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Differential alterations in cardiac adrenergic signaling in chronic hypoxia or norepinephrine infusion

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K. MOHAMMADI,[3] B. CROZATIER,[3] AND J.-P. RICHALET[1,3]
[1]*Laboratoire Réponses cellulaires et fonctionnelles à l'hypoxie, Association pour la Recherche en Physiologie de l'Environuement, Faculté de Médecine, Université Paris XIII, 93017 Bobigny, France;* [2]*Departamento de Ciencias Fisiológicas/Instituto de Investigaciones de la Altura (IIA),Universidad Peruana Cayetano Heredia, Lima 100, Perú;* [3]*Institut National de la Santé et de la Recherche Médicale Unité 400 and* [4]*Unité 99, 94010 Créteil, France*

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León-Velarde F., M.-C. Bourin, R. Germack, K. Mohammadi, B. Crozatier, and J.-P. Richalet. Differential alterations in cardiac adrenergic signaling in chronic hypoxia or norepinephrine infusion. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R274–R281, 2001.—Norepinephrine (NE)-induced desensitization of the adrenergic receptor pathway may mimic the effects of hypoxia on cardiac adrenoceptors. The mechanisms involved in this desensitization were evaluated in male Wistar rats kept in a hypobaric chamber (380 Torr) and in rats infused with NE ($0.3\ mg \cdot kg^{-1} \cdot h^{-1}$) for 21 days. Because NE treatment resulted in left ventricular (LV) hypertrophy, whereas hypoxia resulted in right (RV) hypertrophy, the selective hypertrophic response of hypoxia and NE was also evaluated. In hypoxia, α_1-adrenergic receptors (AR) density increased by 35%, only in the LV. In NE, α_1-AR density decreased by 43% in the RV. Both hypoxia and NE decreased β-AR density. No difference was found in receptor apparent affinity. Stimulated maximal activity of adenylate cyclase decreased in both ventricles with hypoxia (LV, 41%; RV, 36%) but only in LV with NE infusion (42%). The functional activities of G_i and G_s proteins in cardiac membranes were assessed by incubation with pertussis toxin (PT) and cholera toxin (CT). PT had an important effect in abolishing the decrease in isoproterenol-induced stimulation of adenylate cyclase in hypoxia; however, pretreatment of the NE ventricle cells with PT failed to restore this stimulation. Although CT attenuates the basal activity of adenylate cyclase in the RV and the isoproterenol-stimulated activity in the LV, pretreatment of NE or hypoxic cardiac membranes with CT has a less clear effect on the adenylate cyclase pathway. The present study has demonstrated that *1*) NE does not mimic the effects of hypoxia at the cellular level, i.e., hypoxia has specific effects on cardiac adrenergic signaling, and *2*) changes in α- and β-adrenergic pathways are chamber specific and may depend on the type of stimulation (hypoxia or adrenergic).

adrenergic receptors; adenylate cyclase; protein kinase C; G proteins; ventricular hypertrophy

CHRONIC HYPOXIA INDUCES an overall sympathetic stimulation that is reflected in elevated plasma and urine catecholamine concentrations . The stimulation of the adrenergic system induces a progressive blunting of the heart chronotropic response to isoproterenol. This process produces subsequent cardiovascular adaptations to offset a global decrease in tissue oxygen supply (21, 23, 30, 32). These modifications can be related, in part, to alterations in β-adrenergic receptors (β-AR) signal transduction. β-AR are coupled with adenylate cyclase through guanine nucleotide binding proteins (G proteins). Activation of β-AR leads to an increased cAMP production by adenylate cyclase. In intact animal models of chronic hypoxia, a decrease in β-AR density has been observed (12, 18, 20, 34). At the G protein level, $G\alpha_s$ activity was found to be decreased in both ventricles and $G\alpha_{i\text{-}2}$, the inhibitory protein of adenylate cyclase, increased only in the right ventricule (RV). No change was observed in $G\alpha_s$ mRNA levels, but an increase in $G\alpha_{i\text{-}2}$ mRNA has been found in the RV (12).

Hypoxia-induced changes in α_1-adrenergic receptors (α_1-AR) have mainly been studied in vitro during acute episodes. An enhanced inositol triphosphate response to α_1-AR stimulation and a decrease in the receptor affinity were shown to occur in cardiocytes over short periods of exposure to hypoxia (8, 13). With a longer duration of hypoxia, cardiac myocytes showed a differential regulation of the various α_1-AR subtypes (19). Previous studies (6, 19) have reported alterations of both β- and α_1-receptors in compensatory cardiac hypertrophy previous to chronic cardiac failure. Chronic hypoxia imposes an additional load to the right heart, which leads to a RV hypertrophy secondary to pulmonary hypertension (28); therefore, it might be expected that the association of both hypoxia and hypertrophy would further alter the regulation of the receptor system.

Norepinephrine (NE), the major neurotransmitter released by sympathetic nerves, produces positive inotropic responses. It has been proposed that the effects

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of hypoxia on cardiac adrenoceptors may be mediated indirectly, by a desensitizing effect of increased NE (30), due, in part, to an impaired uptake-1 (22). Besides, chronic administration of NE induces an LV hypertrophy and reduces β-receptor coupling to the contractile response without substantially compromising ventricular function (26). Studies in rat heart muscle cells and studies, where animals were exposed to increased catecholamine levels for 7 to 14 days, led to a reduced sensitivity to β-agonists and to a decreased β-AR density. NE-induced desensitization progresses from a homologous to a heterologous form with increased dose and time of exposure to NE. Events distal to the β-AR in the adenylate cyclase cascade are also affected (4, 5, 7, 29). β-AR density has also been found downregulated, and the adenylate cyclase response has been found desensitized or unchanged in in vitro models of catecholamine incubation in neonatal rat cardiac myocytes (7, 14, 15). Chronic infusion of isoproterenol, which also results in myocardial hypertrophy in mice, has been shown to decrease the adenylate cyclase mRNA levels for both isoforms in the heart (type V and VI; Ref. 16).

It has been suggested that the effects of hypoxia on cardiac adrenoceptors may be mediated, in part, by a desensitizing effect of increased catecholamines (12, 30). In fact, AR regulation may result from both elevated catecholamine levels and hypoxia itself. To investigate the possibility that agonist-induced desensitization of the AR pathway can mimic the effects of hypoxia on cardiac adrenoceptors, we tested the effects of prolonged infusion of rats with NE on resting heart rate (HR) and cardiac response to isoproterenol, as well as on the characteristics of β- and α_1-AR and on their effector enzymes. Furthermore, the selective hypertrophic response of hypoxia and NE enabled us to observe the characteristics of cardiac adrenoceptor pathways from hypoxia-induced right hypertrophied ventricles and NE-induced left hypertrophied ventricles.

MATERIAL AND METHODS

Animals. Male Wistar rats (200–250 g) were separated in two normoxic (NX), two hypoxic (HX), and two NE groups. One group of each type was used for the measurement of HR and the response to isoproterenol (ΔHRIso; $n = 7$ in each group), as well as for AR binding (NX, HX, and NE, $n = 7$). The other groups ($n = 7$ in each group) were used for adenylate cyclase studies. HX rats were kept on a 12:12-h light-dark cycle (room temperature, 23 ± 2°C) with free access to food and water. The chamber was brought to normobaria for 30–40 min 3 times/wk for cleaning and food and water replacing. They were exposed to a 5,500-m simulated altitude (380 Torr) for 21 days. After the 3-wk exposure to hypoxia, the animals were killed by cervical dislocation and the hearts were quickly removed and dissected free of fat and large vessels. The ventricles were separated from the atria. The wet weights of the combined LV plus septum and of the RV were determined and rapidly put into liquid nitrogen. They were then immediately placed at −70°C until use. All procedures were performed in agreement with the local rules and with the regulation of the French "Ministère de l'Agriculture" for animal care.

Surgical procedures. Male Wistar rats were obtained from Charles River of France. (-)NE HCl was infused at a rate of 0.3 $mg \cdot kg^{-1} \cdot h^{-1}$ from an Alzet minipump (model 2ML4) for a period of 21 days. The minipump was filled with NE HCl with 0.2% ascorbic acid dissolved in isotonic saline. NE was prepared on the day of implantation. Telemetry measurements (HR) were obtained by a small transmitting sensor (TA 10-EA F40, Data Sciences). A small incision was made in the intrascapular region for implanting the minipumps and in the peritoneum for implanting the transmitting sensors. Both incisions were made subcutaneously under pentobarbital sodium anesthesia (6 g/100 ml ip). Fifteen rats were operated in two different periods; the controls were not sham operated considering that the preliminary in vivo HR measurements in sham-operated rats had shown no differences between operated and controls. None of the rats used in this study showed evidence of infections at the site of the operation. Body weights were measured before implantation of the osmotic minipumps and at *days 11* and *21* after implantation.

HR and response to isoproterenol. Resting HR and ΔHRIso were measured in unanesthetized rats. ΔHRIso is defined as: ΔHRIso = HRIso-resting HR. The signal was sent by telemetry to a receiver placed under the cage. The average of ~150 readings recorded at the same hour each day was taken as the resting HR. Data were collected and analyzed using DATAQUEST III data acquisition system. For the ΔHRIso determinations, the animals were injected with intraperitoneal isoproterenol (0.05 mg/kg) to obtain an HR increase of ~40%. This measurement was made only once at *day 19* of hypoxia and of NE infusion.

Preparation of membrane samples. Preparation of membrane samples from ventricles was performed according to the method of Baker et al. (1) with a minor modification. Briefly, the ventricles were minced and homogenized in 10 vol of ice-cold buffer (*buffer A*: 30 mM Tris·HCl, 100 mM NaCl, 5 mM $MgCl_2$, 1mM EGTA, 500 μg/ml trypsin inhibitor, and 100 μg/ml bacitracin; pH 7.5) with a polytron tissue homogenizer (6 × 5-s bursts). The suspension was diluted with an equal volume of ice-cold *buffer A* and centrifuged at 1,000 *g* for 10 min. Soon after, the supernatant was centrifuged (45,000 *g* × 45 min × 2 times) in 4 vol of ice-cold *buffer A*. The final pellet was resuspended in 3 vol of incubation buffer (50 mM Tris·HCl, 5 mM $MgCl_2$, pH 7.5) and placed at −70 °C until use. The protein content was adjusted to the convenient concentration the day of each assay (2).

α_1- and β_1-AR binding. The radioligand [^{3}H]prazosin was used to label myocardial α_1-AR binding sites, and [^{3}H]CGP-12177 was used to label β-AR. The concentration of [^{3}H]prazosin ranged from 0.02 to 1 nM, and the concentration of [^{3}H]CGP-12177 ranged from 10 to 250 pM. Unlabeled prazosin (1 μM) and propanolol (10^{-4} M) were added to determine nonspecific binding. In displacement experiments, [^{3}H]prazosin concentration was 0.25 nM, corresponding to about twice the receptor apparent affinity (K_d) values of prazosin found in saturation binding experiments. Displacement of [^{3}H]prazosin binding by NE was performed using 12 concentrations (1 nM-1 mM) of the agonist (with 0.1% ascorbic acid).

Triplicate of samples (100 μl; 60–75 μg) of the membrane preparations was incubated for 45 min at 25°C (α_1-AR) and at 37°C (β-AR) in the incubation buffer (final vol: 250 μl). Incubation was terminated by rapid vacuum filtration (Scatron) through adequate filters (1-μm retention; 102 mm length, 256 mm width). The tritiation plaques were rinsed 10 times with ice-cold incubation buffer. The radioactivity retained on the filters was determined by liquid scintillation spectrometry. The binding assays were carried out in tripli-

cate; 10 points were used in each case. Nonspecific binding averaged 7% of total binding.

Adenylate cyclase assay. Adenylate cyclase activity was determined in cardiac membranes according to Johnson et al. (10) with minor modifications as previously described (27). The membrane preparations were incubated (25 μg proteins) in a final volume of 60 μl of reaction buffer (50 mM Tris·HCl, pH 7.6; 5 mM $MgCl_2$; 1 mM EDTA, an ATP-regenerating system consisting of 1 mg/ml creatine kinase and 1 mM phosphocreatine; and 1 mM ATP). A concentration of 1 mM cAMP was used to quench phosphodiesterase activities. Amounts of α–[^{32}P]ATP [10^6 counts/min (cpm)/reaction, specific activity 30 $Ci \cdot min^{-1} \cdot mol^{-1}$] were included to give a specific radioactivity of the incubation cocktail of 40 cpm/pmol ATP. The [^{32}P]cAMP synthetized was recovered by chromatography on an aluminia column. Radiolabeled [^{3}H]cAMP (20,000 cpm/assay, specific activity 20–30 $Ci \cdot min^{-1} \cdot mol^{-1}$) was included to monitor the recovery of each chromatography elution.

After a 10-min incubation period in a shaking water bath at 37°C, the reaction was terminated by adding 200 μl of 0.5 N HCl followed by inmediate boiling for 6 min. The pH of the assay mixture was adjusted to 7.6 with 250 μl of 1.5 M imidazole-HCl. Samples were then eluted with 2 ml of 10 mM imidazole-HCl through an alumina column that retains [^{32}P]ATP. The ^{3}H and ^{32}P activities of the eluate were then counted after addition of a scintillation cocktail. After incubation with [^{32}P]ATP, the level of [^{32}P]cAMP was measured. Basal adenylate cyclase activity as well as the activities after stimulation with 10 mM sodium fluoride (NaF) and 30 μM (-)isoproterenol in the presence of 10 μM GTP and 50 μM forskolin (FRK) were measured. All determinations were performed in triplicate and expressed as picomoles of cAMP synthesized per minute and milligram of protein.

Pertussis and cholera toxin-catalyzed ADP-ribosylation. Pertussis toxin (PT) catalyzes ADP-ribosylation of the α-subunit of G_o and G_i proteins, which act as negative regulators of adenylate cyclases. The modified G_i protein uncoupled from the receptor and the cyclase. Cholera toxin (CT) catalytic subunit by ribosylation of $G\alpha_s$ irreversibly activates all G_s proteins mediating the stimulation of the adenylate cyclases. The effects of PT and CT on the basal and isoproterenol-stimulated adenylate cyclase activities of cardiac membranes were determined according to Sethi et al. (33). The membrane preparations (3 mg/ml) were incubated with or without toxin in a final volume of 100 μl for 60 min at 30°C in a preincubation reaction mixture before the adenylate cyclase assay. The preincubation reaction mixture consisted of 50 mM Tris·HCl (pH, 7.6) containing 1 mM EDTA, 1 mM EGTA, 5 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1 mM ATP, 0.1 mM GTP, 10 mM thymidine, and 1 mM NAD. Activated PT and CT in the preincubation mixture were 10 and 30 μg/ml, respectively.

Data analysis. Radioligand binding data were analyzed with Ligand, a weighted, nonlinear, least-square curve fitting computer program (24). For saturation experiments, equilibrium dissociation constants (K_d) and maximum numbers of binding sites were determined by nonlinear regression fitting. Displacement data were first fitted to a one- and then to a two-site model. The statistical differences between one- or two-site models were determined by comparing the residual variance between the actual and predicted data points, and *F* test analysis was used by the Ligand program to decide whether a model of one- or two-binding site fit was more appropriate. When the *P* value was >0.05, the one-site model was considered as the best fit. Even if the Hill parameter was always lower than 1, Ligand was not able to fit a two-binding fit model.

Statistical analysis. One-way ANOVA (followed by a Tukey's posttest) was used to assess the statistical significance between mean values. The effects of isoproterenol on HR were analyzed by the paired *t*-test because values were obtained from each animal both before and after the isoproterenol administration. The effect of PT and CT before and after membrane treatment was also analyzed by the paired *t*-test. $P < 0.05$ is considered statistically significant.

RESULTS

Physiological data. With NE-infusion at *day 21*, the animals failed to gain weight, whereas with hypoxia, they gained weight but less than the control group (NX, 480 g ± 38 SD; HX, 451 ± 29; NE, 375 ± 9; $P < 0.05$). In regard to the ventricular weight, chronic NE treatment resulted in LV hypertrophy, whereas hypoxia resulted in RV hypertrophy as assessed by the ratio of LV and RV wet weight to body weight (Table 1). No significant change was found between the wet weight of the RV of the NE group when compared with controls.

NE infusion had no effect on HR during the first 4 days of exposure [360 ± 43 beats/min (bpm)], whereas the HX group increased its mean HR at the same period to 465 ± 25 bpm ($P < 0.01$). After a 7-day treatment NE rats started to raise their HRs, but HX animals greatly decreased their initial increased HR progressing toward the NX values at the end of exposure. The NE-infused animals presented the highest average mean HR for *days 18–19* (417 ± 32 bpm; $P < 0.05$) when compared with HX (346 ± 13 bpm) and NX (342 ± 12 bpm) rats. NE and hypoxia produce desensitized responses to stimulation at various levels of the β-receptor pathway (4, 12, 20, 21). Our NE-infused and HX-exposed animals showed as well a clearly desensitized response to acute administration of isoproterenol (Fig. 1). In the NX rats, the ΔHRIso increase at *min 2* of injection was 166 ± 8 bpm. In contrast, ΔHRIso response of NE and HX rats was significantly lower

Table 1. *BWs and LV and RV fresh weights and LV/BW and RV/BW in NX, HX, and NE-infused rats*

	BW_o, g	BW_{21}, g	LV, mg	RV, mg	LV/BW, mg/g	RV/BW, mg/g
NX	394 ± 17	480 ± 38	824 ± 32	206 ± 11	1.72 ± 0.2	0.43 ± 0.06
HX	392 ± 20	451 ± 29*	896 ± 93	364 ± 49†	1.99 ± 0.08	0.81 ± 0.08†
NE	393 ± 7	375 ± 9†	1,001 ± 91†	186 ± 31	2.67 ± 0.1†	0.50 ± 0.08

Values are means ± SD. BW, body wt; BW_o, BW before treatment; BW_{21}, BW at *day 21* of hypoxic (HX) or norepinephrine (NE) treatment; RV and LV, right and left ventricular, respectively; LV/BW and RV/BW, ratios of LV to BW and RV to BW, respectively. *$P < 0.01$ and †$P < 0.001$ for HX and NE vs. normoxic (NX).

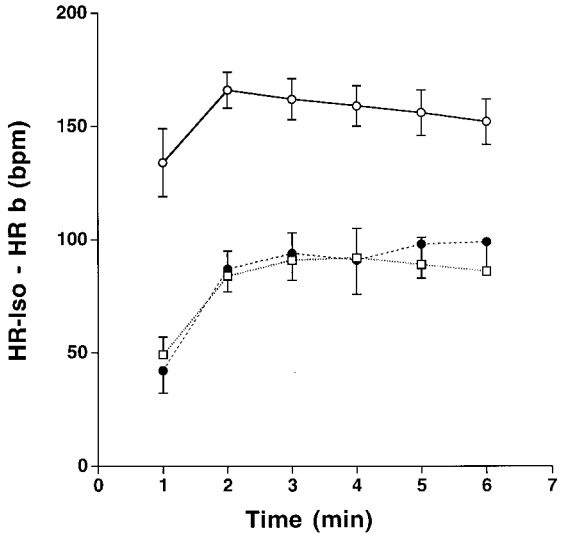

Fig. 1. Change in heart rate (HR) from baseline (b) values on *day 19* of chronic hypoxic (HX; ●) and norepinephrine (NE) infusion (□), and on normoxic animals (NX; ○) in response to isoproterenol (Iso). bpm, Beats/min. $P < 0.05$ HX, NE, vs. normoxic (NX) for all time points.

(NE, 84 ± 11 bpm; HX, 87 ± 10 bpm; $P < 0.0001$ vs. NX).

Density, affinity, and distribution of cardiac adrenoceptors. With NE infusion, no change was found on the density of α_1-AR in the LV, whereas with hypoxic exposure the density of α_1-AR was increased by 35% ($P < 0.05$). Conversely, in the RV of the NE group, α_1-AR density was decreased by 43% ($P < 0.05$), but no changes were found in the HX group. No significant difference was found in the K_d of α_{1A}-AR among NX, HX, or NE rats. It is worthy mentioning that the upregulation of α_1-AR has been found in our particular experimental condition (low K_d), where we have most probable targeted the α_{1A}-AR (6, 19). In regard to β-AR, in NE a 65% decrease in density was found in the LV ($P < 0.001$), whereas a 40% decrease was found in the RV ($P < 0.01$). In hypoxia, a 17% decrease in density was found in the LV and in the RV ($P < 0.05$). No significant difference was found in the affinity of [^{3}H]CGP-12177 for β-AR among the NX, HX, or NE groups. Data are shown in Table 2. In LV, NX, and HX, displacement curves were superimposed, while in RV there was a rightward shift of the HX displacement curves compared with NX. Some displacement curves of [^{3}H]prazosin with NE displayed a biphasic behavior for [^{3}H]prazosin binding sites with pseudo-Hill coefficients less than 1.0, indicating the existence of two different affinity sites for the agonist (LV: HX, −0.86; NX, −0.78. RV: HX, −0.55: NX, −0.77). However, as we were not able to find any statistical difference between one- and two-site affinity models, the data were treated as a one-site model. Hypoxia did not modify the NE calculated dissociation constant in the LV but significantly increased it in the RV (LV: HX, 3.4 μM ± 1.23; NX, 2.7 ± 0.55. RV: HX, 9.6 ± 1.95: NX, 4.4 ± 1.36).

Adenylate cyclase activity. Figure 2 summarizes the results of adenylate cyclase activity in membranes prepared from the hearts of NE and HX rats. Basal activity of adenylate cyclase (pmol·mg^{-1}·min^{-1}) was decreased by NE in the LV (35%) and by hypoxia in the RV (45%). There was a significant decrease in maximal activity of adenylate cyclase with isoproterenol stimulation only in the LV with NE infusion (42%), whereas with hypoxia, both ventricles show a decrease in this parameter (LV, 41%; RV, 36%). Furthermore, there was a significant impairment of activity of adenylate cyclase with NaF and FRK stimulation again in both ventricles with hypoxia but only in the hypertrophied ventricle with NE infusion. It is worthy to emphasize that there was a decrease in adenylate cyclase activity in the nonhypertrophied ventricles (in all situations of stimulation) only in the HX group. When NE and hypoxic hypertrophied ventricles were compared, there was a significant decrease in maximal activity of adenylate cyclase with isoproterenol, NaF, and FRK stimulation in both groups. However, the HX group presented a greater decrease in adenylate cyclase activity for all the conditions of stimulation.

PT- and CT-catalized ADP-ribosylation of G proteins. The functional activities of G_i and G_s proteins in cardiac membranes were assessed by incubation with PT and CT. Figure 3 shows the basal and isoproterenol-stimulated adenylate activity of treated membranes. If there is an increase of G_i, pretreatment of ventricle cells with PT should have a considerable effect in abolishing the decrease in isoproterenol-in-

Table 2. *Effects of 21 days of hypoxia and NE infusion on α_1- and β-AR density and affinity*

	β-AR		α_1–AR	
	B_{max}, fmol/mg protein	K_d, nM	B_{max}, fmol/mg protein	K_d, nM
LV (β-AR, $n = 5$; α_1–AR, $n = 6$)				
NX	94 ± 4	0.2 ± 0.05	76 ± 22	0.07 ± 0.03
HX	78 ± 4*	0.12 ± 0.02	126 ± 29*	0.16 ± 0.05
NE	33 ± 5†	0.11 ± 0.01	75 ± 30	0.11 ± 0.06
RV (β-AR, $n = 4$; α_1–AR, $n = 4$)				
NX	57 ± 11	0.11 ± 0.05	89 ± 22	0.17 ± 0.07
HX	47 ± 14	0.13 ± 0.1	78 ± 8.7	0.10 ± 0.04
NE	34 ± 16*	0.13 ± 0.07	50 ± 7.8*	0.11 ± 0.06

Values are means ± SD for determinations performed in NX, HX and NE rats for [^{3}H]prazosin (α_1-receptors) and [^{3}H]CGP-12177 (β-receptors) studies; n = no. of rats. Density of receptors (B_{max}, fmol/g protein) and dissociation constant (K_d, nM) were determined with the Ligand program. AR, adrenergic receptor. *$P < 0.05$ and †$P < 0.01$ for HX and NE vs. NX.

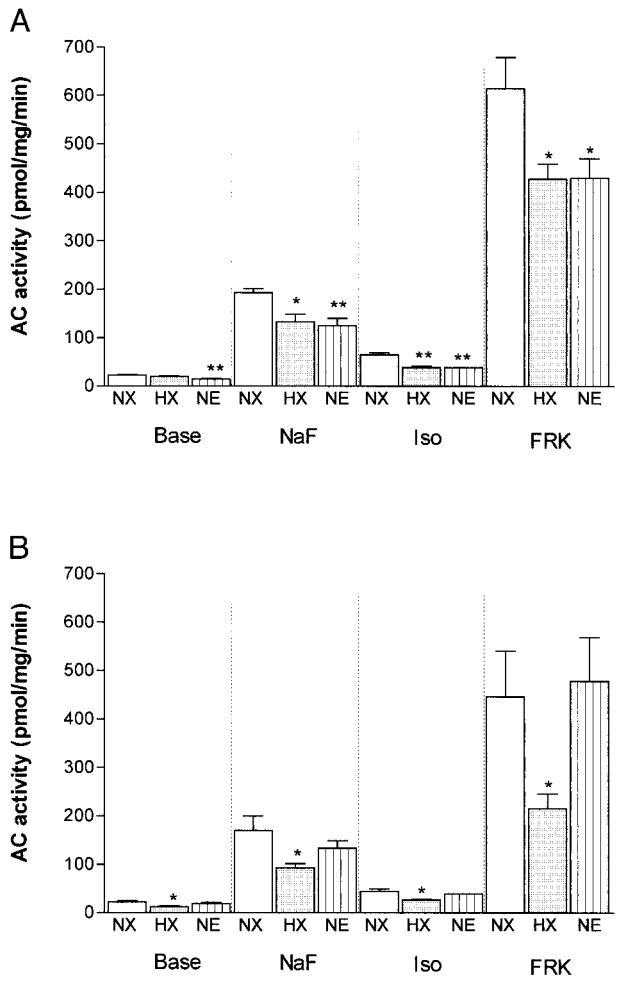

Fig. 2. Effect of chronic HX and NE infusion on adenylate cyclase (AC) activity stimulated by sodium fluoride (NaF, 10 mM), Iso 30 μM + 10 μM GTP, and forskolin (FRK, 50 μM) in left (LV; *A*) and right (RV; *B*) ventricles. AC activity was assayed in heart membranes of NX (open bars), HX (dotted bars), and NE-infused (lined bars) rats. Data are means ± SE for 5 separate experiments. $^{*}P < 0.05$; $^{**}P < 0.001$ for HX and NE vs. NX.

duced stimulation of adenylate cyclase. Incubation of membranes of the LV with PT resulted in a greater basal adenylate activity in NE-infused animals when compared with the respective control group ($P < 0.01$). An increase was also observed in the HX-exposed group, but this change was considerably higher than that of NE-infused rats ($P < 0.05$). Isoproterenol-stimulated adenylate activity was only increased in the HX group ($P < 0.05$). As CT activates all G_s proteins mediating the stimulation of the adenylate cyclases, a diminution of adenylate activity in pretreated cells is an indication of decreased levels of G_s. In LV, CT decreases isoproterenol-adenylate cyclase activity in hypoxia as expected (12). Incubation of the RV membranes with CT decreased the basal activity in the NE and HX groups ($P < 0.05$) but did not produce any change in the isoproterenol-stimulated adenylate activity neither in the NE nor in the HX group.

Table 3 summarizes the results of the study. It shows that there is a differential regulation of α_1- and β-AR by NE infusion and chronic hypoxia. This table describes as well a differential regulation of adenylate cyclase and proteins G_i and G_s. Chronic adrenergic stimulation (NE-RV) does not affect adenylate cyclase sensitivity or functional activity of the G proteins, whereas hypoxic stimulation (HX-LV) produces an attenuated sensitivity of adenylate cyclase to hormone stimulation, an augmentation of G_i functional activity, and a decrease of G_s functional activity. This table also shows that changes in α-AR, β-AR, and adrenergic pathway and signaling converge in someway when both ventricles are exposed to hypertrophy (NE-LV and HX-RV).

DISCUSSION

This work answers for the first time to the question of whether the cardiac changes observed during exposure to chronic hypoxia are secondary to the adrenergic stimulus, or whether they are directly related to the hypoxic stress. For this purpose, we analyzed the changes in the transduction pathway of the β_1-adenylate cyclase cascade and in the α_1-AR in animals subjected to hypoxic and/or adrenergic stimulation. Besides, the modifications observed in hypertrophy secondary to chronic hypoxia and chronic NE infusion were compared. Both treatments proved to provoke the same magnitude of decrease in HR response to isoproterenol. Our hypothesis was that hypoxia would mimic adrenergic activation of the α_1- and β-pathways. The present results demonstrate that in contrast with rats exposed to chronic hypoxia, which shows a differential regulation of α_1- and β-AR in the LV, rats exposed to NE infusion show only a β-AR downregulation. In the hypertrophied RV, hypoxic exposure decreased only the β-AR density, but NE infusion decreased both α_1-AR and β-AR density. This work also describes a differential regulation of adenylate cyclase. Rats exposed to hypoxia showed an attenuated sensitivity of adenylate cyclase to hormone stimulation, whereas chronic adrenergic stimulation (when not associated to hypertrophy, i.e., RV) did not affect adenylate cyclase sensitivity. In NE-infused rats, changes in G proteins do not parallel those observed in rats exposed to hypoxia (Table 3). Even if the attenuated responses to acutely administered isoproterenol confirm that both conditions cause a desensitization of the adrenergic system, the differential results at the level of basal heart rate (higher in the NE group) indicate that in vivo hypoxia has also some specific effects. The regulation of adrenergic receptors in hypoxia may result from the combination of both elevated catecholamine levels and hypoxia itself.

One of the advantages of the chronic hypoxia model is that it allows the comparison between LV, exposed to hypoxia and adrenergic stimulation (HX-LV), and RV, which is additionally subjected to pressure overload due to pulmonary hypertension (HX-RV; Refs. 9, 28). The model of chronic NE infusion allows the comparison between RV (NE-RV), exposed to adrenergic stimulation only, and LV, subjected to both adrenergic stimulation

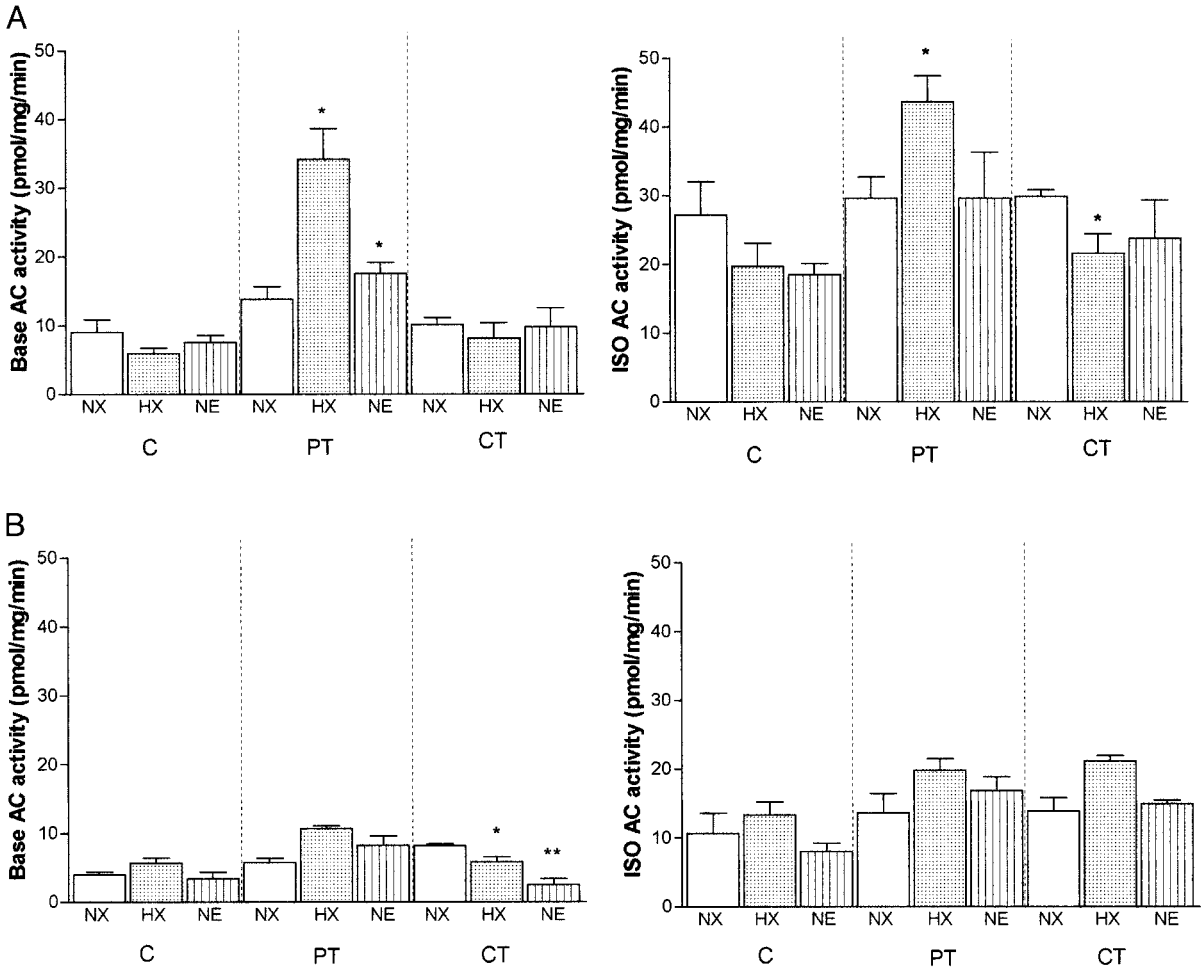

Fig. 3. Basal (Base AC) and Iso-stimulated adenylate activity of pertussis toxin (PT)- and cholera toxin (CT)-treated membranes in LV (*A*) and RV (*B*). PT and CT were assayed in heart membranes of NX (open bars), HX (dotted bars), and NE-infused (lined bars) rats. C, control. Data are means ± SE for 5 separate experiments. $^*P < 0.05$; $^{**}P < 0.01$.

and systemic hypertension (NE-LV). We have used a large dose of NE to avoid the compensation by the regulatory capacity of the system. Chronic treatment with NE caused LV (21%) but not RV hypertrophy. This level of hypertrophy is comparable to that observed in other studies in which similar doses of NE were used, but this response may have been influenced by an enhanced systemic catabolic state, as demonstrated by a decrease in weight gain (3, 17). This selective effect suggests that the hypertrophic stimulus may be an increase in afterload produced by α_1-AR-mediated vasoconstriction and/or β-AR-mediated increase in myocardial contractility. RV hypertrophy caused by chronic hypoxia is due mainly to pulmonary hypertension secondary to vasoconstriction and remodeling of the pulmonary arteries. LV hypertrophy is absent in chronic hypoxia, despite the elevation in catecholamine level; thus RV hypertrophy seems not to be related to high levels of NE. Although we have not measured catecholamine augmentation and blood pressure in our animals, there is a general agreement that plasma or urinary NE is elevated in prolonged hypoxia, as shown in humans staying for more than 1 wk at high altitude (32), and these findings are compatible with increased sympathetic activity. At the dose of NE we presently used, an increase in systemic pressure has been found (125–155 mmHg), which is compatible with the LV hypertrophy found in this study (17, 36).

Table 3. *Differential regulation of α_1- and β-adrenergic receptors and signaling by chronic HX and NE infusion*

	β-AR				α1–AR
	Density	AC	G_i	G_s	Density
LV					
HX	−	−−	+++	−	+
NE	−−−	−−	n.Δ	n.Δ	n.Δ
RV					
HX	−	−−−	n.Δ	n.Δ	n.Δ
NE	−−	n.Δ	n.Δ	n.Δ	−−

Signs indicate changes in density of β- and α-AR, in activated adenylate cyclase (AC) (NaF, isoproterenol, and forskolin-AC) and in G_i, G_s (isoproterenol stimulated). The (−) or (+) signs indicate decrease or increase of HX and NE values when compared with NX. n.Δ means "no change" among 3 groups. Animals in NX were used as reference.

In HX animals, the α_1-AR affinity for NE in the RV was significantly decreased, and the magnitude of decrease in α_1- and β-AR density was higher with NE infusion compared with chronic hypoxia, both in RV and LV. Thus NE may be responsible, in part, for this reduction in intact animals, at least at the receptor level. Our observations on α_1- and β-AR differ from previous reports in cell culture models, most likely because hypoxia-induced changes in α_1- and β_1-AR have been mainly studied in vitro and during acute episodes (7, 19, 31, 36). In contrast, little information is presently available regarding the regulation of these receptors during prolonged hypoxia in vivo . Thus (12, 34) caution should be taken in extrapolating in vitro models with in vivo chronic models, taking into account that in intact animals, circulating catecholamine levels are elevated in response to hypoxia (32).

In 21 days of hypoxia, along with the decrease in β-AR density, the catalytic unit of adenylate cyclase was desensitized, showing a depressed response to the activators tested. However, the magnitude of the depression of response to FSK in LV was less (21%) than that to isoproterenol (33%), suggesting not only an uncoupling of β-AR but also a change distal to the receptor. In RV of rats exposed to NE infusion, the response to the activators was not depressed, and there was an insignificant change in the responses in both conditions, suggesting that with respect to adenylate cyclase activity, hypoxia does not mimic adrenergic activation. In contrast, in the hypertrophied RV in hypoxia, the depression of the response to FSK was greater (13%) than that to isoproterenol (1%), suggesting a decrease in the content of the enzyme itself. In the hypertrophied LV of rats infused with NE, there was a depression in the response to isoproterenol (9%); however, no depression was found when compared with the response to FSK. In fact, only a 42% decrease in the NE group was found in adenylate cyclase maximal activity stimulated by forskolin when expressed per LV, but a 73% decrease in the HX group when expressed per RV. Thus the adenylate cyclase decrease seems to be more dependent on the degree of hypoxic hypertrophy rather than adrenergic hypertrophy.

In chronic hypoxia, we have observed a decrease in the responsiveness to NaF and to isoproterenol stimulation of adenylate cyclase activity. In fact, pretreatment of hypoxic cardiac membranes with PT, which functionally inactivates G_i proteins, not only restored but increased the isoproterenol-induced stimulation of adenylate cyclase in the LV. This strongly suggests an increased activation or level of G_i proteins in desensitized membranes. Several studies (11, 29) support the hypothesis that the regulation of the quantity of $G\alpha_i$ proteins may be a general regulatory mechanism for sensitization and desensitization of adenylate cyclase at the postreceptor level. Additionally, increased activity of G_i could result in reduced levels of cAMP, which might be at the origin of the observed desensitization of adenylate cyclase in hypoxia (25). On the other hand, although NE infusion is associated with a downregulation of β-AR in several animal models (4, 5, 7, 17), pretreatment of the NE ventricle cells with PT failed to restore the isoproterenol stimulation of adenylate cyclase. Our results suggest either that β-AR are less coupled to adenylate cyclase in the NE-stimulated heart or that other adenylate cyclase regulators are more relevant in this model. Pretreatment of NE or hypoxic cardiac membranes with CT, which functionally activates G_s, has less clear effect on the adenylate cyclase pathway. Although pretreatment with CT attenuated the basal activity of adenylate cyclase in the RV and the isoproterenol-stimulated activity in the LV, these results are in line with the less prominent role of G_s compared with G_i in the desensitization of β-AR (11, 29). In chronically failing human hearts, β_2-AR stimulation also induces positive inotropic and lusitropic effects and phosphorylation of regulatory proteins. Inhibition of G_i proteins by PT causes β_2-AR to closely resemble that of β_1-AR (35). Whether the increased level of G_i in HX animals modifies also the β_2-AR/G_i coupling awaits for further study.

In conclusion, we have shown that NE does not mimic the effects of hypoxia at the cellular level, i.e., that hypoxia has specific effects on the transduction pathway of the β_1-adenylate cyclase cascade and on α_1-AR. These results also show that changes in α- and β-adrenergic pathways are chamber specific. The distinct effects of hypoxia and adrenergic stimulation seem to be due to differential responses of the ventricles rather than various degrees of desensitization. On the contrary, the effects of hypertrophy produced by hypoxia and by adrenergic stimulation are more probably due to different degrees of desensitization rather than differential responses of the ventricles. These distinct responses cannot be explained only on the basis of generalized changes in the hormonal profile such as increased levels of blood catecholamines in chronic hypoxia. Thus the differential changes in signal transduction in LV and RV suggest that the regional-specific modifications in signal transduction in the heart may be produced by differences in local conditions such as hemodynamic or hormonal state and/or adrenergic nerve activity.

Perspectives

At high altitude and in experimental chronic hypoxia, a desensitization (blunting) of the adrenergic system and a sensitization of the cholinergic system occur. These changes are present even in animals genetically adapted to life at high altitudes, like guinea pigs, and could contribute to succesful adaptive processes in these animals. Blunting of the chronotropic response to hypoxia, mediated by β-AR and M_2 cholinergic receptors, may be one of the strategies to protect the myocardium. Nevertheless, these kinds of changes have also been found in heart failure. In addition to the interaction between these receptors, an underlying interaction between other types of autonomic receptors might be involved in the regulation of various cardiac functions in hypoxia. Our results show that, besides the contribution of the adrenergic system (via β_1-AR, α_1-AR, A_1-adenosinergic receptors, and the

M_2-receptor systems) to ventricular function at high altitude, hypoxia itself is involved in the cardiovascular changes observed in our model. Exposure to prolonged hypoxia is a useful model to realize how the heart adapts to a stressful environment. Hopefully, the present study will help to understand a rather little known angle of cardiac physiology, i.e., the interaction between different types of autonomic receptors and signaling between both ventricles in conditions of chronic hypoxia. In addition to a better knowledge of the physiological adaptation to hypoxia, these models may help to understand other physiological conditions, particularly ischemia, because in hypoxia, oxygen supply is lower than the myocardial needs.

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REFERENCES

1. **Baker SP, Boyd HM, and Potter LT.** Effect of propanolol on β-adrenoceptors in rats hearts. *Br J Pharmacol* 68: 8–10, 1980.
2. **Bradford MM.** A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 246–254, 1976.
3. **Brown L, Sernia C, Newling R, and Fletcher P.** Cardiac responses after norepinephrine-induced ventricular hypertrophy in rats. *J Cardiovasc Pharmacol* 20: 316–323, 1992.
4. **Chang HY, Klein RM, and Kunos G.** Selective desensitization of cardiac beta adrenoceptors by prolonged in vivo infusion of catecholamines in rats. *J Pharmacol Exp Ther* 221: 784–789, 1982.
5. **Deighton NM, Brown AD, Hamilton CA, and Reid JL.** Regulation of adrenergic receptor number following chronic noradrenaline infusion in the rabbit. *Arch Pharm (Weinheim)* 338: 517–522, 1988.
6. **Deng X-F, Sculptoreanu A, Mulay S, Peri KG, Li JF, Zheng WH, Chemtob S, and Varma DR.** Crosstalk between alpha-1A and alpha-1B adrenoceptors in neonatal rat myocradium: implications in cardiac hypertrophy. *J Pharmacol Exp Ther* 286: 489–496, 1998.
7. **Dong E, Yatani A, Mohan A, and Liang CS.** Myocardial beta-adrenoceptor down-regulation by norepinephrine is linked to reduced norepinephrine activity. *Eur J Pharmacol* 384: 17–24, 1999.
8. **Heathers GP, Evers AS, and Corr PB.** Enhanced inositol triphosphate response to α_1-adrenergic stimulation in hypoxic cardiac myocytes. *J Clin Invest* 83: 1409–1413, 1989.
9. **Hislop A and Reid L.** New findings in pulmonary arteries of rats with hypoxia induced pulmonary hypertension. *Br J Exp Pathol* 57: 542–554, 1976.
10. **Johnson RC, Alvarez R, and Salomon Y.** Determination of adenylyl cyclase catalytic activity using single and double column procedures. *Methods Enzymol* 238: 31–55, 1994.
11. **Kacimi R, Moalic JM, Aldashev A, Vatner D, Richalet J-P, and Crozatier B.** Differential regulation of G protein expression in rat hearts exposed to chronic hypoxia. *Am J Physiol Heart Circ Physiol* 269: H1865–H1873, 1995.
12. **Kacimi R, Richalet J-P, Corsin A, Abousahl E, and Crozatier B.** Hypoxia-induced downregulation of β-adrenergic receptors in heart rat. *J Appl Physiol* 73: 1377–1382, 1992.
13. **Kagiya T, Rocha-Singh KJ, Honbo N, and Karliner JS.** α_1-Adrenoceptor mediated signal transduction in neonatal rat ventricular myocytes: effects of prolonged hypoxia and reoxygenation. *Cardiovasc Res* 25: 609–616, 1991.
14. **Karliner JS, Simpson PC, Honbo N, and Woloszyn W.** Mechanism and time course of beta 1-adrenoceptor desensitization in mammalian cardiac myocytes. *Cardiovasc Res* 20: 221–228, 1986.
15. **Karliner JS and Simpson PC.** β-Adrenoceptor and adenylate cyclase regulation in cardiac myocyte growth. *Basic Res Cardiol* 83: 655–663, 1988.
16. **Kudej RK, Iwase M, Uechi M, Vatner DE, Oka N, Ishikawa Y, Shannon RP, Bishop SP, and Vatner SF.** Effects of β-adrenergic receptor stimulation in mice. *J Mol Cell Cardiol* 29: 2735–2746, 1997.
17. **Laycock SK, McMurray J, Kane KA, and Parrat JR.** Effect of chronic norepinephrine administration on cardiac function in rats. *J Cardiovasc Pharmacol* 26: 584–589, 1995.
18. **León-Velarde F, Richalet J-P, Chavez J-C, Kacimi R, Rivera-Chira M, Palacios JA, and Clark D.** Hypoxia and normoxia-induced reversibility of autonomic control in Andean guinea pig heart. *J Appl Physiol* 81: 2229–2234, 1996.
19. **Li H-T, Long CS, Rokosh DG, Honbo NY, and Karliner JS.** Chronic hypoxia differentially regulates α_1-adrenergic receptor subtype mRNAs and inhibits α_1-adrenergic receptor-stimulated cardiac hypertrophy and signaling. *Circulation* 92: 918–925, 1995.
20. **Maher JT, Denniston SC, Wolfe DL, and Cymerman A.** Mechanism of the attenuated cardiac response to β-adrenergic stimulation in chronic hypoxia. *J Appl Physiol* 44: 647–657, 1978.
21. **Maher JT, Manchanda SC, Cymerman A, Wolfe DL, and Hartley H.** Cardiovascular responsiveness to β-adrenergic stimulation and blockade in chronic hypoxia. *Am J Physiol* 228: 477–481, 1975.
22. **Mardon K, Merlet P, Syrota A, and Mazière B.** Effects of 5-day hypoxia on cardiac adrenergic neurotransmission in rats. *J Appl Physiol* 85: 890–897, 1998.
23. **Mazzeo RS, Wolfel EE, Butterfield GE, and Reeves JT.** Sympathetic responses during 21 days at high altitude (4,300 m) as determined by urinary and arterial catecholamines. *Metabolism* 43: 1226–1232, 1994.
24. **Munson PJ and Rodbard D.** Ligand: a versatile computerized approach for the characterization of ligand binding systems. *Anal Biochem* 107: 220–239, 1980.
25. **Neuman J, Schmitz W, Scholz H, Von Meyerinck L, Doring V, and Kalmar P.** Increase in myocardial Gi-proteins in heart failure. *Lancet* 2: 936–938, 1988.
26. **Patel MB, Stewart JM, Loud AV, Anversa P, Wang J, Fiegel L, and Hintze TH.** Altered function and structure of the heart in dogs with chronic elevation in plasma norepinephrine. *Circulation* 84: 2091–2100, 1991.
27. **Premont RT, Matsuoka I, Mattei MG, Pouille Y, Deger N, and Hanoune J.** Identification and characterization of a widely expressed form of adenylyl cyclase. *J Biol Chem* 271: 13900–13907, 1996.
28. **Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, and Reid L.** Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. *Am J Physiol Heart Circ Physiol* 236: H818–H827, 1979.
29. **Reithmann C, Gierschik P, Sidiropoulos D, Werdan K, and Jakobs KH.** Mechanism of noradrenaline-induced heterologous desensitization of adenylate cyclase stimulation in rat heart muscle cells: increase in the level of inhibitory G-protein alpha-subunits. *Eur J Pharmacol* 172: 211–221, 1989.
30. **Richalet J-P.** The heart and adrenergic system in hypoxia. In: *Hypoxia*: *The Adaptations*, edited by Sutton JR, Coates G, and Remmers JE. Philadelphia, PA: BC Decker, 1990, p. 231–240.
31. **Rocha-Singh K, Honbo NY, and Karliner JS.** Hypoxia and glucose independently regulate the β-adrenergic receptor-adenylate cyclase system in cardiac myocytes. *J Clin Invest* 88: 204–213, 1991.
32. **Rostrup M.** Catecholamines, hypoxia and high altitude. *Acta Physiol Scand* 162: 389–399, 1998.
33. **Sethi R, Elimban V, Chapman D, Dixon IMC, and Dhalla NS.** Differential alterations in left and right ventricle G-proteins in congestive heart failure due to myocardial infarction. *J Mol Cell Cardiol* 30: 2153–2163, 1998.
34. **Voelkel NF, Hegstrand L, Reeves JT, McMurty IF, and Molinoff PB.** Effects of hypoxia on density of β-adrenergic receptors. *J Appl Physiol Respir Environ Exercise Physiol* 50: 363–366, 1981.
35. **Xiao RP, Cheng H, Zhou Y-Y, Kuschel M, and Lakatta EG.** Recent advances in cardiac β2-adrenergic signal transduction. *Circ Res* 85: 1092–1100, 1999.
36. **Yamori Y, Tarazi RC, and Ooshima A.** Effect of β-receptor-blocking agents on cardiovascular structural changes in spontaneous and noradrenaline-induced hypertension in rats. *Clin Sci (Colch)* 59: 457–460, 1980.