Glycine and leucine incorporation into total myocardial proteins of the rat heart in vivo has been reported to decrease already after 1 h of swimming, and to return to the control level 2 h later[2]. The differences in the apparent protein synthesis rates may partially depend on the indicator amino acid used. In the measurements of cardiac muscle protein synthesis, phenylalanine is a very suitable monitor, because it e.g. penetrates the plasma membrane rapidly, it is non-metabolizable in myocardial tissue, and because the specific activity of the phe-tRNA reaches that of the extracellular phenylalanine when the latter is used at concentrations high enough[10,11].

The trigger mechanism of the protein synthetic processes involved in cardiac hypertrophy is not known, and several candidates have been proposed for it, as mentioned above. Nichols and Gonzales[6] proposed that elevated cGMP level could be the trigger in the hypertrophy caused by pressure overload. This is not probable in the case of volume overload, because we did not find any change in the cGMP content of the myocardial tissue after the exercise or during the 4-h recovery period, although the incorporation of phenylalanine into the total cardiac proteins transiently changed during that time.

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* Present address (for reprint requests); Department of Clinical Sciences, University of Tampere, P.O. Box 607, SF-33101 Tampere.

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The effect of hypoxia on hepatic cytochromes and heme turnover in rats in vivo

A. L. Jordi-Racine, E. Alvarez* and J. Reichen

*Department of Clinical Pharmacology, University of Bern, CH-3012 Bern (Switzerland), and *Department of Medicine, University of Colorado Medical School, Denver (Colorado, USA)*
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Summary. We evaluated the effect of hypoxia (7% v/v) on hepatic heme turnover in vivo and microsomal heme protein content in male Sprague-Dawley rats. Hepatic heme protein turnover, measured as ^{14}CO-production during continuous infusion of 5-^{14}C-aminolevulinic acid, a precursor of nonerythrogenic heme, was decreased 60% during hypoxia and returned to control levels promptly after reoxygenation. Hepatic cytochrome P-450 content was decreased in hypoxic and 24-h reoxygenated animals. We conclude that normobaric hypoxia decreases hepatic cytochrome P-450 which could contribute to decreased drug metabolism in hypoxia. This decrease is probably due to heme oxygenase-independent breakdown of hepatic heme.
Key words. Cytochrome P-450; heme oxygenase; cytochrome c reductase; hemoproteins; liver.

Hypoxia affects the handling of different drugs, e.g. of theophylline, in experimental animals[1] and man[2]. Hypoxia also prolongs hexobarbital sleeping time in the rat[3]. In the perfused liver a critical level of 45 mm Hg has been shown to be required before any changes in metabolism occur[4]. Normobaric hypoxia leads to a biphasic response of the hemoprotein class of cytochromes P-450, the main hepatic drug oxidizing system[5]. Hypobaric hypoxia, by contrast, induces cytochrome P-450 together with heme oxygenase, the main heme catabolizing enzyme[6].

How hypoxia affects hepatic heme metabolism is unknown, however. We therefore investigated hepatic heme turnover and cytochrome P-450 content by biosynthetically labeling the hepatic heme pool with 5-^{14}C-aminolevulinic acid and measuring ^{14}CO-output, a specific and sensitive method to measure heme oxygenase-dependent hepatic heme turnover[7,8].

Methods. Male Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories, Wilmington, MA and maintained in temperature and humidity controlled animal quarters on a 12-h light-dark cycle. They were allowed access to standard rat chow and tap water ad libitum. The animals were acclimatized to the altitude of Denver (1610 m) for at least 3 weeks. At the time of the study, they weighed 260–280 g.

For the hypoxia studies, the animals were fitted with a chronic indwelling jugular catheter and kept in Bollman type metabolic cages as described previously[8]. Immediately after surgery, 5-^{14}C-aminolevulinic acid (sp. act. 48.9 mCi/mmol from Research Products International, Elk Grove, IL) was infused over 3 days to label the hepatic heme pool[8]. ^{14}CO-production was measured during continuous chronic infusion of 5-^{14}C-aminolevulinic acid; ^{14}CO was trapped in ethanolamine after catalytic conversion to $^{14}CO_2$ as previously described[8,9]. During a control period of 24 h compressed air (oxygen 21% v/v) was passed through the cages at a rate of 1 l/min. Thereafter, half of the animals were given a mixture of nitrogen/oxygen 93:7 (v/v) while an equal number of control animals continued on room air (n = 6/group). The gas composition was chosen after preliminary experiments had shown that at higher oxygen concentrations (8% v/v) no changes occurred, whereas the animals did not survive oxygen concentrations of 5 or 6% (v/v). Thereafter, ^{14}CO production was measured for a further 72 h. Then, the

animals were sacrificed after determination of arterial pO_2 and microsomes were prepared.

In a second set of experiments, two groups (n = 6/group) were treated identically but after 72 h the hypoxic animals were switched back to room air, ^{14}CO-production measured for an additional 24 h prior to sacrifice.

Microsomes were prepared by differential centrifugation as previously described from this laboratory[10]. Microsomal content of cytochrome P-450, b_5 and activity of cytochrome c reductase, and heme oxygenase were determined by recently described[10] modifications of standard techniques[11-13]. Protein was determined by a modification of the Lowry assay using bovine serum albumin as a standard[14]. Blood gas analyses were performed on a standard membrane electrode type blood gas analyzer (Instrument Laboratories, Yellow Springs, MD).

All results are expressed as mean ± 1 standard deviation. Multiple means were compared by analysis of variance and means of two groups by Student's t-test[15]. $p < 0.05$ was considered statistically significant.

Results. In control animals, body weight increased from 232 ± 16 to 237 ± 11 g (n.s.) while in the hypoxic animals it decreased from 230 ± 18 to 198 ± 11 g ($p < 0.001$). The liver weights did not differ significantly between the two groups, averaging 10.9 ± 1.9 and 9.5 ± 1.5 g in the control and hypoxic group, respectively. Hypoxia induced respiratory acidosis, pH decreasing from 7.43 ± 0.03 to 7.30 ± 0.09 ($p < 0.05$). Arterial pO_2 decreased from 86 ± 4 to 34 ± 8 mm Hg ($p < 0.0002$).

Baseline excretion of ^{14}CO ranged from 185 to 440 dpm/h; to eliminate this interindividual variation each value was divided by the average ^{14}CO excretion observed during the control period. The effects of hypoxia on these normalized ^{14}CO-excretion data is shown in figure 1. In control animals, ^{14}CO-production remained constant throughout the experiment while in the treated animals it significantly decreased by 60% in the 2nd h after switching to low oxygen tension and remained at that level throughout the experiment. The effect of reoxygenation on ^{14}CO-production is shown in figure 2; similarly to the first experiment, ^{14}CO-production decreased in the 2nd h after switching to a low oxygen atmosphere. When the cages were gassed with 21% oxygen again, ^{14}CO-production rose back to control levels within 3 h; a slight overshoot in ^{14}CO-production in the 4th h was observed but did not reach statistical significance.

The effects of hypoxia on microsomal enzymes are given in the table. Cytochrome P-450, but not cytochrome b_5, were significantly reduced after exposure to a hypoxic atmosphere for 3 days. Heme oxygenase activity was almost doubled, while cytochrome c reductase activity did not change (table). The effects of reoxygenation are shown in the table. Qualitatively the same changes, i.e., a decrease in cytochrome P-450 content and an increase in heme oxygenase activity were observed 24 h after reoxygenation. These values had returned back to normal 48 h after reoxygenation (data not shown). Neither hypoxia nor reoxygenation affected microsomal recovery as expressed by an unchanged relative specific activity (4.1 ± 1.3 vs 4.2 ± 1.3) and recovery (30 ± 8 vs 25 ± 5%) of cytochrome c reductase.

Effect of hypoxia (3-d) and hypoxia (3-d) followed by 24 h of reoxygenation on hepatic microsomal enzymes. Mean ± 1 SD (n = 6/group) are given. Treatment effects were evaluated by Student's t-test.

	Hypoxia		Reoxygenation	
	Control	Treated	Control	Treated
Cytochrome P-450 (nmol/mg)	0.82 ± 0.20	0.48 ± 0.09[a]	0.94 ± 0.28	0.63 ± 0.10[b]
Cytochrome b_5	0.27 ± 0.04	0.24 ± 0.07	0.42 ± 0.13	0.41 ± 0.14
Heme oxygenase	1.30 ± 0.36	2.14 ± 0.15[c]	1.11 ± 0.19	2.03 ± 0.32[c]
Cytochrome c reductase	101 ± 46	114 ± 26	110 ± 19	118 ± 29

[a] $p < 0.003$ vs controls; [b] $p < 0.03$; [c] $p < 0.0005$ vs controls.

Discussion. Our investigations demonstrate that hypoxia under essentially normobaric conditions (1610 m above sea level) decreases ^{14}CO-production, a measure of heme oxygenase-dependent heme breakdown. This is associated with a decrease in hepatic cytochrome P-450 content and an increase in heme oxygenase activity. The change in ^{14}CO-production is rapidly reversible upon reoxygenation while the changes in microsomal enzyme content and activity persist for 24 h; they are back to control levels after 48 h.

The present method for measuring nonerythrogenic heme breakdown has been extensively validated[7-10]; it has the major advantage of permitting continuous and noninvasive measurement of hepatic heme breakdown during experimental manipulations. Nonerythrogenic heme breakdown only is measured, since the substrate used does not enter erythrocyte precursor cells[12,16]. The major drawback is that it measures heme oxygenase-dependent heme breakdown only[8-10], while up to 50% of hepatic heme may be broken down by pathways not involving formation of CO[17].

Our finding of a decreased ^{14}CO-production suggests a decreased hepatic bilirubin production since CO and bilirubin are stoichiometrically formed[12]. Direct measurement of bil-

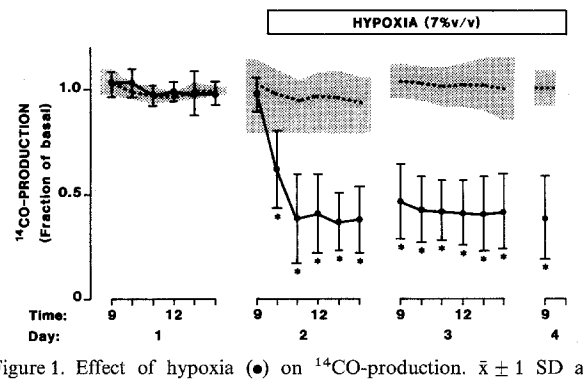

Figure 1. Effect of hypoxia (•) on ^{14}CO-production. $\bar{x} \pm 1$ SD are shown. The control values $\bar{x} \pm 1$ SD are shown as shaded areas. To eliminate interindividual variability each value was divided by its average ^{14}CO-production during the control day (day 1). * denotes a significant treatment effect ($p < 0.05$ or less) as determined by analysis of variance.

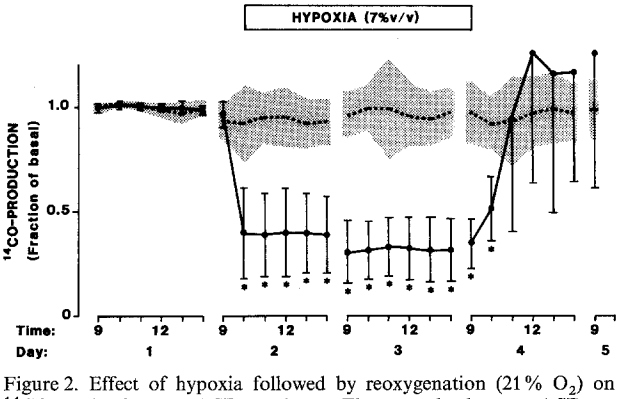

Figure 2. Effect of hypoxia followed by reoxygenation (21% O_2) on ^{14}CO-production. $\bar{x} \pm 1$ SD are shown. The control values $\bar{x} \pm 1$ SD are shown as shaded areas. To eliminate interindividual variability each value was divided by its average ^{14}CO-production during the control day (day 1). * denotes a significant treatment effect ($p < 0.05$ or less) as determined by analysis of variance.

iary bilirubin excretion has demonstrated an increased bilirubin production during hypobaric hypoxia[18]. This apparent discrepancy is easily resolved when one considers the adaptive increase in erythropoiesis induced by hypobaric hypoxia; accordingly, the increased bilirubin formed is of splenic, i.e. erythrogenic origin[18,19].

The finding of an increased heme oxygenase activity (table) is in accordance with the literature[6,19]. This reflects the increased need to catabolize heme from the ongoing hemolysis[19]. Part of this induction could also serve to eliminate heme from catabolism of cytochrome P-450. This is not reflected in an increased production of ^{14}CO, however (fig. 1). Two reasons could account for this: either the hepatic heme being broken down has not been labeled by the infused aminolevulinic acid and/or the extreme hypoxemia shuts down less essential oxidation reactions such as heme oxidation. The former appears rather unlikely since aminolevulinic acid rapidly labels hepatic heme[7,12] and the cytochrome P-450 half-life time is 7–48 h[20]. Therefore, we reason that our pretreatment of 5 days was sufficient to thoroughly label the hepatic heme pool. We interpret out data as reflecting a decrease in hepatic heme oxidation during chronic hypoxia. The heme oxygenase induction is a response to an increase in the regulatory free hepatic heme pool[7,12].

Hypoxia has generally been reported to induce rather than to depress hepatic cytochrome P-450[5,6,21,22]. In some of these experiments longer periods of hypoxia and/or hypobaric hypoxia were studied. Bechtel et al. observed a biphasic response to hypoxia similar to that employed in our experiments in mice: after an initial decrease cytochrome P-450 increased on days 2 and 3, to decrease finally after prolonged periods of hypoxia[5]. In accordance with this, Srivastava et al. described a marked decrease 6 h after acute hypobaric hypoxia[23]. Longmuir and Pashko described an induction of cytochrome P-450 by chronic hypoxemia but also commented that it decreased when the hypoxia was close to the lethal level[21]. We selected the lowest oxygen tension compatible with 100% survival. These divergent results point to the fact that a) hypo- versus normobaric hypoxia and b) the duration of hypoxia will markedly affect the response of the cytochrome P-450 system.

After 3 days of hypoxia, microsomal cytochrome b_5 levels and cytochrome c reductase activity were unaltered; the same held true for the reoxygenated animals (table). The response of cytochrome b_5 in our animals differs from that reported by two other groups[5,23]. The same methodological differences as are discussed above seem to be the reason for this discrepancy. Acute hypobaric hypoxia diminishes microsomal cytochrome c reductase activity[23], whereas in our experiments the enzyme was not affected. Whether this difference is due to the extreme conditions employed[23] or whether cytochrome c reductase recovered after an initial decline cannot be answered from our experiments. The maintenance of the activity of this enzyme is of interest in view of its reported role in heme breakdown not involving formation of CO[24].

Other factors besides hypoxia could have influenced our results. Thus, the animals were in respiratory acidosis. The stress associated with this metabolic alteration could have influenced heme oxygenase activity[25]. Fasting per se stimulates heme oxygenase[25] and heme breakdown[8], and decreases hepatic cytochrome P-450 content[8,26]. It is unlikely that fasting significantly contributed to our results. Thus, although fasting leads to a similar decrease in cytochrome P-450 content, the associated loss in body weight is much more marked than in the present experiment[8]. Furthermore, fasting, in contrast to hypoxia, induces ^{14}CO-formation. Finally, fasting decreases liver weight owing to glycogen depletion, but liver weight was not affected in our experiments.

In conclusion, our studies demonstrate a marked decrease of microsomal cytochrome P-450 content and a decrease in nonerythrogenic heme breakdown during chronic hypoxia at the maximal level compatible with 100% survival. Hepatic heme oxygenase activity is markedly stimulated during and after hypoxia, while cytochrome c reductase activity and cytochrome b_5 content are not affected. These findings differ from hypobaric or less marked hypoxia where an adaptive increase in cytochrome P-450 content has been reported.

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