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J Appl Physiol 89:680-686, 2000.

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XIAOHUI REN,[1] KEITH L. DORRINGTON,[1] PATRICK H. MAXWELL,[2] AND PETER A. ROBBINS[1]

[1]*University Laboratory of Physiology, University of Oxford, Oxford OX1 3PT; and* [2]*The Wellcome Trust Centre for Human Genetics, Oxford OX3 7BN, United Kingdom*

Received 7 January 2000; accepted in final form 7 April 2000

Ren, Xiaohui, Keith L. Dorrington, Patrick H. Maxwell, and Peter A. Robbins. Effects of desferrioxamine on serum erythropoietin and ventilatory sensitivity to hypoxia in humans. *J Appl Physiol* 89: 680–686, 2000.—In cell culture, hypoxia stabilizes a transcriptional complex called hypoxia-inducible factor-1 (HIF-1) that increases erythropoietin (Epo) formation. One hallmark of HIF-1 responses is that they can be induced by iron chelation. The first aim of this study was to examine whether an infusion of desferrioxamine (DFO) increased serum Epo in humans. If so, this might provide a paradigm for identifying other HIF-1 responses in humans. Consequently a second aim was to determine whether an infusion of DFO would mimic prolonged hypoxia and increase the acute hypoxic ventilatory response (AHVR). Sixteen volunteers undertook two protocols: *1*) continuous infusion of DFO over 8 h and *2*) control. Epo and AHVR were measured at fixed times during and after the protocols. The results show that *1*) compared with control, Epo increased in most subjects at 8 h [52.8 ± 57.7 vs. 6.9 ± 2.5 (SD) mIU/ml, for DFO = 4 g/70 kg body wt, $P < 0.05$] and 12 h (63.7 ± 76.3 vs. 7.3 ± 2.5 mIU/ml, $P < 0.001$) after the start of DFO administration and *2*) DFO had no significant effect on AHVR. We conclude that, whereas infusions of DFO mimic hypoxia by increasing Epo, they do not mimic prolonged hypoxia by augmenting AHVR.

ventilation; acute hypoxic ventilatory response

EXPOSURE TO PROLONGED HYPOXIA leads to some remarkable physiological adaptations in humans. Included among these are enhanced erythropoiesis and ventilatory acclimatization to hypoxia (VAH). The enhanced erythropoiesis is characterized by increases in red blood cell count, packed cell volume, and hemoglobin concentration ([Hb]). VAH is characterized by a progressive increase in ventilation with an accompanying hypocapnia. Both of these adaptations may enhance tissue oxygenation by increasing arterial O_2 content (44).

Erythropoiesis is mainly controlled by the production of the hormone erythropoietin (Epo), which is produced principally in the kidneys and, to a lesser extent, in the liver (23). Production of Epo differs with variations in Epo mRNA concentration (33), which is increased in the presence of hypoxia and some metal ions (16). The response is mediated largely at the level of transcription and involves activation of a heterodimeric complex termed hypoxia inducible factor-1 (HIF-1). Although regulated nuclear localization and coactivator recruitment contribute to HIF-1 activation, the dominant mode of regulation appears to be O_2-regulated proteolysis of HIF-1α subunits. Desferrioxamine (DFO), an iron chelator, has been shown to activate HIF-1 in vitro, with kinetics similar to those associated with hypoxia, and to increase expression of HIF-1 target genes, including Epo (43). Like hypoxia, DFO stabilizes HIF-1α subunits, and it has been suggested that the O_2-sensing mechanism might involve O_2-dependent radical production by a local Fenton reaction. Although HIF-1 dependence in tissue culture can now be demonstrated more directly by studying mutant cells that lack HIF-1 components, the similar effects of DFO and hypoxia have provided a useful means of identifying HIF-1-dependent responses.

In vivo, administration of a single dose of DFO to mice has been reported to increase Epo mRNA levels in the kidney (43). In humans, DFO coupled to hydroxyethyl starch (which greatly prolongs plasma half-life) increased serum Epo 4 days after administration (24). However, this preparation is not freely available, and the kinetics appear different from the effects in cultured cells. Therefore, the first aim of this study was to establish whether we could reliably induce Epo in human subjects with an infusion of DFO. If so, infusion of DFO might provide a paradigm for identifying potential HIF-1 responses of a more integrative nature.

VAH is an integrative response of humans that is potentially HIF-1 dependent. As part of VAH, there is an increase in the acute hypoxic ventilatory response (AHVR) (35, 45). This increase is induced by hypoxia per se and not by the hypocapnic alkalosis that normally accompanies exposure to an hypoxic environment (20, 38). The increase in AHVR seems at least partly related to an augmentation of carotid body chemosensory function (2, 29, 40). The second aim of this study was to examine whether an infusion of DFO that

Address for reprint requests and other correspondence: P. A. Robbins, Univ. Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK (E-mail: peter.robbins@physiol.ox.ac.uk).

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provoked an Epo response could also activate an increase in AHVR.

METHODS

Subjects

Sixteen healthy subjects (10 men, 6 women) took part in the study. Average age was 25.6 ± 8.0 (SD) yr with a range of 18–55 yr. Average height was 175.9 ± 11.8 cm, and weight was 71.6 ± 11.8 kg. All were healthy, and none had a history of respiratory or hematologic disease. The experimental procedure was explained to them, but they were naive to the exact purpose of the experiments. All subjects gave informed, written consent before their participation in the study. The study was approved by the Central Oxford Research Ethics Committee.

Each subject visited the laboratory once or twice before undertaking the main experimental protocols. During these visits the subjects were familiarized with the apparatus. Subjects were requested to refrain from alcohol and caffeine-containing drinks on each experimental day. Female subjects only participated in the experiments during the first 14 days of their menstrual cycles.

Protocols

Two main protocols were used with each subject: *1*) continuous intravenous infusion of DFO for 8 h (*protocol DFO*) and *2*) a control protocol without intravenous infusion (*protocol C*). These main protocols were separated from each other by at least 1 wk, and the order was varied between subjects.

Three different doses of DFO were used. The first three subjects that were studied (*group 1*) received 1 g/70 kg body wt of DFO. The second group of three subjects (*group 2*) received 2 g/70 kg body wt of DFO, and the remaining 10 subjects (*group 3*) received 4 g/70 kg body wt of DFO.

Blood was taken for determination of serum Epo at 0, 4, 8, 12, and 24 h after the start of the infusion of DFO. (In *group 1*, samples were taken at 0, 8, and 24 h after the start of the infusion.) Additional blood for determination of iron status was drawn at 0 and 24 h in 7 of the 10 subjects in *group 3*. AHVR was determined at 0, 8, and 24 h after the start of the infusion of DFO. Blood samples were drawn, and AHVR determined at matched time points in *protocol C*.

Procedures

Intravenous infusion of DFO. For administration of DFO, an intravenous catheter was introduced into a vein in the back of the hand or in the forearm. The appropriate dose of DFO (Desferal, CIBA-GEIGY PLC, Cheshire, UK) was dissolved in water (0.1 g/ml) and then diluted with saline to a total volume of 50 ml. An intravenous infusion pump was used to administer this volume at a constant infusion speed over 8 h.

Venous blood samples. Venous blood samples (2 ml) were taken at the appropriate time points in both *protocol DFO* and *protocol C* to determine serum Epo. The samples were left to clot on ice, and the serum was separated and frozen at −70°C until analysis. For samples from which iron status was to be determined, an additional 8 ml of blood were taken. The total blood loss associated with phlebotomy was 10 ml in the subjects in whom only Epo was analyzed (6 ml in *group 1*) and 26 ml in the subjects in whom iron status was determined as well.

Serum Epo levels were determined by enzyme-linked immunoabsorbent assay using a Quantikine human Epo immunoassay kit (R&D Systems). The manufacturer reports a sensitivity for this kit of 0.6 mIU/ml, with intra- and interassay coefficients of variation of 2.8–5.2 and 4.2–8.3%, respectively. The assay was calibrated with standard concentrations of Epo in the range of 2.5–200 mIU/ml.

Iron status was determined from measurements of serum iron, total iron binding capacity (TIBC), ferritin, and soluble transferrin receptor (sTfR). The first three were determined in a clinical laboratory using standard procedures. sTfR was determined using the Quantikine human sTfR immunoassay kit (R&D Systems).

Determination of AHVR. AHVR was determined by measuring ventilatory responses to a set of variations in end-tidal P_{O_2} ($P_{ET_{O_2}}$) that were undertaken while end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) was held constant. $P_{ET_{O_2}}$ was held at 100 Torr for the first 5 min, and this was then followed by six square waves in $P_{ET_{O_2}}$, with $P_{ET_{O_2}}$ varying between 1 min at 50 Torr and 1 min at 100 Torr. $P_{ET_{CO_2}}$ was held at 1–2 Torr above the subject's control value during the measurements. Figure 1 illustrates the protocol employed, with an actual set of measurements of $P_{ET_{O_2}}$, $P_{ET_{CO_2}}$, and ventilation ($\dot{V}_E$) in one subject (*subject 1069*).

Determinations of AHVR were undertaken with the subject seated in an upright position and breathing through a mouthpiece with his or her nose occluded with a clip. Respiratory volumes were measured by a turbine volume-measuring device (22) fixed in series with the mouthpiece. A pulse

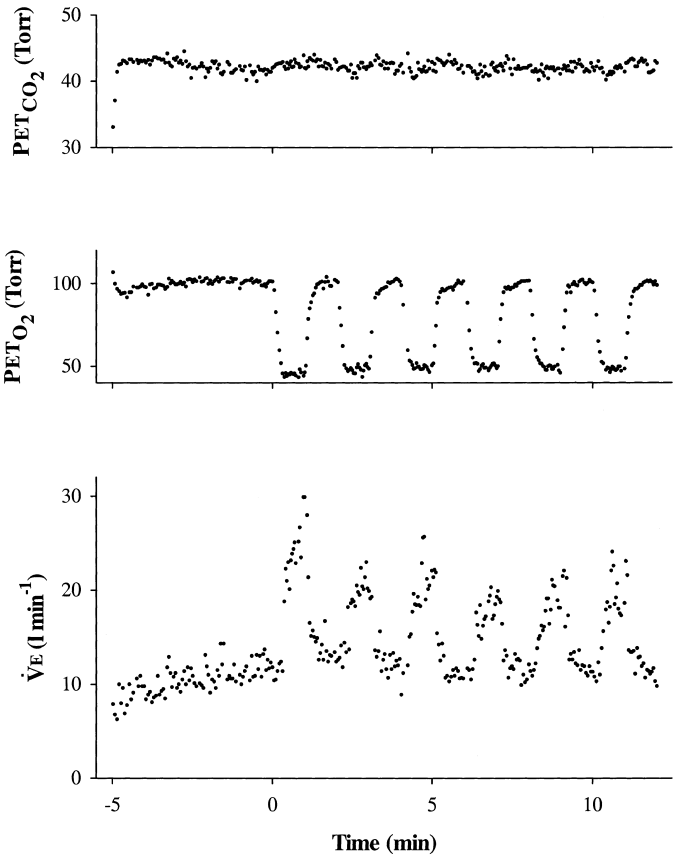

Fig. 1. Example of assessment of acute hypoxic ventilatory response. End-tidal P_{CO_2} ($P_{ET_{CO_2}}$) and P_{O_2} ($P_{ET_{O_2}}$) and ventilation ($\dot{V}_E$) were recorded breath by breath. Data are from *subject 1069* at the start of the control protocol (*protocol C*).

oximeter was attached to the forefinger to monitor the O_2 saturation of the blood. The end-tidal gas profiles were generated using a dynamic end-tidal forcing system. Before the measurements started, a "forcing function" consisting of the predicted inspired gas compositions required to produce the desired levels of end-tidal gases in the subject was calculated. This was entered into a computer that controlled a fast gas-mixing system (21), which was used to generate the inspiratory gas mixture. During the course of the experiment, actual values of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ were passed to this computer from a data acquisition computer. Deviations of these actual values from the desired values were used to modify the inspired gas mixtures using an integral-proportional feedback control scheme. The control scheme has been described in detail previously (34).

Data Analysis

Quantification of AHVR. To obtain numerical values for AHVR, a single-compartment model was used to fit the data from the six square waves in hypoxia, as described by Clement and Robbins (*model 3*) (6). In this model, total ventilation is divided into hypoxia-independent (central; $\dot{V}_c$) and -dependent (peripheral; $\dot{V}_p$) components. $\dot{V}_c$ was assumed constant in the assessments of AHVR as isocapnia was maintained. As the hypoxic stimulus changed over time, $\dot{V}_p$ was represented by a dynamic first-order model

$$\tau \frac{d\dot{V}_p}{dt} + \dot{V}_p = G_p[100 - S(t - T_d)]$$

where t is time, G_p is hypoxic sensitivity, τ is the time constant for the peripheral chemoreflex, T_d is the time delay for the peripheral chemoreflex, and S is the percent saturation of arterial blood, calculated from $P_{ET_{O_2}}$ as described by Severinghaus (37).

To fit the model to the data, a difference equation was first obtained from the model to describe the model output for the current breath in terms of the model output for the previous breath, the input function, and the parameters of the model. These calculations have been described in detail elsewhere (6). The parameters of the model (G_p, $\dot{V}_c$, τ, and T_d) could then be estimated by nonlinear regression. This was undertaken by using the Numerical Algorithms Group (Oxford, UK) FORTRAN library routine E04FDF to minimize the sum of squares of the residuals.

Statistical analysis. ANOVA was used to test for possible differences in the parameters between the two exposures, in which protocol and time of measurement were treated as fixed factors and subjects were treated as a random factor. The analysis was performed using the SPSS software package.

RESULTS

Subjects

All of the subjects completed the experiments. None of them reported discomfort from the DFO infusion or from the measurements of AHVR.

Serum Epo concentrations

Measured values for serum Epo concentration for all subjects at the various time points for *protocols DFO* and *C* are shown in Table 1. The sample at $t = 24$ h from *subject 981* in *protocol C* was missing because the subject was unable to attend on that day. Average values at each time point for each group of subjects are illustrated in Fig. 2. The data in Table 1 suggest that most of the subjects had a response to DFO infusion. Serum Epo appears to have increased by the end of the DFO infusion ($t = 8$ h) and this rise appears to persist at 4 h after the cessation of the infusion ($t = 12$ h). On the second morning ($t = 24$ h), serum Epo concentrations of most subjects appear to have returned to levels close to preexposure values. Only in two subjects (*subjects 1118* and *1125*), who appear to have had the most vigorous response to DFO infusion (serum Epo >200 mIU/ml), do the Epo concentrations appear to have remained higher than the preexposure values. ANOVA revealed that the differences between *protocol DFO* and *protocol C* were significant ($P < 0.005$). Subsequent analysis revealed that serum Epo was significantly higher at $t = 8$ and 12 h in *protocol DFO* than at

Table 1. *Serum Epo levels in all subjects for both protocol DFO and protocol C*

DFO Infusion, g/70 kg body wt	Subjects		*Protocol DFO*					*Protocol C*				
	No.	Gender	$t = 0$	$t = 4$	$t = 8$	$t = 12$	$t = 24$	$t = 0$	$t = 4$	$t = 8$	$t = 12$	$t = 24$
1	981	M	7.0		57.2		11.4	7.9		14.0		
	1023	M	3.9		4.5		4.2	4.0		2.9		6.7
	1091	M	<2.5		3.5		<2.5	<2.5		<2.5		<2.5
2	1004	M	4.9	4.1	5.8	4.1	2.6	5.3	2.6	3.3	3.4	3.7
	1056	M	12.3	14.1	23.3	24.1	10.6	17.5	16.1	16.0	15.4	17.4
	1099	F	<2.5	<2.5	31.9	37.2	4.3	2.9	3.4	3.5	<2.5	<2.5
4	1069	F	6.7	8.2	41.5	42.2	6.5	9.8	7.0	8.7	9.9	7.7
	1109	M	4.2	5.2	20.2	10.5	<2.5	3.0	3.8	5.7	6.1	4.9
	1110	F	5.8	12.6	80.5	92.7	8.7	8.5	5.0	4.8	5.0	8.9
	1115*	F	9.7	9.3	21.1	15.7	5.4	4.2	3.4	2.8	4.0	3.3
	1116*	M	8.4	3.3	12.6	7.6	4.2	6.7	7.2	9.4	5.7	7.3
	1117*	M	8.3	6.6	42.2	41.7	10.0	7.1	5.3	5.0	5.5	9.4
	1118*	F	10.1	10.2	80.2	>200.0	45.4	11.5	9.5	11.6	11.7	10.9
	1119*	M	5.6	6.1	15.4	10.9	5.8	6.8	5.1	6.5	6.8	5.9
	1124*	M	3.1	4.8	14.7	15.2	5.9	5.5	3.5	7.0	9.4	5.0
	1125*	F	4.9	16.1	>200.0	>200.0	26.3	5.5	8.0	8.0	8.5	9.1

Serum erythropoietin (Epo) levels (mIU/ml) in control (C) and desferrioxamine (DFO) protocols at 0, 4, 8, 12, and 24 h after start of DFO infusion of 1, 2, or 4 g/70 g body wt. *t*, Time; M, male; F, female. *Subjects whose iron status was evaluated.

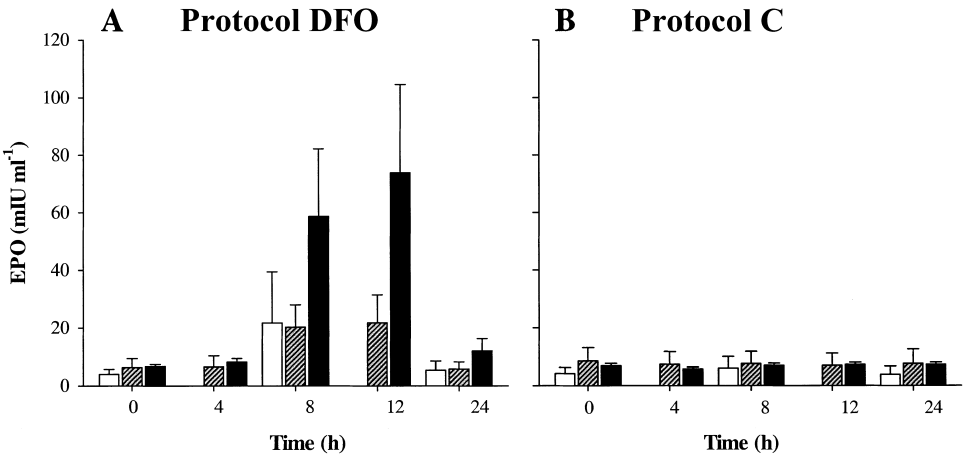

Fig. 2. Mean values for serum erythropoietin (Epo) at different time points for desferrioxamine (DFO) infusion protocol (*protocol DFO; A*) and *protocol C (B)*. Open bars, *group 1*; hatched bars, *group 2*; solid bars, *group 3*. Error bars, SE.

other time points ($P < 0.05$ and $P < 0.001$, respectively). In *protocol C*, no significant change was detected at any time point.

Iron status

Blood samples taken at $t = 0$ and $t = 24$ h from seven subjects in *group 3* were analyzed for serum iron, TIBC, ferritin, and sTfR, as described in METHODS. The percent saturation of the serum transferrin (serum iron/TIBC) was also calculated. ANOVA revealed no significant differences between the protocols or between the values at $t = 0$ and 24 h. Thus the four measurements for each variable in each subject were averaged (Table 2). Inspection of these data shows that *subjects 1118* and *1125*, who had the most marked increase in Epo, also had the lowest ferritin levels. Conversely, *subject 1116*, with the highest ferritin level, showed the least increase in Epo. However, statistical analysis failed to detect any significant correlation of these variables with the changes observed in Epo.

AHVR

Measured G_p values for all subjects in both protocols at each time point are listed in Table 3. ANOVA revealed no significant differences in G_p between protocols or, indeed, in any of the other parameters of the model. The mean overall value for AHVR was 0.87 ± 0.50 (SD) $l \cdot min^{-1} \cdot \%^{-1}$ (between subjects).

Table 2. *Iron status of 7 subjects in group 3*

Subject	Serum Iron, μmol/l	TIBC, μmol/l	Saturation of Transferrin, %	Ferritin, μg/l	sTfR, nmol/l
1115	10.1	66.0	15.6	26.8	19.5
1116	20.5	48.0	42.9	222.3	28.3
1117	9.9	64.5	15.3	50.3	26.9
1118	8.3	78.0	10.6	5.0	20.0
1119	18.4	55.9	32.8	28.3	19.7
1124	11.4	54.8	20.7	111.0	31.3
1125	12.6	55.5	22.9	10.5	16.0

TIBC, total iron binding capacity; sTfR, soluble transferrin receptor.

DISCUSSION

This study established that a single infusion of DFO, administered in a straightforward way, raises serum Epo concentration in most humans over a matter of hours. This provided us with a protocol to examine whether other human adaptive responses to hypoxia are, like Epo production, activated by iron chelation. Using this protocol, we failed to obtain any evidence that the increase in AHVR associated with VAH could also be generated in this manner.

Effects of DFO on serum Epo: comparison with other studies in vivo

Whereas this is the first study to demonstrate that a single infusion of DFO raises serum Epo concentration over a matter of hours in humans, there are other studies which suggest that DFO may have long-term effects on Epo concentration. Kling et al. (24) studied DFO that had been covalently conjugated with hydroxyethyl starch to form a complex of large molecular weight (HES-DFO). Such a complex increases serum DFO concentration and prolongs the half-life of the drug in blood (11). In their study, Kling et al. (24) found that serum Epo concentration did not change significantly, except with the highest dose of HES-DFO (equivalent to 150 mg/kg body wt of DFO, in terms of iron chelating capacity). With the highest dose of HES-DFO, serum Epo concentration was unchanged in the first 2 days after administration of HES-DFO. However, by the fourth day, serum Epo concentration had increased onefold, and this rise persisted to the seventh day after administration of HES-DFO. The highest dose in the study of Kling et al. is approximately three times the highest dose used in our study. This suggests that, whereas the conjugation of DFO with hydroxyethyl starch enables higher concentrations of iron chelator to be maintained in the plasma for longer

Table 3. *Hypoxic sensitivity measured at different time points for protocols DFO and C*

		Protocol DFO			*Protocol C*		
	Subject	$t = 0$	$t = 8$	$t = 24$	$t = 0$	$t = 8$	$t = 24$
Group 1	981	0.98	1.22		1.13	1.20	
	1023	1.64	2.11	2.16	1.20	1.31	2.58
	1091	1.33	1.36	1.76	2.09	1.20	1.47
Group 2	1004	0.97	0.85	1.13	0.84	1.18	1.30
	1056	0.40	0.72	0.58	0.72	1.06	1.00
	1099	0.69	0.44	0.49	0.50	0.59	0.52
Group 3	1069	0.71	0.73	0.88	0.64	0.62	0.78
	1109	0.85	0.71	0.93	0.85	0.44	0.68
	1110	0.40	0.36	0.24	0.33	0.33	0.51
	1115	0.78	0.83	0.70	0.85	0.79	0.93
	1116	0.32	0.35	0.22	0.68	0.28	0.21
	1117	0.42	0.55	0.44	0.73	1.11	0.46
	1118	1.05	0.92	1.36	0.81	0.66	1.00
	1119	1.71	1.42	1.16	1.60	1.75	2.08
	1124	0.68	0.55	0.95	0.74	0.53	0.60
	1125	0.35	0.22	0.28	0.17	0.23	0.21
Mean ± SD		0.83 ± 0.44	0.83 ± 0.49	0.88 ± 0.57	0.87 ± 0.47	0.83 ± 0.44	0.96 ± 0.67

Hypoxic sensitivity ($l \cdot min^{-1} \cdot \%^{-1}$) measured at 0, 8, and 24 h for *groups 1–3* in *protocols DFO* and *C*.

periods of time, the conjugated form of DFO is less effective in terms of inducing a response in Epo production.

Previous studies in patients with chronic anemia resulting from different disease processes have also reported an improvement in erythropoiesis after treatment with DFO (1, 15, 41). However, this was usually associated with long-term administration of DFO over weeks or months and was reported in the form of a decrease in transfusion requirement or an increase in [Hb] (15, 41).

In an animal study, Wang and Semenza (43) administered a single, intraperitoneal dose of DFO to mice. They found that Epo mRNA in the kidney was significantly enhanced after 22 h.

Effects of DFO on Epo: mechanisms of action

In cell culture, hypoxia-induced Epo gene expression has been intensively studied in human hepatoma cell lines (Hep 3B, Hep G2) that express Epo in an O_2-regulated fashion (17). This led to the identification of an O_2-responsive enhancer lying 3′ to the Epo gene (32) and the HIF-1 complex which binds it in hypoxia (42). DFO has been shown to induce both HIF-1α and HIF-2α at the protein level at 4 and 6 h (46); the kinetics are similar to those associated with induction by hypoxia (36, 43).

The effects of DFO in vitro almost certainly relate to its iron chelation function per se because other iron chelators also activate the system (14), and the induction of Epo by either DFO or an antitransferrin receptor antibody can be suppressed by the addition of extra iron to the culture medium (24). In our study, we did not observe a significant correlation between Epo response and any of the variables relating to iron status. However, it is interesting to note that the largest response in terms of Epo production was seen in the two subjects with the lowest levels of ferritin. The in vitro data suggest that intracellular iron removal is responsible for the induction of Epo by DFO (24).

In relation to the O_2-sensing process, it has been proposed that some form of heme protein is a likely candidate for the O_2 sensor (16). The ability of certain transition metals to stimulate Epo production has been postulated to result from their ability to substitute themselves for iron in the synthesized porphyrines and lock the heme protein into the deoxy form (3, 8, 13, 16). In relation to the effects of DFO on Epo production, it was originally suggested that DFO might prevent incorporation of iron into newly synthesized heme molecules (16).

An alternative hypothesis in relation to the effects of DFO on Epo gene expression relates to the proposal that both O_2 sensing and signal transduction may involve the detection of H_2O_2 and/or other reactive O_2 species (3, 5, 8, 13). By depleting the free iron pool, DFO may influence the generation of reactive O_2 intermediates (4, 12, 13), possibly by interfering with the iron-mediated degradation of H_2O_2 (12, 13).

Recently, it has been shown that the tumor suppressor protein VHL physically interacts with HIF-1 and is necessary for the destruction of HIF-1α subunits. DFO breaks this interaction (26). Although the underlying reason for this is not yet established, it approaches a molecular mechanism for the effect of DFO on HIF-1.

DFO and AHVR

Unlike the vigorous response of Epo to DFO infusion, there was no significant change in AHVR. This could simply imply that there are different underlying mechanisms generating the responses of AHVR and Epo to prolonged hypoxia. Alternatively, DFO may simply be ineffective at the carotid body or may require some additional complementary factors to exert its effects on AHVR. A further possibility is that response was present but was too weak to be detected.

In vitro, in addition to the Hep 3B cell line, a pheochromocytoma cell line (PC-12) has also been studied with respect to hypoxic regulation of gene expression. From the point of view of the long-term effects of hypoxia on AHVR, PC-12 cells are interesting because they have many similarities with carotid body type I cells (3, 7). After a period of chronic hypoxia, PC-12 cells show an enhanced catecholamine secretory response to acute hypoxia (39). This is associated with increased activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis (3, 4, 8). In the carotid body, after a period of sustained hypoxia, there also appears to be an increased level of dopamine synthesis and release (10, 18, 19) that is associated with an enhanced level of TH activity (4, 9, 18). It should, however, be noted that these aspects of the carotid body's response to sustained hypoxia do not necessarily underlie the increase in AHVR, as the physiological role of dopamine within the carotid body remains unclear. In fact, low-dose infusion of dopamine suppresses AHVR (31).

In PC-12 cells, DFO, like hypoxia, has been shown to be capable of inducing TH mRNA (25). This suggests that there are at least some common elements to O_2-sensitive gene expression in both chemosensory and Epo-producing cells. Other similarities exist as well. In PC-12 cells, addition of low levels of carbon monoxide to the hypoxic gas mixture substantially reverses the inhibition of K^+ currents induced by low Po_2 and chemosensory nerve discharge (4, 47). In Hep 3B cells, hypoxia-induced Epo gene expression can be inhibited by treatment with carbon monoxide (3, 4, 8). In addition, it has been suggested that H_2O_2 may serve as an intermediate signal transduction molecule in regulation of both TH and Epo genes during hypoxia (8, 12, 13, 25). Moreover, the sequences that confer O_2 responsiveness of the TH gene contain a potential HIF-1 response element (3, 8, 30).

However, despite the similarities, there are also differences between the HIF-1 response, as recognized in other cells and oxygen sensing in the carotid body and/or PC-12 cells. In particular, the latter are excitable cells that depolarize and enable Ca^{2+} influx in response to acute hypoxia (3, 8). Whereas cytosolic Ca^{2+} does not appear to play a major role in the HIF-1 response, in the case of TH gene expression, the rise in cytosolic Ca^{2+} associated with hypoxia may be an essential component (3, 8, 27, 28). It also remains possible that DFO is not inducing TH gene expression by activating HIF-1 (13, 25).

Extrapolation of in vitro findings to in vivo conditions has to be done with care. For instance, although DFO and hypoxia both induce TH mRNA in cell culture, there is no strong reason to suppose that we have achieved this in vivo or that such increases in TH mRNA in the carotid body would necessarily result in an increase in AHVR in the intact organism. More of the cellular physiology that underlies the modulation of hypoxic responsiveness of the carotid body needs to be understood.

We thank D. O'Connor for skillful technical assistance.

This study was supported by The Wellcome Trust. X. Ren holds an ORS Award and a K.C. Wong Scholarship.

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