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In Vivo Expression of mRNAs Encoding Hypoxia-Inducible Factor 1

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Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric basic helix-loop-helix transcription factor that regulates genes whose products play key roles in maintaining O_2 homeostasis. We have previously demonstrated that HIF-1 mRNA, protein, and DNA-binding activity are induced when mammalian tissue culture cells are subjected to hypoxia. In this paper, we report our analysis of HIF-1 mRNA expression *in vivo.* We demonstrate expression of HIF-1α and HIF-1β (ARNT) mRNA in all human, rat, and mouse organs assayed and show for the first time that HIF-1 mRNA expression was induced in brain, kidney, and lung when rats or mice were exposed to reduced ambient O_2 concentrations for 30 to 60 min. The ubiquitous *in vivo* expression of HIF-1α and HIF-1β (ARNT) mRNA is consistent with the proposed role of HIF-1 in coordinating adaptive transcriptional responses to hypoxia.

Many different homeostatic mechanisms allow adaptation to changes in tissue oxygenation in humans and other mammals. Studies performed primarily in cultured cells indicate that the basic helix-loop-helix (bHLH) protein hypoxia-inducible factor 1 (HIF-1) activates transcription of genes whose products participate in these homeostatic responses (reviewed in ref. 1). HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β (ARNT) subunits (2). Whereas the HIF-1α subunit is unique to HIF-1, HIF-1β (ARNT) was also isolated as a component of the mammalian aryl hydrocarbon receptor complex (3) and can heterodimerize with other bHLH proteins (4). The human *ARNT* and *HIF1A* genes have been mapped to chromosome 1q21 and 14q21-24, respectively (5, 6).

When human Hep3B hepatoblastoma cells were exposed to 1% O_2, both HIF-1α and HIF-1β (ARNT) mRNA and protein expression were markedly induced (2). Subsequent studies indicated that in hypoxic tissue culture cells HIF-1 activates transcription of genes encoding erythropoietin and vascular endothelial growth factor which are essential for erythropoiesis and angiogenesis, respectively (7, 8). HIF-1 has also been implicated in the coordinate transcriptional activation of genes encoding glycolytic enzymes in hypoxic cells, which provide an alternative means of energy product under conditions of limited oxygen availability (9, 10). These data suggest that HIF-1 plays a general role in coordinating adaptive physiologic responses to hypoxia at the level of transcription. As a first step toward determining the *in vivo* role of HIF-1 as a mediator of homeostatic responses to hypoxia in mammals, we have now analyzed the expression of HIF-1α and HIF-1β (ARNT) mRNA in various organs of humans, rats, and mice under normoxic and hypoxic conditions.

MATERIALS AND METHODS

Animal studies. All experiments were performed according to protocols approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine. Adult male Sprague-Dawley rats (225-275 g) (Harlan Industries, Cumberland IN) or C57BL/6J mice (20-30 g) (Jackson Laboratory, Bar Harbor ME) were placed in a

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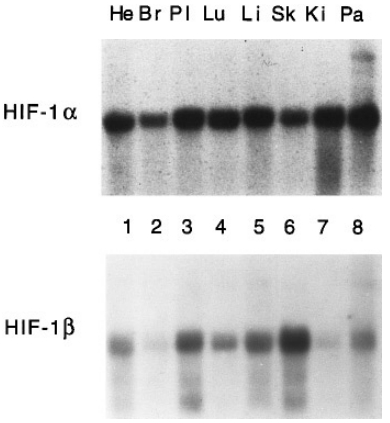

FIG. 1. Expression of HIF-1 mRNAs in human organs. A Northern blot containing 2 μg aliquots of poly A(+) RNA isolated from human heart (He), brain (Br), placenta (Pl), lung (Lu), liver (Li), skeletal muscle (Sk), kidney (Ki), and pancreas (Pa) was hybridized to a human HIF-1α cDNA probe (*top panel*). The blot was then stripped of radioactivity and hybridized to a human HIF-1β (ARNT) cDNA probe (*bottom panel*).

custom-made 3.7-cubic-foot plexiglass normobaric hypoxia chamber for 0.5 to 2 h. O_2 and CO_2 tensions were monitored continuously (Beckman OM-15 and LB-1) and maintained at 6.4-7.4% O_2 and $<$0.25 Torr CO_2, respectively, by infusing N_2 or air. Animals were supplied with food and water ad libitum. At each time point, animals were removed from the chamber and sacrificed immediately by decapitation. Organs were rapidly harvested and frozen in liquid N_2 for RNA extraction. Control littermates breathing room air (21% O_2) were sacrificed in the same manner. Total cellular RNA was isolated from rat and mouse organs by acid guanidium thiocyanate-phenol-chloroform extraction (11) using a commercial kit (Stratagene).

Blot hybridization. For analysis of HIF-1 expression in humans, a Northern blot containing 2 μg of poly A(+) RNA isolated from human organs was obtained from Clontech. For analysis of HIF-1 expression in rodents, 15 μg aliquots of total RNA were fractionated by 2.2 M formaldehyde/ 1.4% agarose gel electrophoresis and transferred to nitrocellulose or nylon membranes. Gels were stained with ethidium bromide and RNA was visualized and photographed under UV illumination both before and after transfer to assess gel loading and membrane transfer. For use as probes, the 3.4-kb human HIF-1α cDNA insert from pBluescript/HIF-1α3.2-3 or the 0.85-kb human HIF-1β (ARNT) cDNA insert from pGEX-2T/ARNT-C (2) were labelled using a random primer synthesis kit (BRL GIBCO) and α-^{32}P-dCTP (Dupont NEN). Blots were hybridized in Quik-Hyb (Stratagene) at 67°C, washed in 0.1 $\times$ SSC/0.1% SDS at 50°C, and analyzed by autoradiography. An oligonucleotide complementary to 18S rRNA, 5′-ACGGTATCT-GATCGTCTTCGAACC-3′, was 5′-end-labelled with T4 DNA kinase (BRL GIBCO) and γ-^{32}P-dATP (Dupont NEN) and hybridizations were performed at 65°C and washed at 45°C as described above.

RESULTS AND DISCUSSION

A Northern blot containing poly A(+) RNA isolated from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas was hybridized with a human HIF-1α cDNA probe (2). HIF-1α mRNA was strongly expressed in all organs (Fig. 1, *top panel*). The blot was then stripped of radioactivity and hybridized to a HIF-1β (ARNT) cDNA probe. HIF-1β (ARNT) mRNA was also present in all organs (Fig. 1, *bottom panel*), but the relative level of expression varied between organs to a greater extent than was observed for HIF-1α. Taken together, these results suggest that HIF-1α and HIF-1β (ARNT) mRNA are expressed in most, if not all, organs of the human body.

In order to determine whether HIF-1 mRNA expression is induced by hypoxia *in vivo*, rats were placed in a chamber that was continuously flushed with room air (21% O_2) or a hypoxic gas mixture (7% O_2). Total cellular RNA was isolated from lung, heart, liver, spleen, kidney, and brain and 15 μg aliquots were analyzed by sequential blot hybridization using the human HIF-1α and HIF-1β (ARNT) cDNA probes. HIF-1 mRNAs were detected in all organs of a

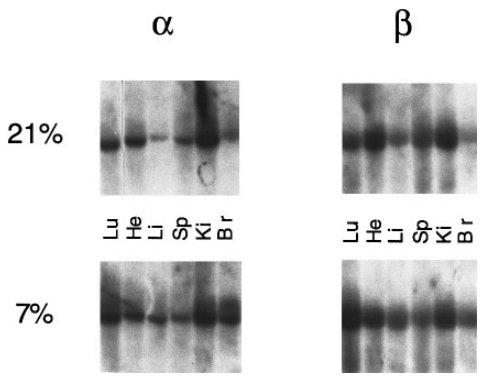

FIG. 2. Expression of HIF-1 mRNAs in rat organs. Rats were exposed to 21% O_2 (*top panels*) or 7% O_2 (*bottom panels*) for 60 min prior to sacrifice. Northern blots containing 15 μg of total RNA from lung (Lu), heart (He), liver (Li), spleen (Sp), kidney (Ki), and brain (Br) were hybridized to human HIF-1α and HIF-1β (ARNT) cDNA probes.

rat exposed to 21% O_2 (Fig. 2, *top panels*). In response to breathing 7% O_2 for 60 min prior to sacrifice, HIF-1 mRNA levels were modestly but reproducibly increased in brain, lung, and kidney (Fig. 2, *bottom panels*). Ethidium bromide staining of the agarose gels prior to transfer indicated that these results were not due to differences in RNA loading (data not shown).

To pursue these observations further, total cellular RNA was isolated from brain, lung, and kidney of mice exposed to 21% O_2 (non-hypoxic) or 7% O_2 (hypoxia) for 30 min prior to sacrifice. In these mice, blot hybridization experiments (Fig. 3) revealed that the expression of murine HIF-1α mRNA under non-hypoxic conditions was considerably lower than in the rats previously analyzed. Whether the difference in expression in the rats and mice studied was due to species or strain variation was not determined. However, the lower basal expression allowed a clearer demonstration of hypoxia-induced HIF-1α mRNA expression in brain, lung, and kidney of genetically-identical mouse littermates.

These results represent the first demonstration of regulated HIF-1 mRNA expression *in vivo.* Several important conclusions can be drawn from these data. First, *HIF1A* and *ARNT* gene expression appear to be ubiquitous as mRNA was detected in every human, rat, and mouse organ analyzed including brain, heart, kidney, liver, lung, pancreas, placenta, skeletal muscle,

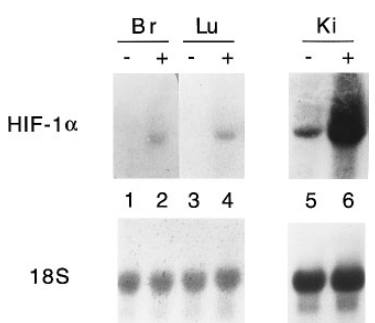

FIG. 3. Expression of HIF-1 mRNAs in mouse organs. Mice were exposed to 21% O_2 (non-hypoxic; lanes 1, 3, and 5) or 7% O_2 (hypoxic; lanes 2, 4, and 6) for 30 min prior to sacrifice. Northern blots containing 15 μg aliquots of total RNA were hybridized to the human HIF-1α cDNA probe (*top panels*). The blots were then stripped of radioactivity and hybridized to an oligonucleotide probe complementary to 18S rRNA (*bottom panels*). The kidney samples were analyzed in a hybridization experiment separate from the brain and lung samples and the relative intensity of hybridization therefore cannot be compared.

and spleen. In addition, a BLAST search (12) of the expressed sequence tag database (dbEST) (13) identified HIF-1α sequences in cDNA libraries prepared from bone, fetal and adult brain, white blood cells, pancreatic islet, placenta, retina, and uterus. The ubiquitous expression of HIF-1α and HIF-1β (ARNT) mRNA is consistent with the proposed role of HIF-1 in coordinating many of the body's responses to hypoxia.

Second, expression of HIF-1 mRNA was induced by hypoxia in several organs. Although induction of HIF-1α mRNA was demonstrated in mouse brain, lung, and kidney, no induction could be detected in liver and spleen from the same animals (data not shown). However, these data do not rule out the possibility that HIF-1 mRNA was induced in a small population of cells within these organs. Further studies will be necessary to determine to what extent HIF-1α mRNA expression shows cell type specificity or regional variation within an organ (for example, based upon proximity to an arterial blood supply).

Third, HIF-1α mRNA expression was induced in mouse organs within 30 min and was maximal at 60 min (data not shown). It is not known whether this induction reflects transcriptional and/or posttranscriptional regulation. While this manuscript was under review, exposure of C57BL/6 mice to 0.1% carbon monoxide for 4 h as a hypoxic stimulus was reported to have no effect on HIF-1α mRNA levels in kidney, liver, lung, muscle, and testis (14). The lack of induced expression of HIF-1α mRNA in kidney and lung may have been due to the nature of the hypoxic stimulus or the length of hypoxic exposure. In our studies, HIF-1α mRNA levels were increased at 30 and 60 min but returned to baseline after 4 h of continuous exposure to 7% O_2, indicating that the hypoxic induction of HIF-1α mRNA expression is transient. Although a modest increase in steady-state mRNA levels was documented by the data presented in our report, further studies will be necessary to determine whether HIF-1 mRNA levels correlate with protein levels or whether regulation of protein synthesis and/or stability also plays an important role in determining HIF-1 activity *in vivo.*

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REFERENCES

1. Wang, G. L., and Semenza, G. L. (1996) *Curr. Opin. Hematol.* **3,** 156–162.
2. Wang, G. L., Jiang, B.-H., Rue, E. A., and Semenza, G. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5510–5514.
3. Hoffman, E. C., Reyes, H., Chu, F.-F., Sander, F., Conley, L. H., Brooks, B. A., and Hankinson, O. (1991) *Science* **252,** 954–958.
4. Swanson, H. I., Chan, W. K., and Bradfield, C. A. (1995) *J. Biol. Chem.* **270,** 26292–26302.
5. Johnson, B., Brooks, B. A., Heinzmann, C., Diep, A., Mohandas, T., Sparkes, R. S., Reyes, H., Hoffman, E., Lange, E., Gatti, R. A., Xia, Y.-R., Lusis, A. J., and Hankinson, O. (1993) *Genomics* **17,** 592–598.
6. Semenza, G. L., Rue, E. A., Iyer, N. V., Pang, M. G., and Kearns, W. G. (1996) *Genomics* **34,** in press.
7. Jiang, B.-H., Rue, E., Wang, G. L., Roe, R., and Semenza, G. L. (1996) *J. Biol. Chem.* **271,** in press.
8. Forsythe, J. A., Jiang, B.-H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., and Semenza, G. L. (1996) *Mol. Cell. Biol.* **16,** in press.
9. Firth, J. D., Ebert, B. L., and Ratcliffe, P. J. (1995) *J. Biol. Chem.* **270,** 21021–21027.
10. Semenza, G. L., Roth, P. H., Fang, H.-M., and Wang, G. L. (1994) *J. Biol. Chem.* **269,** 23757–23763.
11. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
12. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
13. Boguski, M. S., Lowe, T. M. J., and Tolstoshev, C. M. (1993) *Nat. Genet.* **4,** 332–333.
14. Wenger, R. H., Rolfs, A., Marti, H. H., Guenet, J.-L., and Gassmann, M. (1996) *Biochem. Biophys. Res. Commun.* **223,** 54–59.