Desensitization of the Pulmonary Adenylyl Cyclase System
A Cause of Airway Hyperresponsiveness in Congestive Heart Failure?
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OBJECTIVES
This study was designed to investigate whether the adrenergic signal transduction in the lung and the responsiveness of airway smooth muscle to adrenergic stimulation are modulated in congestive heart failure.

BACKGROUND
Wheezing and airway hyperresponsiveness are often present in heart failure. In the failing heart, chronic adrenergic stimulation down-regulates beta-adrenergic receptors and adenylyl cyclase. We hypothesized that airway dysfunction in heart failure could be due to a similar modulation of pulmonary adrenergic signal transduction.

METHODS
Heart failure was induced in rats by aortic banding, resulting in increases in plasma norepinephrine, lung wet weight indicating congestion and left ventricular end diastolic pressure after four weeks. Beta-receptor densities in pulmonary plasma membranes were measured by radioligand binding using [125I]iodocyanopindolol. The G protein levels were determined by Western blot. Adenylyl cyclase activities in lung membranes were quantified as [32P]cAMP (cyclic adenosine-5'-monophosphate) synthesis rate. To functionally assess airway smooth muscle relaxation, carbachol-precontracted isolated tracheal strips were used.

RESULTS
Beta-receptor density was significantly decreased in heart failure from 771 ± 89 to 539 ± 44 fmol/mg protein without changes in receptor affinities. The beta1-/beta2-subtype ratio, however, remained constant. The Gia and Gs alpha protein expression was unchanged. Adenylyl cyclase activity stimulated directly with forskolin was decreased by 28%. Relaxation of tracheal strips in response to isoproterenol and forskolin, but not to papaverin, was diminished by 30%.

CONCLUSIONS
In heart failure, the down-regulation of pulmonary beta-receptors and concomitant decrease in adenylyl cyclase activity result in a significant attenuation of cAMP-mediated airway relaxation. These mechanisms may play a pivotal role in the pathogenesis of "cardiac asthma." (J Am Coll Cardiol 1999;34:848–56) © 1999 by the American College of Cardiology

Dyspnea is one of the most distressing symptoms of congestive heart failure (CHF). Its clinical features often resemble asthma attacks, having led to the term “cardiac asthma” originally coined by Hope in 1835 (1). Since then, airway obstruction has been observed to occur in heart failure both clinically and experimentally (2). Airway function in CHF has been thoroughly studied in the past (2), and there is accumulating clinical evidence that one crucial component of bronchial narrowing in CHF is hyperresponsiveness to cholinergic stimuli with subsequent constriction of airway smooth muscles (3–7).

Various mechanisms have been proposed to be involved in the airway hyperresponsiveness associated with CHF, including constriction by vagal reflexes, unspecific bronchial C-fiber activation, thickening of bronchial walls, changes in epithelial sodium and water transport and increased endothelin levels (2,8). The potential role of alterations in the adenylyl cyclase (AC)-dependent signal transduction system of the respiratory tract, however, has seldom been taken into consideration. This pathway, which is pivotal for the regulation of airway smooth muscle tone, is known to be extensively regulated in the failing myocardium.

Heart failure is associated with an early and progressive sympathetic nervous hyperactivity (9), resulting in down-regulation of myocardial beta-adrenergic receptors and a decrease in their mRNA levels (10–13). It has long been known that the beta-receptor-mediated inotropic responses of the heart are attenuated in CHF (14). Beyond the level of adrenergic receptors, CHF results in an increased expression...
of cardiac inhibitory G proteins (15,16) and a selective reduction in the mRNA levels of the myocardial AC isoform type V (17), a finding associated with a diminution of receptor-independent cardiac AC activity (17). These changes have been characterized for the cardiac signal transduction system pathway, but respective changes in the respiratory tract have not been investigated. It is conceivable that in CHF, an imbalance in the AC signal transduction system of airway smooth muscles paralleling the changes occurring in the myocardium might ensue a reduction in the bronchial relaxation mediated by beta-receptors and, as a consequence, a sensitization to constrictive stimuli.

A rapid desensitization and uncoupling of beta-adrenergic receptors in lungs from rats treated with catecholamines was described previously (18,19). More recently, chronic administration of catecholamines has been found to induce down-regulation and functional uncoupling of pulmonary beta-receptors, a finding associated with diminished airway relaxant responses to adrenergic agonists (20,21) and with reduced pulmonary mRNA levels for beta2-adrenergic receptors (21,22). In obstructive lung diseases, chronic administration of beta-adrenergic drugs is known to cause homologous desensitization of beta-adrenergic receptors, a finding associated with increased bronchial responsiveness to cholinergic challenge in asthmatics (23). These data support the hypothesis that in CHF the AC signal transduction system in respiratory tissue might play a pivotal role in the development of bronchial hyperresponsiveness. However, this issue has not been clarified thus far.

In the present study, the regulatory mechanisms of the pulmonary AC signal transduction system and the subsequent functional changes in airway smooth muscle responsiveness were investigated in a rat model of CHF induced by aortic banding.

**METHODS**

**Reagents.** Thiopental-Na+ was purchased from BYK-Gulden (Konstanz, Germany), ketamine-HCl was from Parke-Davis (Berlin, Germany) and diazepam was from Hoffmann-La Roche (Grenzach-Wyhlen, Germany). Alprenolol, benzamidine, bovine serum albumin, carbachol (carbamylcholine chloride), forskolin, GTP, Gpp(NH)p, isobutylmethylxanthine, (−)-isopropenol-(−)-bitartrate, papaverin, phenylmethylsulfonylfluoride and Tween-20 were purchased from Sigma (Munich, Germany). The ICI 118,551 was from Tocris/Biotrend (Cologne, Germany). The NaF was from Aldrich (Milwaukee, Wisconsin). Dithiothreitol (DTT) and EGTA were obtained from Serva (Heidelberg, Germany). Bradford reagent was bought from Bio-Rad (Munich, Germany). Cyclic adenosine-5′-monophosphate (cAMP), creatine, phosphocreatine and creatine kinase were purchased from Boehringer (Mannheim, Germany). Both [125I]iodocyanopindolol and [α-32P]ATP were from NEN (Dreieich, Germany). The ECL chemoluminescence Western blotting detection reagent was bought from Amersham (Little Chalfont, UK). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

**Animal experiments.** Male Wistar rats (90 g) obtained from Thomae (Biberach/Riss, Germany) were anesthetized by intraperitoneal injection of ketamine (50 mg/kg body weight) and diazepam (5 mg/kg) and ventilated with oxygen. As described previously (24), the left hemithorax was opened, the ascending aorta was partially occluded using tantalum hemostatic clips with a defined internal diameter of 0.711 mm (Edward Weck, Research Triangle Park, North Carolina) and the wound was sutured (n = 34). Sham-operated animals (n = 23) underwent the same surgical procedure without insertion of the clip. The perioperative mortality ranged at ~10%.

After 3, 7 or 28 days the animals were again anesthetized with thiopental (50 mg/kg i.p., dissolved in isotonic saline). A femoral artery was canulated using a 2F PE 50 line, and a 3.5-ml EDTA blood sample was taken for catecholamine measurements. The rats were tracheotomized and mechanically ventilated at ~50 cycles/min (Animal Respirator 4600, Rhema-Labortechnik, Hofheim/Ts., Germany) with air at a peak pressure of ~15 cm of water. The thorax was opened, and a 0.8-mm stainless steel cannula connected to a pressure transducer was inserted through the free left ventricular wall to record left ventricular pressures on a polygraph (PRC 21 and MVO-0600, FMI, Seeheim/Oberbeerbach, Germany; WeKagraph 350R, WKK, Kaltbrunn, Switzerland). Lungs were rapidly excised, rinsed in ice-cold isotonic saline, quickly blotted, weighed and frozen in liquid nitrogen. Hearts were processed in the same manner after rapid dissection of left and right ventricles. Tissues were stored at −80°C until further analysis.

All animal experiments were performed with official approval by the authorities of the Regierungspräsidium Karlsruhe, Germany.

**Membrane preparation.** For the preparation of plasma membranes, hearts or lungs were homogenized in 35-ml buffer A containing (mmol/liter): Tris-HCl 50; EDTA 5; EGTA 2 (pH 7.2, 4°C), using a Polytron (Kinematika, Lucerne, Switzerland; 10,000 rpm, 3 × 10 s). The proteinase inhibitors benzamidine (1 mmol/liter) and phenylmeth-
ylsulfonylfluoride (1 mmol/liter) were used in all buffers for lung membrane preparation. After sedimentation (350g, 10 min, 4°C) the supernatant was filtered through two layers of cheesecloth. Plasma membranes were collected by centrifugation (48,000g, 10 min, 4°C) and washed twice in 35-ml buffer A by centrifugation (48,000g, 10 min, 4°C). The final membranes were resuspended in 50 mmol/liter Tris-HCl (pH 7.4), to give a final protein concentration of \(-2\) mg/ml. Aliquots were stored at \(-80°C\).

**Radioligand binding.** The density of beta-adrenergic receptors was determined by radioligand binding using the radiolabeled beta-antagonist \([^{125}I]\)iodocyanopindolol (25). For saturation isotherms the membranes were resuspended in 50 mmol/liter Tris-HCl (pH 7.4). Increasing concentrations of the radioligand (10 to 320 pmol/liter) in 50 mmol/liter Tris-HCl, 2 mmol/liter EDTA, and 1 mmol/liter EGTA were incubated in duplicates (pH 7.5, 60 min, 30°C) with plasma membranes (lung: 5 to 10 \(\mu\)g; heart: 15 to 30 \(\mu\)g). For competition binding assays the membranes were resuspended in 75 mmol/liter Tris-HCl, 12.5 mmol/liter MgCl, and 1 mmol/liter EDTA (pH 7.4). The radioligand \([^{125}I]\)iodocyanopindolol was added to give a final concentration of 50 pmol/liter. Increasing concentrations of the beta-agonist isoproterenol (10 pmol/liter to 0.1 mmol/liter) were added in the absence or in the presence of the non-hydrolyzable GTP-analogue Gpp(NH)p (0.1 mmol/liter). To determine the ratio of adrenergic receptors the membranes were washed (3×4 ml, 50 mmol/liter Tris-HCl, pH 7.5) and counted in a \(\gamma\)-counter (Multicrystal, Berthold, Munich, Germany) with a counting efficiency of 84%. Nonspecific binding was determined as the residual binding in the presence of 10 pmol/liter alprenolol.

**Adenyl cyclase activity.** Adenyl cyclase activity was determined according to the method of Salomon et al. (26) modified according to Jakobs et al. (27). Final assay concentrations were: \([\alpha-^{32}P]ATP\) 0.5 mmol/liter (\(\approx 200,000\) cpm/tube), 75 mmol/liter Tris-HCl (pH 7.5), 12.5 mmol/liter MgCl, 1.0 mmol/liter EDTA, 0.1 mmol/liter GTP, 1.0 \(\mu\)mol/liter DTT, 0.1 mmol/liter cAMP, 1.0 mmol/liter isobutylmethylxanthine, 20 mmol/liter phosphocreatine and 10 units/tube creatine kinase. To stimulate AC (−) isoproterenol (0.1 mmol/liter), NaF (10 mmol/liter) or forskolin (0.1 mmol/liter) was added as indicated. The incubation was started by the addition of plasma membranes (lung: \(\approx 10\) \(\mu\)g; heart: \(\approx 60\) \(\mu\)g protein/tube) and stopped after 10 min at 37°C by the addition of 500 \(\mu\)l of NaHCO\(_3\) (120 mmol/liter) and 500 \(\mu\)l zinc acetate (125 mmol/liter). The radiolabeled cAMP was isolated according to the method of Jakobs et al. (27) using aluminum oxide columns. For quantification, Cerenkov radiation was determined with a counting efficiency of 50%.

**Immunoblot analysis of the alpha-subunit of \(G_\alpha_1\) and \(G_\alpha_2\) proteins.** Plasma membrane preparations were subjected to gel electrophoresis (100 \(\mu\)g protein/lane) according to the method of Laemmli et al. (28). The proteins were transferred to nitrocellulose according to Towbin et al. (29). Rabbit polyclonal antibodies directed against the C-terminus of the alpha-subunit of \(G_\alpha_1\) and \(G_\alpha_2\) proteins and against the C-terminus of the alpha-subunit of \(G_\alpha_1\) and \(G_\alpha_2\) proteins were used as primary antibodies (30). Immunocomplexes were visualized by the ECL system. Densitometric analysis of the autoradiograms (X-Omat AR 5 film, Kodak, Rochester, New York) was performed with a laser densitometer (Ultrascan, LKB Pharmacia, Freiburg, Germany) by direct comparison of controls and lung membranes derived from animals with aortic banding.

**Catecholamines.** Norepinephrine levels were measured in deproteinized plasma samples by radioenzymatic assay and electrochemical detection, as described previously (31).

**Protein assay.** Membrane protein concentrations were determined according to the method of Bradford using bovine serum albumin as standard (32).

**Organ bath tracheal contraction experiments.** For functional studies, tracheae were rapidly dissected and allowed to recover for 1 h at room temperature in a modified Krebs-Henseleit buffer containing (mmol/liter): NaCl 116; KCl 4.7; MgSO\(_4\) 1.1; KH\(_2\)PO\(_4\) 1.17; NaHCO\(_3\) 24.9; CaCl\(_2\) 2.52; glucose 8.3; pyruvate 2.0; and EDTA 0.03. The medium was equilibrated with O\(_2\):CO\(_2\) 95%:5%. As described by Lemoine and Overlack (33), tracheal strips, each encompassing three to four cartilaginous rings, were prepared and mounted in 10 ml organ baths containing gassed Krebs-Henseleit buffer at 37°C. Rings were allowed to relax maximally for 30 min under a tension adjusted to 10 mN. At that time, equilibrium conditions were reached. Changes in tension during addition of pharmacologic agonists were recorded under isometric conditions using a force-displacement transducer (Grass, Quincy, Massachusetts), a preamplifier (Hellige, Freiburg, Germany) and a strip chart recorder (Heath, Benton Harbor, Michigan). Carbachol at a concentration of 1 \(\mu\)mol/liter (which in preliminary experiments was found to elicit 80% of maximal contraction) was used to precontract tracheal strips. After 15 min, no further increase in tension was observed. In preliminary experiments, no decline in the contractile response to carbachol had been noted, making subsequent corrections for baseline tone unnecessary. Relaxation in response to isoproterenol added to the organ bath at concentrations from 10 pmol/liter to 10 \(\mu\)mol/liter, to forskolin (1 mmol/liter) and to papaverin (0.1 mmol/liter) was measured under steady-state conditions.
Data analysis. Data reported are mean ± SEM. Statistical comparison of group means was performed using the two-tailed Student t test (34). Saturation and competition curves were established using least-square fitting based on the mass action law (35). Concentration response curves were obtained from multiple regression analysis with variable hillslopes. Statistical comparison of the saturation and concentration response curves was performed by analysis of variance (two-way ANOVA).

RESULTS

Induction of heart failure and neuroendocrine activation. Banding of the ascending aorta resulted in progressive left ventricular hypertrophy, which became highly significant as early as three days after the intervention (Fig. 1A). In addition, significant right ventricular hypertrophy was present after four weeks, reflecting an increase in pulmonary artery pressure due to left ventricular backward failure (Fig. 1B). Left ventricular end diastolic pressure (LVEDP), which was obtained in nine animals after four weeks, increased from 4.6 ± 1.1 to 11.1 ± 1.3 mm Hg (p ≤ 0.005) as compared with control animals. Pulmonary wet weight was also significantly increased after four weeks (Fig. 1C). The increase in lung wet weight was due to pulmonary congestion and not to an increase in cellular elements because total membrane protein content was not different between lungs from animals with CHF and control animals (8.5 ± 1.0 vs. 8.2 ± 0.4 mg/lung). In 20% of the animals, pleural effusions, pericardial effusions and ascites were observed. As a marker of neuroendocrine activation, resting plasma levels of norepinephrine were significantly elevated in CHF. Mean ± SEM. *p ≤ 0.05; **p ≤ 0.0005 (two-tailed t test).

Figure 1. Development of myocardial hypertrophy and heart failure after aortic banding. Organ wet weights are shown relative to body weight (BW). (A) Progressive left ventricular (LV) hypertrophy occurs early after aortic banding (closed squares) as compared with sham-operated rats (open squares). (B) Right ventricular (RV) weight increases in animals with banded aorta as left heart failure develops. (C) Twenty-eight days after aortic banding, lung wet weight is increased significantly, indicating congestive heart failure (CHF). Mean ± SEM. *p ≤ 0.05; **p ≤ 0.0005 (two-tailed t test).

Regulation of beta-adrenergic receptors. The density of beta-adrenergic receptors (Bmax) in lung plasma membranes tended to decrease during the development of CHF, and a significant reduction by 30% was observed after four weeks (Fig. 2). There was no change in the affinity (Kd) of the beta-adrenergic receptor for the radiolabeled antagonist [125I]iodocyanopindolol. Similarly, the affinities of the beta-receptor for the agonist isoproterenol remained unaltered in CHF. Lung homogenates from rats with CHF (n = 3) had a high affinity binding site, with an EC50 of 10.5 nmol/liter versus 15.0 nmol/liter in sham-operated rats (n = 3; NS). The low-affinity binding site had an EC50 of 46.8 μmol/liter in CHF and of 82.7 μmol/liter in controls (NS). The fraction of pulmonary beta-adrenergic receptors forming the agonist-promoted high-affinity state was 55% in CHF (n = 3) versus 52% in control animals (n = 3). The ratio of beta1:beta2-adrenergic receptor subtypes was 20:80 in pulmonary membranes derived from rats with and without CHF. Regression analysis revealed that the decrease in total pulmonary beta-receptor density was dependent on the severity of CHF. Bmax showed a significant negative linear correlation to LVEDP (Fig. 3A). Similarly, Bmax correlated negatively with lung wet weight but was remarkably constant in control animals (Fig. 3B). The decrease of pulmonary beta-adrenergic receptors was paralleled by similar changes of myocardial beta-receptors. After four weeks Bmax was 57 ± 5 fmol/mg membrane protein in the failing myocardium (n = 14) as compared with 68 ± 8 fmol/mg in controls (n = 8; mean ± SEM; p ≤ 0.001). Again, no difference in Kd values was detected (data not shown).

Regulation of G proteins. To assess whether CHF induced a regulation of pulmonary G proteins, expression of the alpha-subunits of Gs and Gi proteins was determined by Western blot. As opposed to the regulation of beta-adrenergic receptors, relative G protein levels were unchanged in lung membranes from rats with CHF. When compared with sham-operated animals, Gi protein levels were 105 ± 20%, 97 ± 3% and 104 ± 2% after 3, 7 and 28 days after aortic banding (n = 3, measured in triplicates). Similar data were obtained for Gs proteins (Fig. 4).

Regulation of AC activity. Total AC activity was determined in pulmonary membrane preparations under basal conditions and after stimulation of the enzyme by 0.1 mmol/liter isoproterenol, by 10 mmol/liter NaF or by 0.1 mol/liter forskolin. At the earlier stages of myocardial hypertrophy (i.e., after three and seven days) no differences in AC activities were observed (data not shown). After 28 days, however, when signs of CHF had developed, lung AC
activity (n = 6) was significantly reduced as compared with control animals (n = 7; Fig. 5). This was true for basal AC activity (p = 0.004) as well as stimulation with isoproterenol (p = 0.002) and forskolin (p = 0.013). The decrease in NaF-stimulated activity remained just below significance (p = 0.056).

As opposed to these alterations in lung membranes, the decreased responsiveness of myocardial AC activity occurred very early in the development of CHF. Forskolin-stimulated enzyme activity in the heart was reduced by 39% after 3 days (n = 5; p = 0.004), by 44% after 7 days (n = 5; p = 0.023) and by 39% after 28 days (n = 13; p = 0.0001). All AC assays were performed in the presence of GTP (see Methods), because control experiments’ omission of GTP reduced cAMP yield by −40% without influencing relative changes (data not shown).

Airway responsiveness to inhibition and stimulation of the adenyl cyclase system. Exposure of tracheal strips to carbachol (1 μmol/liter) induced a submaximal increase in tension (80% of the maximal tension obtained with carbachol at 0.3 mmol/liter). No significant difference between both groups was seen in the response to carbachol. Developed force increased to a similar extent from 5.1 ± 0.6 to 29.6 ± 2.6 mN in rats 28 days after aortic banding (n = 12) and from 6.5 ± 1.3 to 25.1 ± 3.1 mN in controls (n = 12). The response to isoproterenol (10 pmol/liter to 10 μmol/liter) is depicted in Figure 6. Isoproterenol reduced the

Figure 2. Radioligand binding assay for beta-adrenergic receptors in lung membrane preparations from rats with CHF after aortic banding and from control animals was performed using [125I]iodocyanopindolol (A). Saturation isotherms established by the least-squares method are from single data referring to CHF and control animals 28 days after aortic banding. Nonspecific binding (bottom) was determined as the residual binding in the presence of 10 μmol/liter alprenolol. (B) Density (Bmax) and affinity (Kd) of pulmonary adrenergic receptors from rats at 3 days (n = 6), 7 days (n = 11) and 28 days (n = 12) after aortic banding (hatched bars) are compared with respective controls (n = 5, 9 and 10, respectively) (open bars). Mean ± SEM; *p ≤ 0.001 (two-way ANOVA). Kd values (shown in numbers) were not significantly different. (C) Ratio of adrenergic receptor subtypes as determined by two-site competition curves obtained with the beta1-selective receptor antagonist bisoprolol (Biso) and the beta2-selective antagonist ICI 118,551 (ICI). No differences in the beta1-/beta2-subtype ratio of 20:80 were observed between lungs from rats with CHF (after 28 days; n = 3) and from sham-operated control animals (n = 3).

Figure 3. Linear regression analysis (±95% CI) of pulmonary beta-adrenergic receptor density (Bmax) and parameters of left ventricular failure in rats with CHF 28 days after aortic banding. For comparison, sham-operated control are also shown. (A) Left ventricular end diastolic pressure: r = 0.62 (n = 9; p ≤ 0.05). (B) Lung wet weight: r = 0.71 (n = 12; p ≤ 0.05).
tension in strips from CHF rats to a minimum of 19.9 ± 1.2 mN versus 14.7 ± 2.1 mN in control animals (p < 0.05), whereas the EC50 for isoproterenol remained unchanged. Administration of forskolin (1 mmol/liter) in the presence of isoproterenol (0.1 mmol/liter) further relaxed precontracted tracheal strips to 13.6 ± 1.2 mN in CHF rats as compared with 9.9 ± 1.3 mN in controls. The effect of forskolin was significantly (p < 0.05) reduced in CHF, indicating that airway smooth muscle AC activity during direct stimulation was also attenuated (Fig. 7). In contrast, tracheal relaxation after the addition of the direct smooth muscle relaxant papaverin (0.1 mmol/liter) fully reversed the effect of precontraction with carbachol, showing no difference between strips from rats with CHF (5.0 ± 0.7 mN; n = 8) and controls (7.0 ± 2.1 mN; n = 8) (Fig. 7).

DISCUSSION

The present study indicates for the first time that the chronic neuroendocrine activation in CHF may contribute to changes in airway function by means of a regulation of the pulmonary AC signal transduction cascade with a reduction of beta-adrenergic receptors and of AC activity.

Pulmonary beta-adrenergic receptors in CHF. Four weeks after aortic banding in the rat, increases in LVEDP, in pulmonary wet weight suggesting pulmonary congestion and in plasma norepinephrine levels indicated frank CHF. Concomitantly, the density of pulmonary beta-adrenergic receptors is decreased. The extent of this receptor down-regulation in individual animals is significantly correlated to the degree of left ventricular dysfunction.

For the failing myocardium, it is well known that beta-adrenergic receptors are down-regulated (10–13), resulting in a blunted inotropic response of the heart to adrenergic stimuli (14). The present data demonstrate that a similar regulation process may occur concomitantly in CHF in a noncardiovascular organ system (i.e., the lung). The decrease in pulmonary beta-receptor density is similar to that observed during pharmacologic stimulation using beta-adrenergic agonists (20–22). In this study, changes in receptor density could not be attributed directly to specific cell types, but there is evidence that in CHF, beta-receptors are significantly down-regulated in airway smooth muscle cells, resulting in marked changes in airway function. The experiments performed on tracheal strips showed an ∼30% decrease in maximal relaxant response of airway smooth muscle to the beta-adrenergic agonist isoproterenol, comparable to effects observed after desensitization with high doses of isoproterenol (20) or norepinephrine (21).

Autoradiographic studies have shown unanimously that in most species (including humans), beta2-receptors and beta2-receptor mRNA prevail in most pulmonary cell populations, including airway smooth muscle (20,36). The ratio of beta1:beta2-receptor subtypes of 20:80 in pulmonary membranes was not affected by the development of CHF, similar to findings after high-dose catecholamine treatment (21,22). Therefore, the functional and biochemical data of the present investigation give evidence that pulmonary beta2-adrenergic receptors are down-regulated in CHF. This finding is opposite to the concept of selective down-regulation of beta1-receptors, which had been observed in the failing ventricle (10) and has remained controversial (11).

However, as shown recently both by radioligand binding assay and by determination of subtype-specific mRNA levels, a down-regulation of myocardial beta2-receptors in the failing myocardium cannot be easily demonstrated (12).
and may be missed owing to relatively low abundance of that receptor subtype in the myocardium. Therefore, the present data clearly show that beta2-adrenergic receptors, not only the beta1-receptor subtype, may be regulated in CHF.

Analysis of pulmonary beta-receptor binding characteristics revealed that the K_D of the radiolabeled beta-receptor antagonist \([125I]\)iodocyanopindolol was not altered in CHF, which is in accordance with data obtained after chronic catecholamine treatment (21). On the functional level, the EC_{50} or the slopes of dose-response curves to isoproterenol were not altered in CHF, supporting the notion that the affinity of the remaining pulmonary beta-receptor population for the beta-agonist isoproterenol was unchanged. Because chronic sympathetic stimulation during CHF did not change the affinities and the fraction of high-affinity binding sites to the adrenergic agonist isoproterenol, the rapid uncoupling of beta-adrenergic receptors observed after short-term adrenergic stimulation (18,19) appears to be of minor importance as compared with changes in receptor density. However, the possibility that an initial uncoupling of the receptor before down-regulation takes place in the early phase of receptor regulation in CHF cannot be ruled out.

Muscarinic receptor densities were not studied in the present investigation. Although a sensitization to muscarinic agonists has been observed in patients after long-term treatment with a highly selective beta2-adrenergic receptor agonist (23), there are no data available suggesting any regulation of muscarinic M2 or M3 receptors in the lung during CHF. In the present study, no sensitization of airway smooth muscle toward the muscarinic agonist carbachol could be demonstrated on a functional level.

Pulmonary G protein levels in CHF. No change in the expression of the alpha-subunit of stimulatory and inhibitory G proteins (G_{s\alpha}, G_{i\alpha}) in the lung was detected by Western blot analysis. This finding contrasts with the increased G_i protein and mRNA levels in the failing myocardium (15,16). However, little is known about the regulation of pulmonary G protein expression and function during adrenergic stimulation (37). Therefore, the role of the apparent differences in myocardial and pulmonary regulation of G proteins during CHF remains uncertain.

Pulmonary AC activity in CHF. In addition to the desensitization of pulmonary beta-adrenergic receptors, the present investigation shows a marked reduction of total pulmonary AC activity in CHF. The reduction of the enzyme activity in CHF is not confined to receptor-mediated stimulation; it is also observed in the basal state and after direct stimulation of the enzyme using the diterpene forskolin. Hence, these data indicate that chronic neuroendocrine stimulation in CHF also induces a regulation of the pulmonary AC at the postreceptor level.

In the myocardium, a similar reduction of forskolin-stimulated AC activity is observed (17), although this occurs earlier, during the development of "compensated" hypertro-
phy. The differential time-course of AC activity in pulmonary and myocardial tissue during the development of CHF may be due to systemic overflow of norepinephrine during “decompensation,” thus becoming only operative in the lung at this later stage.

In the lung, little is known about the regulation of AC during adrenergic stimulation. Therefore, the marked decrease in receptor-independent enzyme activity described in this study illustrates for the first time that the pulmonary AC isoforms are subject to pathophysiologic regulation processes comparable with the heart. This indicates that CHF may induce postreceptor changes in the adrenergic signal transduction system extending to other organ systems in a generalized fashion. The functional importance of this pathophysiologic regulation in CHF is underlined by the significantly diminished relaxation of tracheal strips in response to forskolin.

In the past, forskolin has been found to be a relatively weak relaxant of airway smooth muscle in guinea pigs (38). In contrast, the present data indicate that direct stimulation of airway AC with forskolin is quite effective. In control animals, the maximal relaxation of carbachol-precontracted tracheal strips achieved with 10 μmol/liter isoproterenol could be augmented by 57% when forskolin was added. Because this effect was even greater in CHF (106%), it may be concluded that, in CHF, the functional antagonism mediated by beta-receptors is diminished to a greater extent than that due to direct activation of AC. This appears conceivable because airway beta-adrenergic receptors may mediate adrenergic stimuli not only by activation of AC but also through various cAMP-independent mechanisms such as opening of membrane KCa and maxi-K channels (37).

Finally, the changes in airway function observed in CHF were not due to intrinsic changes in smooth muscle function, because the maximal relaxant effect of papaverin, bypassing the adrenergic signal transduction cascade, was found unchanged in CHF. This finding indicates that, in CHF, the regulation of the AC system in airway smooth muscle occurs without changes beyond the signal transduction system.

In summary, the data of this study provide experimental evidence that the desensitization of beta-adrenergic receptors and of the AC in CHF is not confined to the heart, but that the lung is a second functionally important target organ for these neuroendocrine regulation processes. They involve both beta2- and beta1-receptor subtypes. Clinically, these newly described mechanisms of impaired pulmonary signal transduction may contribute to the bronchial hyperresponsiveness and to airway narrowing in patients with heart failure—that is, the syndrome of “cardiac asthma.”

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