cells and used for in vitro CaMK II kinase assay.
IH Induces Expression of the HIF-1 Target Gene TH—Expression of the TH gene encoding tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, is induced when PC12 cells are subjected to continuous hypoxia, an effect that is mediated by HIF-1 (39). Because catecholamine levels are elevated in recurrent apnea patients experiencing chronic IH (40, 41), we examined TH mRNA expression in PC12 cells subjected to IH. TH mRNA expression was increased 4.6-fold in PC12 cells subjected to IH and KN93 blocked this response (Fig. 8A). In contrast, KN93 did not attenuate the increased TH mRNA expression induced in cells subjected to continuous hypoxia for 6 h (Fig. 8A). Consistent with the inhibitory effects of KN-93 on IH-induced TH mRNA, over-expression of constitutively active form of CaMK II with HIF-1α leads to robust activation of TH mRNA. TH and β-actin mRNA were analyzed by Northern blot assay (n = 3). **, p < 0.01 compared with control (N); N.S., not significant. C, inhibitors of HIF-1α hydroxylases increase TH mRNA expression in a KN93-independent manner. PC12 cells pretreated with KN93 (10 μM) for 30 min were treated with 1 mM DMOG or 1 mM desferrioxamine (DFO) for 12 h at 20% O₂.

DISCUSSION

HIF-1 plays a major role in coordinating physiological responses to continuous hypoxia (9–13), and recent data suggest involvement of HIF-1 in responses to IH (16–20). In this study, we have directly demonstrated for the first time the induction of HIF-1 activity in response to IH. The results presented above indicate that IH induces HIF-1α protein expression and transcriptional activity, respectively (10–15, 42). The hydroxylases contain Fe(II) in their catalytic sites and utilize O₂ and 2-oxoglutarate (α-ketoglutarate) as reaction substrates. DMOG, a competitive inhibitor of 2-oxoglutarate, and desferrioxamine (DFO), a Fe(II) chelator, induce HIF-1 activity by inhibiting the hydroxylases. Exposure of PC12 cells to DMOG or DFO enhanced the expression of TH mRNA similar to that seen with continuous hypoxia, but KN93 failed to block the response (Fig. 8C). Thus, IH and continuous hypoxia activate TH mRNA expression by distinct mechanisms, similar to their effects on HIF-1 activity.

Fig. 8. TH mRNA expression is induced by IH and continuous hypoxia via different mechanisms. A, KN93 inhibits TH mRNA expression induced by IH but not by continuous hypoxia. PC12 cells were pretreated with KN93 (10 μM) for 30 min and then exposed to IH (120 cycles) or 6 h of continuous hypoxia (CH). B, overexpression of constitutively active form of CaMK II with HIF-1α leads to robust activation of TH mRNA. TH and β-actin mRNA were analyzed by Northern blot assay (n = 3). **, p < 0.01 compared with control (N); N.S., not significant. C, inhibitors of HIF-1α hydroxylases increase TH mRNA expression in a KN93-independent manner. PC12 cells pretreated with KN93 (10 μM) for 30 min were treated with 1 mM DMOG or 1 mM desferrioxamine (DFO) for 12 h at 20% O₂.
The induction of transactivation function is caused by increased CaMK II activity in cells subjected to IH. Our data suggest that the effects of CaMK II are mediated by phosphorylation of the co-activator p300. CaMK IV has previously been shown to phosphorylate CBP (43), which is structurally and functionally similar to p300. In several cell types, ERK signaling has been shown to induce phosphorylation of p300, which in turn increases its interaction with the transactivation domain of HIF-1α, thus stimulating HIF-1 transcriptional activity (31, 34–46). Although IH increased phosphorylated ERK1 and ERK2, ERK activity appears to be dispensable for IH-induced HIF-1 transcriptional activity in PC12 cells. PI3K, JNK and PKC also do not appear to play major roles in mediating HIF-1 activation in response to IH. Taken together, our data suggest that IH induces CaMK II activity, phosphorylation of p300, and increased HIF-1α-mediated transactivation.

In contrast to IH, cells exposed to continuous hypoxia, inhibition of CaMK II activity had no effect on the increased HIF-1 transcriptional activity, which occurs as a result of decreased O2-dependent hydroxylation of Asn803 in TAD-C (15). These observations are consistent with an earlier report that IH induces CaMK II activity, phosphorylation of p300, and PKC also do not appear to play major roles in mediating HIF-1 activation in response to continuous hypoxia. In contrast to IH, in cells exposed to continuous hypoxia, inhibition of CaMK II activity had no effect on the increased HIF-1 transcriptional activity, which occurs as a result of decreased O2-dependent hydroxylation of Asn803 in TAD-C (15). These observations are consistent with an earlier report that IH induces CaMK II activity, phosphorylation of p300, and PKC also do not appear to play major roles in mediating HIF-1 activation in response to continuous hypoxia. In contrast to IH, in cells exposed to continuous hypoxia, inhibition of CaMK II activity had no effect on the increased HIF-1 transcriptional activity, which occurs as a result of decreased O2-dependent hydroxylation of Asn803 in TAD-C (15). These observations are consistent with an earlier report that IH induces CaMK II activity, phosphorylation of p300, and PKC also do not appear to play major roles in mediating HIF-1 activation in response to continuous hypoxia.
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