Alterations of Myocardial Sympathetic Innervation in Response to Hypoxia

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The effects of altitude hypoxia on myocardial sympathetic nerve function were assessed in rats using metaiodobenzylguanidine (MIBG). **Methods:** To estimate the change in uptake-1 function induced by hypoxia, three sets of rats were submitted to 5-, 7- and 21-day hypoxia (hypobaric chamber at 410 Torr) and one set of control rats was injected with 25 μCi of ^{123}I-MIBG. Four hours later, the rats were killed and ^{123}I activity was counted in both ventricles. The proportion of MIBG fixed in the myocardium through the norepinephrine (NE) transporter (uptake-1) was evaluated indirectly in 5-day hypoxic and controls rats by the injection of desipramine before ^{123}I-MIBG administration. Myocardial perfusion was evaluated in 5-day hypoxic rats and controls by ^{201}Tl injection. **Results:** Myocardial ^{123}I-MIBG activity was 0.253% ± 0.036% kg dose/g^{-1} in controls and was decreased (0.188% ± 0.029% kg dose/g^{-1}, $p = 0.001$) in 5-day hypoxic rats. This decrease was not related to a change in cardiac perfusion. The decrease in MIBG uptake existed before the appearance of cardiac hypertrophy. Desipramine decreased MIBG uptake by 48% in controls and 17% in hypoxic rats, suggesting that the decrease predominantly affected MIBG uptake by the NE transporter. **Conclusion:** Chronic hypoxia leads to a decrease in myocardial NE uptake-1 function. This finding suggests that altered tissue oxygen supply could play a role in the decreased cardiac MIBG uptake reported in human cardiomyopathies.

Key Words: iodine-123-metaiodobenzylguanidine; uptake-1; hypoxia; desipramine

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Cardiac sympathetic nerve terminals release norepinephrine (NE) into the synaptic cleft. NE increases the rate and force of contraction of myocytes through β-adrenergic receptors. Most of the released NE is taken back in the presynaptic nerves by a specific NE transporter (uptake-1). This membrane protein is inhibited by desipramine (*1*).

Chronic exposure to hypoxia is a potent stressor that affects the adrenergic system. In response to chronic hypoxia, a global increase in adrenergic drive occurs which is reflected in part by elevated plasma and urine catecholamine concentrations (*2*). By contrast the maximal heart rate obtained during exercise is diminished (*2*) suggesting local modifications of the myocardial adrenergic neurotransmission. In particular, the down-regulation of cardiac β-adrenergic receptors found in chronic hypoxia (*3,4*) could explain the decreased maximal heart rate. This down-regulation could be a consequence of presynaptic modifications such as an increased NE production and release or a decreased neuronal NE reuptake.

The cardiac sympathetic nerve function can be explored by metaiodobenzylguanidine (MIBG), a NE analog (*5–7*). MIBG enters the neuronal cells with the NE transporter (*8*) and shares NE storage in intraneuronal vesicles (*6*). However, MIBG also shares NE nonspecific extraneuronal uptake, which is not inhibited by desipramine (*7,9*). Extraneuronal MIBG has a rapid clearance (*9,10*) and myocardial MIBG uptake 4 hr after injection appears to best represent intraneuronal MIBG (*11*). Decreased cardiac MIBG uptake has been widely reported in heart diseases associated with sympathetic hyperactivity (*12,13*).

Decreased ^{123}I-MIBG myocardial uptake has been found also in humans exposed to altitude hypoxia by scintigraphic evaluation (*14*). However, this in vivo evaluation did not provide information concerning the exact mechanism of this modification. In particular, the role of potential variations of either myocardial blood flow or nonspecific uptake, the importance of circulating NE levels and regional variations of adrenergic innervation in the myocardium have not been addressed. This study was undertaken to access some of the pathophysiological mechanisms involved in the decreased MIBG uptake observed in conditions of high sympathetic activity using a model of chronically hypoxic rats.

MATERIALS AND METHODS

Iodine-123-MIBG Uptake Quantification

Male Wistar rats, weighing 250 ± 50 g were used. Control animals were maintained at laboratory level (300 m above sea level). Hypoxic rats were kept in a hypobaric pressure chamber with a barometric pressure of 410 Torr. This pressure corresponds to an atmospheric P_{O2} of 85 mmHg (alveolar P_{O2} of 51 mmHg) and is equivalent to an altitude of 4350 m. All animals received food and water ad libitum and were submitted to a 12-hr day/night cycle.

Six 5-day hypoxic rats, eight 7-day hypoxic rats and six 21-day hypoxic rats were used for ^{123}I-MIBG uptake quantification. Six normoxic animals of the same age were studied with each hypoxic group. On the day of the experiment, hypoxic and normoxic animals were injected in the tail vein with 25 μCi of ^{123}I-MIBG (CIS-Bio International, Saclay, France) in 0.2 ml of saline solution. Hypoxic animals were immediately replaced in the hypobaric chamber. All animals were killed 4 hr after ^{123}I-MIBG injection by intraperitoneal injection of sodium pentobarbital (60 mg/kg^{-1}). Heart, lungs, liver and muscles (soleus and extensor digitorum longum) were excised and blood was drawn. Right and left ventricles were separated. All samples were weighed (wet weight). Their ^{123}I activity was counted in a well counter (KONTRON 2000, Paris, France). Activity was counted for 1 min, using a peak-energy setting of 160 keV (15% window). Corrections were made for radioactive decay and counter efficiency. To normalize for differences in animal weights, tissue concentrations were expressed in percent kilogram dose per gram (% kg dose/g^{-1}) (*15*).

Iodine-123-MIBG Nonspecific Uptake Estimation

Six control and six 5-day hypoxic rats were used for ^{123}I-MIBG nonspecific uptake estimation. They received 10 mg/kg^{-1} of desipramine (a potent uptake-1 inhibitor) intraperitoneally 2 hr before ^{123}I-MIBG intravenous injection (*7*) in order to evaluate the proportion of myocardial MIBG uptake related to the NE transporter. Cardiac ^{123}I-MIBG activity in these animals was assumed to be entirely nonspecific. Hypoxic animals were replaced in the hypobaric chamber between the two injections and after ^{123}I-MIBG

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injection. All animals were killed 4 hr after ^{123}I-MIBG injection. Right and left ventricles were separated, weighed and their ^{123}I activity was counted. Counting technique, corrections and units were similar to those used in ^{123}I-MIBG uptake quantification.

Myocardial Blood Flow Quantification

Nine normoxic and six 5-day hypoxic rats were used for myocardial blood flow quantification. Animals were injected in the tail vein with 15 μCi of ^{201}Tl. They were killed 10 min after ^{201}Tl injection by intraperitoneal injection of sodium pentobarbital. Left and right ventricles were separated, weighed and counted in a well counter (KONTRON 2000, Paris, France). Ventricular activity was counted for 1 min, using peak-energy setting at 70 keV (20% window). Corrections were used for radioactive decay and counter efficiency. The unit used was % kg dose/g^{-1}.

Plasma Catecholamine Concentration

Six of the normoxic and six of the 5-day hypoxic rats were selected. Two milliliters of blood were drawn from the aorta immediately before the death of the animals. Samples were centrifugated (3000 rpm for 10 min). The supernatant was collected and stored at −80°C. Plasma norepinephrine and epinephrine concentration were measured by HPLC method adapted from Anton and Sagro (*16*), using an ESA plasma catecholamine kit (ESA, Bedford, MA).

Statistical Analysis

Values are mean values ± s.d., except when otherwise indicated. Statistical analysis was performed using paired and unpaired Student's t-tests. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Five-Day Hypoxia and Iodine-123-MIBG Uptake

Five-day hypoxic rats had lower global cardiac ^{123}I-MIBG than normoxic rats (0.188% ± 0.029% kg dose/g^{-1} versus 0.253% ± 0.036% kg dose/g^{-1}, $p = 0.001$ (Fig. 1).

Myocardial 123I-MIBG uptake was not significantly different between left and right ventricle in hypoxic rats (Table 1). After 5 days of hypoxia, ^{123}I-MIBG uptake decreased by 37% in the right ventricle and 20% in the left ventricle. This contrasted with the results observed in normoxic rats where right ventricular ^{123}I-MIBG uptake was greater than left ventricular uptake ($p < 0.001$). In the other sampled organs, ^{123}I-MIBG uptake was lower than in the heart and did not change between the two conditions (Fig. 1).

Secondary Cardiac Hypertrophy and Cardiac Iodine-123-MIBG Uptake

Since myocardial hypertrophy may alter MIBG cardiac uptake (*18*), the additional role of hypertrophy was evaluated by studying rats before and after the appearance of hypertrophy. No ventricular hypertrophy was detected after 5 days of hypoxia (Table 2). A biventricular hypertrophy was found after 7 days of hypoxia. However, only right ventricular weight increase reached statistical significance at this time. Left ventricular weight increase also reached statistical significance after 21 days of hypoxia. However, the percentage of right ventricular weight increase (+48%) still remained greatly superior to the left ventricular weight increase (+16%).

Seven-day and 21-day hypoxic rats had significantly decreased cardiac ^{123}I-MIBG uptake compared to normoxic animals (Table 1). However, there was no significant difference in cardiac MIBG uptake between rats submitted to different durations of hypoxia (5, 7, 21 days).

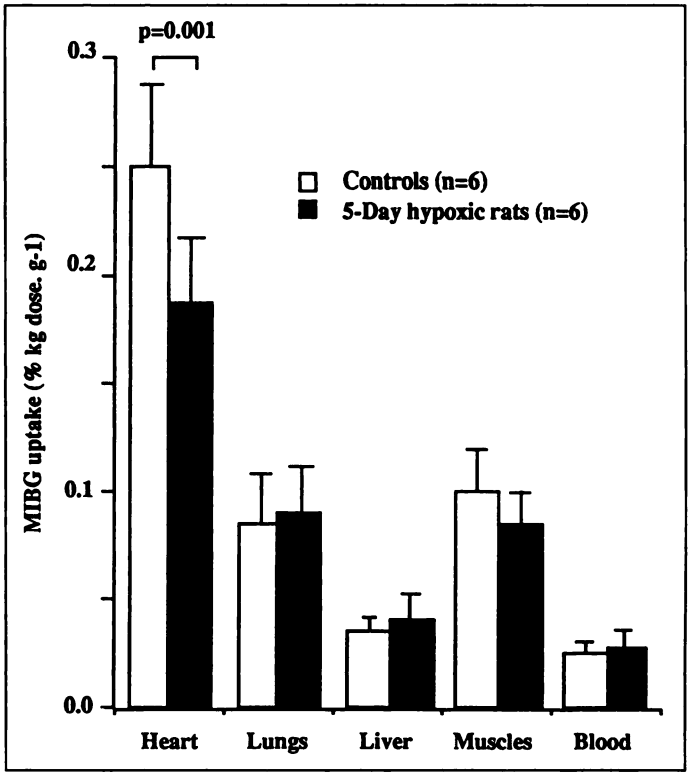

FIGURE 1. Tissular 123I-MIBG uptake in normoxic and 5-day hypoxic rats. Values are mean ± s.d. Cardiac MIBG uptake was decreased by 26% in 5-day hypoxic rats. No significant changes in other organs was observed.

Nonspecific Cardiac Iodine-123-MIBG Uptake

In normoxic rats, cardiac ^{123}I-MIBG uptake remaining after desipramine injection was 0.132% ± 0.023% kg dose /g^{-1} (Fig. 2). This indicates a 52% nonspecific uptake in the total cardiac MIBG uptake.

After 5 days of hypoxic exposure, nonspecific cardiac MIBG uptake (0.155% ± 0.023% kg dose/g^{-1}) was not significantly different from nonspecific cardiac MIBG uptake in normoxia. However, it accounted for 83% of the total cardiac MIBG uptake in 5-day hypoxic rats.

Desipramine injection in 5-day hypoxic rats induced a 17% decrease in cardiac MIBG uptake (0.155% ± 0.023% kg dose/g^{-1} versus 0.188% ± 0.029% kg dose/g^{-1}, $p = 0.04$).

Myocardial Blood Flow

In normoxic rats, ^{201}Tl uptake did not differ between both ventricles (1.204% ± 0.341% kg dose/g^{-1} for the right ventricle versus 1.239% ± 0.326% kg dose/g^{-1} for the left ventricle).

After 5 days of hypoxic exposure, ^{201}Tl concentration in the whole heart (1.304% ± 0.257% kg dose/g^{-1}), in the right ventricle (1.333% ± 0.264% kg dose/g^{-1}), in the left ventricle (1.291% ± 0.263% kg dose/g^{-1}) did not differ significantly from the normoxic condition.

Plasma Catecholamine Level

Plasma norepinephrine concentration was increased in 5-day hypoxic rats compared to controls (2.63 ± 1.33 ng/ml^{-1} versus 0.66 ± 0.23 mg/ml^{-1}, respectively, $p < 0.01$). Plasma epinephrine concentration was not altered (1.83 ± 0.34 ng/ml^{-1} versus 1.82 ± 0.34 ng/ml^{-1}). The data are shown in Table 3.

DISCUSSION

This experimental study shows that chronic hypoxia induces a sympathetic hyperactivity associated to a reduced in myocardial MIBG uptake due to a decrease in uptake-1 function.

TABLE 1
Iodine-123-MIBG Concentration in Normoxic and Hypoxic Rat Heart

	Whole (% kg dose/g^{-1})	Right ventricle (% kg dose/g^{-1})	Left ventricle (% kg dose/g^{-1})
Normoxic rats (n = 18)	0.253 ± 0.036	0.306 ± 0.043	0.234 ± 0.037
5-day hypoxic rats (n = 6)	0.188 ± 0.029†	0.192 ± 0.022‡	0.186 ± 0.033*
7-day hypoxic rats (n = 8)	0.171 ± 0.038‡	0.162 ± 0.031‡	0.175 ± 0.043‡
21-day hypoxic rats (n = 6)	0.168 ± 0.037‡	0.160 ± 0.039‡	0.171 ± 0.038‡

Values are mean ± s.d.
*p = 0.011; †p = 0.001; ‡p < 0.001 hypoxia versus normoxia.

Significance of Altered Cardiac MIBG Uptake in Hypoxia

Decreased cardiac MIBG uptake has been found using scintigraphy in normal volunteers after 8 days at 4350 m (*14*). This finding suggested that hypoxia may alter myocardial adrenergic innervation and functioning. However, although MIBG is captured by the specific NE transporter (*8*), it also shares NE extraneuronal uptake (*7,9*). Furthermore, a passive, nonspecific neuronal uptake has been suggested in the heart (*7*) and shown in adrenomedullary cells (*5,18*). A decrease in cardiac MIBG uptake might have been explained by a decrease in its nonspecific uptakes. In this study, desipramine pretreatment was used to differentiate specific neuronal MIBG uptake by NE transporter from nonspecific uptakes. Cardiac MIBG uptake after desipramine pretreatment was similar in normoxia and hypoxia, suggesting unchanged proportions of nonspecific MIBG uptake. The decreased cardiac MIBG uptake observed in hypoxia therefore seems related to reduced uptake-1 function.

Nevertheless, other factors may have participated in the reduction of MIBG uptake. Cardiac hypertrophy, which could by itself decrease cardiac MIBG uptake (*15*) was not detected in 5-day hypoxic rats while the decrease in myocardial MIBG uptake was still present. No change in either global or regional coronary blood flow was found in 5-day hypoxic rats. Norepinephrine competitively inhibits MIBG specific neuronal uptake in vitro (*5,8,18*) and could decrease MIBG cardiac uptake by this mechanism. Inhibition could take place because of elevated circulating NE levels (*19*). Although this mechanism has been suggested in pheochromocytoma (*19*), NE levels required to produce significant inhibition of MIBG uptake (*5,8,18*) seem to be much higher than those reached in hypoxia.

On the other hand, a contribution of locally increased NE release by the cardiac sympathetic nerves cannot be excluded. MIBG would then be captured but rapidly released by neuronal extremities, decreasing its uptake 4 hr after injection. Support for this hypothesis has been found in animals and humans with cardiomyopathy (*11,20*).

TABLE 2
Cardiac Weights

	Right ventricle (mg)	Left ventricle (mg)
Normoxic rats (n = 17)	190 ± 55	548 ± 97
5-day hypoxic rats (n = 30)	211 ± 43	529 ± 52
7-day hypoxic rats (n = 12)	240 ± 56*	570 ± 110
21-day hypoxic rats (n = 18)	281 ± 49‡	636 ± 91†

Values are in mean ± s.d.
*p < 0.02; †p = 0.008; ‡p < 0.001 hypoxia versus normoxia.

Potential Mechanisms of Decreased Uptake-1 Function

The exact mechanism of decreased uptake-1 function is unclear. A loss of noradrenergic nerves as reported in heart failure (*21*) could explain a decrease in cardiac MIBG uptake. However, this process is unlikely in hypoxia considering the rapid normalization in MIBG uptake previously observed in subjects submitted to high altitude after return to normoxic conditions (*14*). Elevated interstitial norepinephrine concentrations could lead by itself to a decrease in norepinephrine reuptake. In rats infused with norepinephrine for 5 days, we found a similar decrease in left ventricular MIBG and tritiated norepinephrine uptake that was related to a loss in uptake-1 carrier assessed by tritiated mazindol in vitro binding (*22*). This finding suggests a down regulation process of the uptake-1 carrier protein in response to increased level of norepinephrine similar to that observed for postsynaptic β-adrenergic receptors. This down regulation of the norepinephrine transporter would

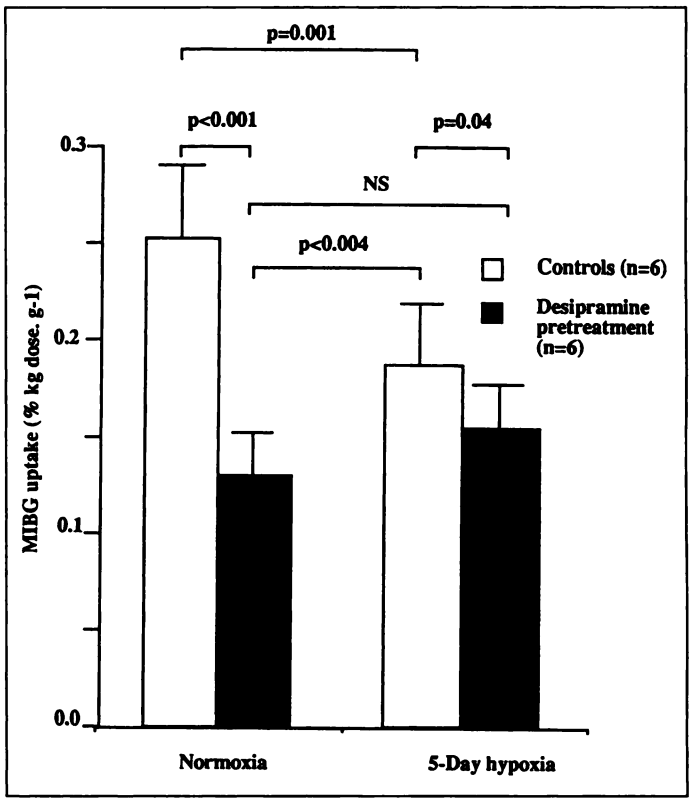

FIGURE 2. Cardiac ^{123}I-MIBG uptake after pretreatment with desipramine, in normoxic and 5-day hypoxic rats. Values are mean ± s.d. In normoxic animals, desipramine pretreatment inhibiting MIBG uptake by the NE transporter induced a 48% decrease in total cardiac ^{123}I-MIBG uptake. In 5-day hypoxic rats, the decrease was only 17%, suggesting altered MIBG uptake by the NE transporter.

TABLE 3
Plasma Catecholamine Levels in Normoxia and After 5 Days of Hypoxia

	Norepinephrine (ng/ml^{-1})	Epinephrine (ng/ml^{-1})
Normoxic rats (n = 6)	0.66 ± 0.23	1.82 ± 0.34
5-day hypoxic rats (n = 6)	2.63 ± 1.33*	1.83 ± 0.34

Values are mean ± s.d.
*$p < 0.01$ hypoxia versus normoxia.

be a consequence of either increased circulating norepinephrine or increased myocardial norepinephrine release or both.

Alternatively, conditions of energy depletion can induce a reverted transport of NE from the axon towards the interstitial space by the neuronal NE transporter (*23*). Chronic hypoxia could create a similar energy depletion and induce a comparable phenomenon.

Pathophysiological Implications of Altered Uptake-1 Function

In this study, postsynaptic function has not been explored. Although β-receptor down regulation has been shown in chronic hypoxia (*3,4*), a link between presynaptic and postsynaptic function cannot be established. This link has been shown in healthy animals, in which the cardiac response to exogenous NE is prolonged by uptake-1 blockade but unchanges by uptake-2 blockade (*24*). Furthermore, in heart failure, another condition where a global increase in sympathetic activity has been widely reported (*25,26*), decreased uptake-1 function has been found (*27–31*) and related to both myocardial overexposure to NE (*27*) and decreased myocardial β-receptors (*27–30*).

CONCLUSION

This study shows that the sympathetic hyperactivity caused by chronic hypoxia leads to a decrease in cardiac MIBG uptake in the rat heart. This decrease is related to an altered uptake-1 function. This suggests that a decrease in MIBG uptake may reflect the level of adrenergic hyperactivation of the myocardium due to either increased adrenergic drive or elevated plasma norepinephrine. Such modifications of presynaptic function may be involved in the alterations of β-adrenoceptor pathway previously reported in hypoxia (*3,4*). Similar findings have been reported in heart failure and a link between the decrease in uptake-1 function and β-adrenoceptor desensitization has been widely reported in this disease (*27–30*). Finally, the similarities of changes in adrenergic function observed between chronic hypoxia and heart failure suggest that impaired tissue oxygen supply may participate in the disorders of the sympathetic innervation of the failing myocardium.

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