Association of Decreased Myocardial β-Receptors and Chronotropic Response to Isoproterenol and Exercise in Pigs Following Chronic Dynamic Exercise

H. Kirk Hammond, Francis C. White, Laurence L. Brunton, and John C. Longhurst

The effects of chronic dynamic exercise on myocardial β-adrenergic and muscarinic cholinergic receptors and chronotropic sensitivity to isoproterenol were studied in 5 Yucatan minilswine. Right atrial and left ventricular biopsies, heart rate responses to isoproterenol, and maximal exercise treadmill testing were obtained before and after 10–19 weeks of treadmill running. Radioligand studies using 125I-iodocyanopindolol (ICYP) and 3H-quinuclidinyl benzilate (QNB) were used to determine the number of β-adrenergic and muscarinic cholinergic receptors. Maximal oxygen consumption increased from 52 ± 5 to 65 ± 7 ml/kg/min (mean ± SD; p<0.02), maximal workload from 530 ± 111 to 1,074 ± 179 KPM/min (p<0.01), resting heart rate decreased from 91 ± 13 to 62 ± 4 beats/min (p<0.01), heart rate at 75% of pretraining maximal workload decreased from 253 ± 15 to 196 ± 12 beats/min (p<0.01), and maximal exercise heart rate decreased from 273 ± 6 to 254 ± 9 beats/min (p<0.01). Decreased heart rate responsiveness to adrenergic stimulation was observed following chronic exercise. Maximal isoproterenol-stimulated heart rate decreased from 225 ± 13 to 185 ± 28 beats/min (p<0.05) and the slope of the isoproterenol dose-response relation decreased from 63 ± 16 to 40 ± 16 (p<0.05). Radioligand studies revealed a decrease in β-receptor number in the right atrium following chronic exercise (61 ± 9 vs. 34 ± 8 fmol/mg; p<0.02), but receptor number in membranes from the left ventricle did not change (60 ± 9 vs. 62 ± 4 fmol/mg). There was no change in muscarinic cholinergic receptor number in right atrial membranes following the training period (204 ± 73 vs. 230 ± 92 fmol/mg). Thus, chronic dynamic exercise results in down-regulation of β-adrenergic receptors in the right atrium and is associated with reduced chronotropic responsiveness to exercise and isoproterenol stimulation. (Circulation Research 1987;60:720–726)

Heart rate is altered by chronic dynamic exercise. Resting bradycardia has been observed in most species studied.1–3 Furthermore, heart rate response to submaximal workloads is decreased,4,9 and maximal heart rate also may be diminished.5,6 Withdrawal of vagal tone is complete at low workloads, and further increases in heart rate are achieved by increased sympathetic neural activity and circulating catecholamines. Thus, decreased heart rate response at high workloads reflects either diminished adrenergic tone or decreased responsiveness. Although lower levels of catecholamines are found at matched moderate workloads in humans following chronic exercise, circulating catecholamines are increased or unchanged at high workloads.7,8 Therefore, decreased responsiveness to adrenergic stimulation must occur to account for relative bradycardia at high intensity effort.

An inverse relation between receptor number and level of agonist stimulation has been observed.9 Although β-receptor down-regulation is a compelling explanation of the paradox of decreased heart rate in the presence of increased catecholamines at high workloads following chronic dynamic exercise, no evidence for down-regulation of myocardial β-adrenergic receptors is available.10,11 However, previous studies have measured receptor number in homogenates of left ventricle or whole heart. Since depolarization of the sinoatrial node determines heart rate, tissue from the right atrium may best reflect receptor-dependent changes in heart rate.

Work from our laboratory has established the pig (Sus scrofa) as amenable to long-term treadmill running. Pigs respond to chronic dynamic exercise with significant reductions in chronotropic response to submaximal and maximal workloads.12 Furthermore, the pig heart is sufficiently large to permit serial biopsy samples of right atrium for the study of myocardial β-receptor response to chronic dynamic exercise.

This study was designed to test the hypothesis that chronic dynamic exercise decreases β-adrenergic receptors in the right atrium of pigs and that this decrease is associated with a decreased heart rate response to both exercise and isoproterenol stimulation in con-
scious animals. Additionally, the effects of exercise training on right atrial muscarinic cholinergic receptor number were determined.

Materials and Methods

Five sexually mature Yucatan miniswine (Sus scrofa; 3 males, 2 females), 9 ± 1 months of age, weighing 26 ± 5 kg, were studied before and after chronic dynamic exercise. Three additional female pigs, 11 ± 4 months old, weighing 34 ± 8 kg, served as sedentary controls for the pharmacologic and β-adrenergic receptor studies.

Surgical Procedure

Animals were sedated with thiamylal sodium (25 mg/kg i.v.), intubated, and anesthetized with 1% halothane. Catheters were placed aseptically in an external jugular vein. Distal ends of the catheters were tunneled subcutaneously toward the spine and exited in the interscapular area. A left thoracotomy was performed, the right atrial appendage located, and the distal portion excised (500 mg wet weight), cleared of blood, wrapped in foil, and put into liquid nitrogen (−70°C).

Left ventricular tissue was obtained from the anterior or free wall by means of a high speed electrical drill fitted with a hollow cylindrical bit with an inside diameter of 0.5 cm and a depth of 1.5 cm, suitable for taking transmural sections (400 mg wet weight). After removal of the drill, the hole was closed with a purse-string suture. The sample was put in foil and stored in liquid nitrogen. Prophylactic antibiotics (gentamicin and cefonicid) were given prior to surgery and continued for 2 days. Testing began following recovery from surgery (about 5 days). When the initial pharmacologic tests were complete, the animals were anesthetized with ketamine (50 mg/kg i.m.), and venous catheters were removed. After 8–12 weeks of treadmill running, venous catheters were replaced. The terminal procedure to obtain myocardial tissue occurred after all pharmacologic tests and maximal treadmill tests were complete, either 72 or 96 hours after the last training run. The conditions present when initial myocardial samples were obtained were duplicated. The heart was removed and placed in saline (0°C). The initial biopsy site on the right atrial appendage was located and another sample obtained from an adjacent area, making certain that no scar tissue was included in the sample. An area of the left ventricle in the region of the previous biopsy site that excluded scar tissue was excised. The left ventricle (septum included) was weighed, and tissue samples were stored in liquid nitrogen.

Pharmacologic Testing

Pharmacologic tests were conducted between 5:00 and 8:00 a.m. in a quiet, dark laboratory (21°C). With rare exception, the animals were asleep throughout the testing; restless animals were not tested.

Intrinsic Heart Rate. Intrinsic heart rate, the rate following autonomic blockade with atropine and propranolol, was determined. This measurement is thought to reflect intrinsic myocardial chronotropic function independent of influence from the autonomic nervous system. Previous work from our laboratory has shown that dosages of 0.2 mg/kg propranolol and 0.075 mg/kg atropine significantly block the influences of adrenergic and parasympathetic nerves on resting heart rate.14 Measurements of intrinsic heart rate were made by administering propranolol over 2 minutes, waiting 5 minutes, and then injecting atropine over 1 minute. The heart rate 5 minutes after atropine injection was recorded as the intrinsic heart rate. Reported results are from 2 or 3 studies in both control and trained states.

ISOProterenol Dose-Response Relations. Isoproterenol dose-response relations were assessed by bolus i.v. injection of 1-isoproterenol with doses ranging from 0.01 to 2.0 μg/kg. The heart rate response was recorded until there was no further increase (usually 30 seconds). The 6-second period showing the largest increase in heart rate was defined as the maximal response. The heart rate was allowed to return to baseline between each dose of 1-isoproterenol. Injections were given until there was less than a 5% increase in change in heart rate; the preceding dose was chosen as the one that caused a maximal response. The relation between change in heart rate and the logarithm of the dose of 1-isoproterenol was examined by linear regression analysis. Two or 3 tests conducted on separate days were combined into a single linear regression in the control and trained state in each animal. Correlation coefficients ranged from 0.82 to 0.99 (r = 0.95 ± 0.04). To correct for weight gain, 1-isoproterenol doses were calculated as μg/kg of blood volume. Blood volume was calculated from the body weight using previously published regression equations for the pig.15 The slopes of the linear regression equation were compared between control and trained conditions. Additionally, the maximal heart rates obtained by isoproterenol administration were compared.

Maximal Exercise Protocol

Following completion of pharmacologic testing, the animals were run to exhaustion on a motor-driven treadmill using a graded protocol while heart rate was monitored and expired air collected with a closed system by mask.16 Initial exercise tests were obtained within 2 weeks of the pharmacologic tests. Maximal effort was assessed by observing a plateauing of the relation between workload and heart rate as workload was increased. This was confirmed by a similar plateauing of oxygen consumption. Each test was repeated 2 or more times in control and trained states. During each exercise protocol, animals ran at a fixed speed, and grade was increased by 3% increments every 2 minutes. Expired air was collected during the final minute of higher stages. Heart rates were recorded during the last 20 seconds of each stage.

Exercise Training Protocol

After completion of pharmacologic and maximal exercise testing, chronic treadmill running was begun.
Initially, treadmill speed was 81 m/min and runs consisted of 5-minute stages at 3, 6, and 9% grade. Each week several minutes were added to the 9% grade stage so that each animal eventually ran 5 minutes at 3% and 6%, and 50 minutes at 9%. This protocol was repeated 5–6 times per week. Three animals were trained for 19 ± 1 weeks and 2 for 10 weeks, but running time was increased more rapidly in these 2 animals so that the number of weeks of running 60 minutes per day was not markedly different (9 ± 1 vs. 7 ± 1 weeks, respectively). Heart rates were monitored weekly, and treadmill speed was increased as necessary to achieve training heart rates 75% of control maximal heart rates. Mean speed for training runs was 97 ± 3 m/min, and workloads averaged 300 KPM/min per session. When animals showed a decline in heart rate at matched workloads and had trained at least 6 weeks at 60 min/day, venous catheters were replaced for final pharmacologic testing. Drug tests were performed in the morning and training runs were conducted after the drug tests. The minimal interval between training runs and pharmacologic testing was 20 hours.

The relation between workload and heart rate was assessed by linear regression. This relation was also examined independently of weight by comparing heart rate with product of grade and speed. Heart rates were compared at a workload 400 KPM/min and at an 18% grade at 81 m/min. Correlation coefficients for the linear regressions ranged from 0.68 to 0.96 (r = 0.85 ± 0.09).

Blood samples for serum catecholamine measurements were obtained in the control and trained state during treadmill running. Samples were drawn after the animals were nearly exhausted, which occurred at 15 minutes in the control state and 60 minutes in the trained state. Serum epinephrine and norepinephrine were measured using the method of Durrett and Ziegler. 17

Radioligand Binding

For preparation of membranes, frozen tissue was powdered and then homogenized in glass–glass homogenizing tubes (Kontes) and filtered through Nitex cloth (149 μm). Since nonspecific radioligand binding to membranes collected from this homogenate was high (90% of total signal), the contractile protein extraction described by Baker et al 18 was performed. Homogenates were diluted with an equal volume of 1 M KCl, incubated at 4° C for 10 minutes, and then centrifuged (48,000g for 20 minutes). The pellet was suspended in homogenizing buffer and used directly in the binding assays. The final yield of protein, determined by the method of Bradford, 19 was 3.75 mg per 100 mg of frozen powder, about half the particulate proteins having been removed by the KCl extraction, with no loss of β-adrenergic receptors.

Beta-receptor binding assays were performed using L-[^3]H-cyanopindolol (ICYP), prepared by the method of Engle et al. 20 Assays were performed in triplicate by incubating 150 μl of membrane suspension with 50 μl of ICYP (2,200 Ci/mmol; 10 to 1,200 pM) and 50 μl of various β-adrenergic agonists and antagonists for 60 minutes at 30° C in an oscillating water bath (120/min). Assays were terminated by the addition of 10 milliliters of hypotonic buffer with a mM concentration of Tris CI10, ethylenediaminetetraacetic acid (EDTA) 1, pH 7.5. Bound and free ligand were separated by filtration over glass fiber filters (Whatman GF/C) followed by an additional wash of 10 ml. Radioactivity retained on the filters was quantified by gamma counting; replicate variation was <5%. Nonspecific binding was defined experimentally as radio-label bound in the presence of 10^-6 M d,l propranolol, except values were corrected for the presence of 10^-5 M d,l propranolol when the concentration of radiolabel exceeded 800 pM. Specific binding (total minus nonspecific) accounted for 65% of total ligand bound at 100 pM ICYP. Determinations of agonist affinity were performed by incubating 100 pM ICYP with isoproterenol (10^-4–10^-10 M) in the presence of 100 μM GTP. The apparent Kd for isoproterenol was 176 ± 47 nM for the l-isomer and 2,927 ± 296 nM for the d-isomer (n = 3). Propranolol also showed stereo-selectivity, apparent Kd being 11 nM for the l-isomer and 149 nM for the d-isomer. The racemate competed for ICYP with an apparent Kd of 16 ± 1 nM (n = 3). The binding of ICYP to myocardial membranes was saturable and of high affinity in both the control and trained states. Scatchard analysis of the data resulted in a single line, with high mean values for r^2 (0.90 ± 0.08) suggesting one class of binding sites. 3H-quinuclidinyl benzilate (QNB) (30 Ci/mmol; New England Nuclear, Boston, Mass.) was incubated in a final volume of 3 ml with membranes in an equilibrium binding assay for 120–240 minutes at 32° C. Bound and free QNB were separated by filtration over GF/C filters (Whatman). Filters were washed twice with 10 ml of ice-cold Eagle basal medium diluted 1:10 with water. Nonspecific binding, defined as binding of QNB occurring in the presence of 10^-6 M atropine, was subtracted from total binding to calculate specific binding. Nonspecific binding was <10% of total binding at QNB concentrations near its Kd. Preliminary experiments demonstrated that binding reached equilibrium by 60 minutes and was stable over a 240-minute period. Binding was fully reversible. Less than 15% of added QNB was bound to receptors.

Data Analysis

Data are expressed as the mean ± 1 SD. Specific measurements before and after training were compared using Student’s t test for paired data. The null hypothesis was rejected when p < 0.05 (two-tailed). Linear regression analyses were performed on the isoproterenol dose-response data, on data relating workload and heart rate, and on data relating degree of β-receptor down-regulation with heart rate at rest and at maximal exercise and with duration of training. The Pearson product-moment correlation coefficient (r) is reported as a measure of the strength of association between 2 variables. Data from myocardial β-receptor assay experiments were examined by Scatchard analysis 17 and
Table 1. Heart Rate, Maximal Systemic Oxygen Consumption, and Maximal Workloads Before and After Chronic Dynamic Exercise

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting HR (beats/min)</td>
<td>91 ± 13</td>
<td>62 ± 4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HR@400 KPM/min (beats/min)</td>
<td>253 ± 15</td>
<td>196 ± 12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maximal HR (beats/min)</td>
<td>273 ± 6</td>
<td>254 ± 9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maximal VO₂ (ml/kg/min)</td>
<td>52 ± 5</td>
<td>65 ± 7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Maximal workload (KPM/min)</td>
<td>530 ± 111</td>
<td>1074 ± 179</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± 1 SD. HR, heart rate; KPM, kilopond meters; VO₂, systemic oxygen consumption.

fit best with a single component model. The equilibrium affinity constant (Kₐ) and maximal number of binding sites (Bₘₐₓ) for ICYP were determined; r² is reported as a measure of the goodness of fit of the data with the linear transformation. Receptor number is reported in fmol/mg protein. Competitive binding experiments were analyzed with the nonlinear regression program LIGAND,²² using a model assuming a single class of binding sites.

Results

Evidence for Training Effects

Animals showed physiologic adaptations typical of those associated with chronic dynamic exercise (Table 1). Resting heart rates and heart rate responses at matched workloads were decreased, and maximal workloads and systemic oxygen consumption were increased. Since matched treadmill speed and grade did not represent matched workloads because of significant weight gain in the trained condition (from 26 ± 5 to 40 ± 7 kg, p < 0.001), the relation between workload and heart rate was compared. Heart rate at 400 KPM/min, which represented a control relative workload of 75%, was significantly reduced following chronic dynamic exercise (Table 1). Heart rates at 21% grade and 81 m/min were reduced as well (260 ± 15 vs. 212 ± 12 beats/min, p < 0.001). Maximal heart rate also was decreased significantly. The changes in heart rate at rest and at maximal exercise were highly correlated with the amount of β-receptor down-regulation: for resting heart rate r = 0.90, p < 0.005; for maximal exercise heart rate, r = 0.89, p < 0.005. Maximal oxygen consumption increased by 26 ± 14% (p < 0.01), and maximal workload more than doubled. The average maximal speed and grade were 81 ± 0 m/min and 24 ± 3% in the control period and 103 ± 5 m/min and 29 ± 1% following exercise training.

Serum catecholamines were not significantly increased in trained animals at near exhaustive effort. Epinephrine levels were 454 ± 386 and 838 ± 834 pg/ml, while norepinephrine levels were 1,007 ± 500 and 2,742 ± 3,729 pg/ml before and after training, respectively. The left ventricle to body weight ratios (gm/kg) were not significantly increased compared to mean values in weight-matched sedentary control pigs studied previously in our laboratory (3.8 ± 0.7 vs. 3.4 ± 0.3 gm/kg, respectively.²³

Pharmacologic Testing

Pharmacologic testing revealed significant changes in heart rate response following chronic exercise. Maximal isoproterenol-stimulated heart rate was lower, and the slope of the linear regression equation relating isoproterenol dose and heart rate response was less steep after the exercise period (Figure 1). The intrinsic heart rate decreased from 147 ± 7 to 122 ± 12 beats/min (p < 0.001), and the heart rate following administration of atropine decreased from 189 ± 21 to 141 ± 9 beats/min (p < 0.01) after training.

Receptor Studies

From plots like those shown in Figure 2, the maximal number of binding sites (Bₘₐₓ) and the affinity constants for ICYP binding (Kₐ) were determined from right atrial and left ventricular membranes before and after chronic exercise in each of the animals (Table 2). These results demonstrated a significant down-regulation of right atrial β-adrenergic receptors following chronic exercise in all animals with a mean decrease of 42 ± 22% (p < 0.02; Figure 3). Animals trained for longer periods experienced greater down-regulation (56 ± 11%) than animals trained for shorter periods (20 ± 10%). The amount of down-regulation was significantly correlated with duration of training.

FIGURE 1. Isoproterenol stimulation demonstrates a significant decline in maximal heart rate (A) and slope of dose-response relationship (B) after training. Closed circles with bars represent mean ± 1 SD. Longitudinal data from 5 animals.
Training reduced β-receptors from 75 to 27 fmol/mg (r = 0.87; p < 0.01). Exercise training did not alter the affinity constant for ICYP (0.16 ± 0.07 vs. 0.09 ± 0.02 nM) or the affinity of right atrial membranes for l-isoproterenol (135 ± 34 vs. 101 ± 56 nM; Table 2). Changes in β-receptor number of the left ventricle were variable with 1 animal showing a 26% increase, 1 a 21% decrease and the others changing only slightly. Mean changes were not different (60 ± 9 vs. 62 ± 4 fmol/mg), and the affinity constants for ICYP were similar before and after the exercise period (0.09 ± 0.02 vs. 0.10 ± 0.02 nM). Scatchard analysis of QNB binding data demonstrated that radioligand affinity was invariant with exercise training (28 ± 6 vs. 30 ± 5 pM). Chronic exercise caused no significant change in muscarinic receptor density in right atrial membranes (204 ± 73 vs. 230 ± 20 fmol/mg; Table 2).

To see if the observed pharmacologic and receptor changes were truly the result of exercise or simply the consequence of maturing, β-receptor number was determined and the pharmacologic tests repeated in 3 sedentary pigs before and after a 10-week interval (Table 3). No statistically significant change was found in any of these measurements. Maximal isoproterenol stimulated heart rate increased from 235 ± 7 to 251 ± 18 beats/min, and the slope of the linear regression derived from isoproterenol dose-response data increased 66 ± 18 to 84 ± 28. Intrinsic heart rate decreased from 186 ± 12 to 177 ± 17 beats/ min, and β-adrenergic receptor density increased from 64 ± 10 to 84 ± 20 fmol/mg over the 10-week period.

Discussion

The major new finding of this study was reduced numbers of β-adrenergic receptors in the right atrium following chronic dynamic exercise. Significant reductions in heart rate responses to exercise and isoproterenol stimulation suggests that the observed down-regulation of receptor number is an important mechanism underlying diminished chronotropic responsiveness.

Receptor Changes

Reduction of right atrial β-adrenergic receptor number was observed in each of the 5 animals following chronic exercise, with a mean reduction of 46 ± 22% (p < 0.02). The extent of down-regulation was related directly to the duration of training (r = 0.87; p < 0.01). This is the first demonstration of decreased myocardial β-adrenergic receptor number following chronic exercise.

Previous work on the effects of exercise training on myocardial β-receptor number, 10-11,24-25 while carefully performed, has two major limitations. First, these studies did not look at myocardial receptor number before and after exercise in the same animal but compared separate groups of control and trained animals. Because of individual variation in receptor number, such studies are more prone to type II statistical errors. Second, since most previous studies examined homogenates of whole heart or left ventricle rather than right atrium, they may have missed important changes present only in the atria. It was our belief that β-receptors in the region of the sinus node would be most tightly linked to chronotropic response. Williams et al 25 examined the atria from swimming-trained female rats in a cross-sectional manner but noted no difference in number of binding sites between the 2 groups of animals. However, this result may represent a difference be-

Table 2. Radioligand Binding Results From Animals Before and After Training

<table>
<thead>
<tr>
<th></th>
<th>BAR atrium (fmol/mg)</th>
<th>BAR ventricle (fmol/mg)</th>
<th>MCR atrium (fmol/mg)</th>
<th>K_D-ISO atrium (nM)</th>
<th>K_D-ICYP atrium (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-training</td>
<td>61 ± 9</td>
<td>60 ± 9</td>
<td>204 ± 73</td>
<td>135 ± 34</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>Post-training</td>
<td>34 ± 8*</td>
<td>62 ± 4</td>
<td>230 ± 92</td>
<td>101 ± 56</td>
<td>0.09 ± 0.02</td>
</tr>
</tbody>
</table>

*p < 0.02.
BAR, beta adrenergic receptors; MCR, muscarinic cholinergic receptors; K_D-ISO, affinity constant of receptor for l-isoproterenol; K_D-ICYP, affinity constant at receptor for ICYP.
between species, the type or extent of training, or a type II statistical error.

Small reductions in ventricular $\beta$-receptor number may have gone undetected because of our small sample size and the inherent limitations of radioligand binding techniques. However, there are other possible explanations why this study (and others) have failed to find such changes in the ventricle following chronic exercise. For example, basic differences in innervation between atrium and ventricle may have influenced the extent of adrenergic receptor regulation. Adrenergic nerve terminals are more numerous in atrial than ventricular myocardium. Different responses of $\beta$-adrenergic receptors in atrium and ventricle may reflect less neurotransmitter release in the ventricle and therefore less receptor down-regulation. Such speculation implies that the level of circulating catecholamines is not as important in receptor regulation as local transmitter release. Also, since the atrium has more cholinergic innervation than the ventricle, increased local concentrations of acetylcholine may have influenced $\beta$-receptor number. A recent study showed down-regulation of $\beta$-receptor number in tissues incubated with carbachol, a muscarinic cholinergic agonist.

Heart Rate at Rest and Maximal Exercise

The dependence of training-associated resting bradycardia on a normally innervated myocardium implies an important central component for the bradycardia but does not distinguish between sympathetic or parasympathetic mechanisms. The current study cannot discern the relative importance of increased parasympathetic tone or decreased adrenergic response with respect to resting bradycardia. However, the QNB binding data suggests that a change in the number of muscarinic cholinergic receptors in the right atrium does not cause training-associated resting bradycardia. Finally, the amount of $\beta$-receptor down-regulation was significantly correlated with the reduction in both resting and maximal exercise heart rates, suggesting that right atrial $\beta$-receptor number and heart rate responses are closely linked.

Heart Rate Response to Isoproterenol

It can be argued that changes in chronotropic sensitivity to isoproterenol stimulation reflect variable blood pressure responses and, therefore, variable reflex modulation before and after exercise training. Although blood pressure response during isoproterenol testing was not monitored, previous data suggest an increased vasodilator response to catecholamines in trained compared to sedentary animals. This would serve as an added chronotropic stimulus by reflex vagal withdrawal at the sinus node, thus underscoring our finding of reduced isoproterenol sensitivity in the trained state. Furthermore, data from the maximal exercise studies, where vasodilation in exercising muscle beds is maximal, suggest that diminished chronotropic responsiveness is independent of variations in peripheral vascular tone.

Other investigators studying the chronotropic sensitivity to pharmacologic stimulation following chronic exercise have not detected significant alterations. Both studies showed a tendency toward a decreased responsiveness in trained subjects, but because of individual variability in responses, differences were not statistically significant. Our study has circumvented many of these problems by examining animals in a longitudinal fashion and examining the entire isoproterenol dose-response relation rather than responsiveness to a single dose.

Limitations of Study

Limited amounts of tissue precluded adenylate cyclase stimulation studies and competition experiments in the absence of guanosine triphosphate (GTP). It is possible that decreased chronotropic responsiveness was due to uncoupling of receptors from the regulatory protein or to a change in hormonal responsiveness of adenylate cyclase, independent of receptor number. However, the close correlation between receptor number and chronotropic response favors the interpretation that receptor number and chronotropic response are tightly linked.

Since initial biopsy samples from the sinoatrial node sufficiently large to perform radioligand assays would have destroyed sinus rhythm, the assumption was made that $\beta$-receptor number in the right atrium would reflect $\beta$-receptor number in the sinoatrial node. Beta-receptor number in these 2 regions may, however, be under separate control. Nevertheless, since adrenergic nerve terminals are denser in the sinoatrial node than elsewhere in the atrium and since all measures of chronotropic response were decreased following train-

<table>
<thead>
<tr>
<th>BAR</th>
<th>ISO</th>
<th>Intrinsic</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrium</td>
<td>slope</td>
<td>HR</td>
<td>HR</td>
</tr>
<tr>
<td>Trained (n = 5)</td>
<td>Initial</td>
<td>61 ± 9</td>
<td>63 ± 16</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>34 ± 8†</td>
<td>40 ± 16*</td>
</tr>
<tr>
<td>Sedentary (n = 3)</td>
<td>Initial</td>
<td>61 ± 10</td>
<td>66 ± 18</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>83 ± 19</td>
<td>84 ± 11</td>
</tr>
</tbody>
</table>

Statistical tests compare trained animals before and after chronic exercise: *$p<0.05$, †$p<0.02$, §$p<0.01$, ‡$p<0.001$.

BAR, $\beta$-adrenergic receptor number; ISO slope, slope of the linear relation between isoproterenol dose (log) and heart rate response; ISO max, maximal isoproterenol-stimulated heart rate; HR, heart rate.
ing, the inference that sinoatrial node β-receptor num-
ber decreased seems reasonable.
It is possible that the observed down-regulation of β-adrenergic receptor number was caused simply by internalization of receptors, as has been described follow-
ing agonist infusion,31 and that similar changes may have occurred following a single exercise bout. However, post-training tissue demonstrating down-
regulation was obtained 72–96 hours after the last ex-
nercise bout, a period of time sufficient to allow inter-
nalized receptors to return to the cell surface.31 Therefore, our data suggest that receptor down-regula-
tion occurred by an alteration in the synthesis or degra-
dation of receptor protein.

Conclusions
In this study, chronic dynamic exercise decreased β-
adrenergic receptors in the right atrium of pigs. This finding was associated with decreased chronotropic responsiveness to isoproterenol stimulation in the trained state, as well as reduced resting, submaximal, and maximal chronotropic responses to exercise. These data offer compelling evidence linking β-adrenergic receptor down-regulation with training-associated decreases in heart rate.

Acknowledgment
The authors are very grateful to Jeff Drum and Matt Spellman who assisted in these studies, to Michael Ziegler who performed the catecholamine determina-
tions, to Joan Kantor and Mike Watson who performed the QNB binding studies, and to Joann Fürse who prepared the manuscript.

References
2. Ordway GA, Charles JB, Randall DC, Billman GE, Weckstein DR: Heart rate adaptation to exercise training in cardiac dener-
3. Frick MH, Eloviano RO, Sommer T: The mechanism of bradycar-
dia evoked by physical training. Cardiologia 1967;51:46–54
4. Sigvardson K, Svanfeldt E, Kilbom A: Role of the adrenergic nerv-
ous system in development of training-induced bradycar-
5. Pechar GS, Mc Ardle WD, Katch FI, Magel JR, DeLucia J: Speci-
cificity of cardio-vascular adaptations to bicycle and tread-
8. Ziegler MG, Milano AJ, Hull E: The catecholaminergic re-
9. Motsilsky HJ, Insel PA: The study of cell surface receptors with radioligand binding: Methodology, data analysis, and ex-
10. Moore RL, Riedy M, Gollnick PD: Effect of training on β-
12. Sanders M, White FC, Peterson RM, Bloor CM: Effects of endurance exercise on coronary collateral blood flow in mini-
16. McKirnan MD, White FC, Guth BG, Longhurst JC, Bloor CM: Validation of a respiratory mask for measuring gas ex-
19. Bradford MM: A rapid and sensitive method for the quantifica-
23. White FC, Sanders M, Peterson T, Bloor CM: Ischemic myocar-
27. Limas CJ, Limas C: Carbachol induces desensitization of car-
28. Wiegmans DL, Harris PD, Joshua JG, Miller FN: Decreased wa-
scular sensitivity to norepinephrine following exercise train-
29. Harri MNE: Physical training under the influence of β-adreno-
30. Lehman M, Dickroil HH, Schmidt P, Portig H, Keul J: Plasma catecholamines, beta-adrenergic receptors, and isoproterenol sen-
sitivity in endurance trained and nonendurance trained vol-
31. Snively MD, Ziegler MG, Insel PA: A new approach to deter-
mine rates of receptor appearance and disappearance in vivo. Mol Pharmacol 1984;27:19–26

Key Words • exercise training • adrenergic sensitiv-
ity • β-adrenergic receptors • intrinsic heart rate • exer-
cise bradycardia • muscarinic cholinergic receptors
Association of decreased myocardial beta-receptors and chronotropic response to isoproterenol and exercise in pigs following chronic dynamic exercise.

H K Hammond, F C White, L L Brunton and J C Longhurst

*Circ Res.* 1987;60:720-726
doi: 10.1161/01.RES.60.5.720

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/60/5/720