β-Adrenergic and Cholinergic Receptors in Hypertension-Induced Hypertrophy

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SUMMARY Perinephritic hypertension was produced in dogs by wrapping one kidney with silk and removing the contralateral kidney 1 week later. Mean arterial pressure rose from 104 ± 3 to 156 ± 11 mm Hg, while left ventricular free wall weight, normalized for body weight, was increased by 49%. Muscarinic, cholinergic receptor density measured with [3H]-quinuclidinyl benzilate, fell in hypertensive left ventricles (181 ± 19 fmol/mg, n = 6; p < 0.01) as compared with that found in normal left ventricles (272 ± 16 fmol/mg, n = 8), while receptor affinity was not changed. The β-adrenergic receptor density, measured by binding studies with [3H]-dihydroalprenolol, rose in the hypertensive left ventricles (108 ± 10 fmol/mg, n = 7; p < 0.01) as compared with that found in normal left ventricles (68.6 ± 5.2 fmol/mg, n = 15), while β-adrenergic receptor affinity decreased in the hypertensive left ventricles (10.4 ± 1.2 nM) compared with that found in the normal left ventricles (5.0 ± 0.7 nM). Plasma norepinephrine levels were similar in the two groups, but myocardial norepinephrine levels were depressed (p < 0.05) in dogs with hypertension. Moderate left ventricular hypertrophy induced by long-term aortic banding in dogs resulted in elevations in β-adrenergic receptor density (115 ± 14 fmol/mg) and decreases in affinity (10.4 ± 2.2 nM) similar to those observed in the dogs with left ventricular hypertrophy induced by hypertension. Thus, our results suggest that perinephritic hypertension in the dog induces divergent effects on cholinergic and β-adrenergic receptor density. The increased β-adrenergic receptor density and decreased affinity may be a characteristic of left ventricular hypertrophy rather than hypertension.

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KEY WORDS • β-adrenergic receptor • muscarinic, cholinergic receptor • renal hypertension
Methods

Preparation of Model

After recording baseline levels of arterial pressure in the conscious state for 3 consecutive days by either a catheter implanted in the aorta or a catheter acutely introduced into the femoral artery using local anesthesia with 2% lidocaine HCl, perinephric hypertension was induced with a modification of the method of Page.10 Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with a respirator. The left kidney was exposed through a left flank incision and stripped of fat and fascia. Raw, unbleached silk was wrapped around the kidney and sutured in place. Care was taken to avoid constriction of the renal pedicle and parenchyma. Plastic wrap (Saran Wrap, Dow Chemical Company, Indianapolis, IN) was placed over the silk to prevent the formation of adhesions with other tissues in proximity to the kidney. The animals were allowed to recover for 1 week before the second operation. Pressure again was recorded in the conscious dog, which then underwent anesthesia (sodium pentobarbital, 30 mg/kg). The right kidney was then removed through a right flank incision. Two sham-operated dogs underwent similar operations in which the kidney was stripped of fascia and plastic wrap was laid down beside it during the first operation and nephrectomy was performed 1 week later. In addition, 13 normal dogs were used for the remainder of the control group.

Arterial pressures were measured weekly with the implanted catheter and Statham P23ID strain gauge manometers (Statham Instruments Inc., Oxnard, CA). Blood samples for plasma catecholamine levels were drawn from the implanted catheters in conscious dogs. The data were recorded on a tape recorder (Honeywell RC-2, DuPont Instruments, Inc., Brussels, DE) at 1000g for 15 minutes, and the pellet was resuspended in buffer B (100 mM Tris, 5 mM MgCl₂, 1 mM EDTA, pH 7.2, 4°C) with a glass homogenizer. The homogenization and 45,000g centrifugation were repeated twice. The pellet was resuspended to a protein concentration of 2 to 3 mg/ml and stored at −70°C until assayed. At the time of assay, the membranes were again washed in buffer B and centrifuged at 45,000g for 15 minutes. All studies were performed in triplicate.

For the determination of β-adrenergic receptor density, 100 μL of the cardiac membrane preparation (2–3 mg of protein/ml) was incubated at 37°C for 30 minutes with increasing concentrations (1–30 nM) of [³H](−)dihydroalprenolol ([³H]-DHA; New England Nuclear, Boston, MA), with or without unlabeled d/-propranolol (10 μM), in a final reaction volume of 150 μL. A concentration of 10 μM propranolol was required to inhibit specific binding of ligand competing at the higher concentration of [³H]-DHA. Following incubation, the 150 μL of reaction mixture was rapidly filtered under vacuum onto Whatman GF/C glass fiber filters (Whatman, Inc., Clifton, NJ). The filters were quickly washed (<10 seconds) three times with 4 ml of buffer B, 4°C. The filters were counted for 5 minutes in 10 ml of Hydrofluor (New England Nuclear) in a Delta 300 scintillation counter (Searle Analytic Inc., Des Plaines, IL) with a counting efficiency of 45%. Analysis of saturation binding assays was performed according to the method of Scatchard.13 The data were also analyzed with the iterative curve-fitting program Ligand of Munson and Rodbard.14

The muscarinic, cholinergic receptors were analyzed with binding studies with increasing concentrations (0.05–6.0 nM) of [³H]-quinuclidinyl benzilate ([³H]-QNB, New England Nuclear), with or without atropine, 1 μM. Methacholine, 100 μM, provided results similar to those obtained with atropine. Assay conditions were the same as for the [³H]-DHA binding studies.

The Na-K-ATPase activity was determined by the method of Harris and Popat.15 This is a colorimetric method that measures the hydrolysis of inorganic phosphate from adenosine triphosphate.

Plasma epinephrine and norepinephrine levels in the conscious dogs, as well as tissue norepinephrine levels obtained from LV samples before the animals were killed, were measured using the method of DaPrada and Zurcher.16 The protein concentrations for each membrane assay were determined by the method of Lowry et al.15

Comparison with Left Ventricular Hypertrophy Induced by Aortic Banding

A previous study from this laboratory16 found that LV hypertrophy induced by banding the aorta in puppies resulted in an increase in β-adrenergic receptor density but a decreased receptor affinity (a larger dissociation constant, i.e., Kᵦ); however, the degree of hypertrophy was greater in that group. Accordingly, a subset of these dogs was selected for comparison in...
which the ratio of LV free wall weight to body weight increased by 61%, a value similar to that observed in the current study with LV hypertrophy induced by perinephritic hypertension. The data for the subgroup of dogs with LV hypertrophy induced by aortic banding are compared with data from the dogs with perinephritic hypertension in Table 1.

Statistical Analysis

Data are expressed as means ± SEM. Data were stored in a digital computer (PDP-11/34, Digital Equipment Corp., Maynard, MA), and statistical evaluation was performed by Student’s t test for grouped comparisons.

Results

Hemodynamic Studies

The increases in mean arterial pressure over the course of the development of renal hypertension are shown in Figure 1. Mean arterial pressure rose from control values of 104 ± 3 mm Hg to 129 ± 5 mm Hg following the wrapping of one kidney. There was a further increase in mean arterial pressure to 142 ± 9 mm Hg 1 week after nephrectomy. After 4 weeks, the mean arterial pressure stabilized at 156 ± 11 mm Hg. Mean arterial pressure did not change significantly with time in the control group. There were no differences in heart rate between the hypertensive (101 ± 8.0 beats/min) and control (99 ± 4.0 beats/min) animals.

Pathological Studies

After the animals had been killed, the degree of hypertrophy was determined by comparing the LV free wall weight to body weight ratio in the normal dogs (4.98 ± 0.36 g/kg, p < 0.01) with values from the hypertensive animals (4.98 ± 0.36 g/kg, p < 0.01). The 49% rise in this ratio indicates a moderate hypertrophic process accompanying the renal hypertension. The LV free wall weights from the hypertensive dogs were also significantly greater than those obtained in normal dogs. The normal dogs had an LV free wall weight of 83.4 ± 5.1 g and the hypertensive dogs had an LV free wall weight of 111 ± 7.8 g (p < 0.01).

Muscarinic, Cholinergic Receptor Binding Studies

Specific [3H]-QNB binding to the myocardial membranes was saturable and best characterized by a single binding site according to the iterative nonlinear computer analysis of Munson and Rodbard. The affinity of the normal and renal hypertensive hearts for [3H]-QNB was similar (Kp = 0.30 ± 0.04, n = 6, versus 0.38 ± 0.04 nM, n = 8), whereas cholinergic receptor number was decreased in the renal hypertensive group (181 ± 19 fmol/mg, p < 0.01) compared with the values obtained in the control group (272 ± 16 fmol/mg; Figure 2).

Myocardial β-Adrenergic Receptor Binding Studies

Specific [3H]-DHA binding to the myocardial membrane preparation was saturable and yielded a single component. Typical computer analyses of [3H]-DHA binding to normal LV myocardial membranes and LV hypertensive membrane preparations are shown in Figure 2. The affinity for [3H]-DHA was decreased in these membranes, as demonstrated by the increase in Kp, in the LV preparations of the hypertensive heart (Kp = 10.4 ± 1.2 nM, n = 7) as compared with that of the normal LV preparations (Kp = 5.0 ± 0.7 nM, n = 15, p < 0.01). The density of binding sites, determined by the computer analysis, was significantly decreased.
FIGURE 2 Scatchard analyses of β-adrenergic receptor saturation binding with 3H-DHA (left) and muscarinic, cholinergic receptor saturation binding with 3H-QNB (right) are shown for a normal left ventricle (triangles) and a left ventricle from a dog with pernephritic hypertension (circles). The β-adrenergic receptor showed an increase in density and decrease in affinity, whereas the cholinergic receptor showed a decrease in density with no change in affinity.

greater in the LV preparations from the animals with hypertension (108 ± 10 fmol/mg of protein, p < 0.01) as compared with that of the normal membranes (68.6 ± 5.2 fmol/mg of protein).

To determine whether the increase in β-adrenergic receptor density in the hypertensive LV membranes reflected a difference in the content of plasma membrane, the membrane preparations were assayed for their content of another plasma membrane-associated protein uninvolved in receptor ligand binding. The membrane-associated activity of ouabain-inhibitable Na-K-ATPase activity fell slightly in the hypertensive preparation (2.85 ± 0.19 to 2.30 ± 0.13 µmol P/mg/hr).

Catecholamine Studies

Before the animals were killed, plasma levels of norepinephrine (204 ± 29 pg/ml) and epinephrine (105 ± 19 pg/ml) in hypertensive dogs were similar to plasma levels of norepinephrine (244 ± 32 pg/ml) and epinephrine (110 ± 13 pg/ml) in normal, conscious dogs. In addition, in anesthetized dogs, LV norepinephrine levels were significantly less (266 ± 66 pg/mg wet weight; p < 0.05) in the renal hypertensive group than in the normal group (493 ± 50 pg/mg wet weight; Figure 3).

Comparison with Left Ventricular Hypertrophy Induced by Aortic Banding

When the groups with LV hypertrophy (pernephritic hypertension and aortic banding) with similar increases in LV free wall weight to body weight ratio were compared, similar increases in β-adrenergic receptor density and K_D were observed in both models of LV hypertrophy (Table 1).

Discussion

The results from previous studies on the effects of chronic hypertension on β-adrenergic receptors are controversial. Even the same model of hypertension in the same species has produced opposite results.1-5 The majority of work in this field has been conducted in rodents, however, and no change or decreases in β-adrenergic receptor density have been found.2-4,7-9 The present investigation examined hearts from dogs with chronic pernephritic hypertension, a model that has been characterized extensively by other investigators.10-21 In contrast to what was observed by the majority of studies in rodents, the density of β-adrenergic receptors increased (57%) in the hearts from dogs with chronic pernephritic hypertension. The increase in β-adrenergic receptor number observed in the present investigation could not be attributed to a generalized reduction in sarcolemma content of the membranes, as the level of other membrane markers (e.g., muscarinic receptors and Na-K-ATPase) actually fell.

It was considered that the upregulation in β-adrenergic receptor number could have been secondary to changes in catecholamines. Because the plasma levels of norepinephrine and epinephrine were similar in the control and hypertensive groups of conscious dogs before they were killed, the change in myocardial β-adrenergic receptor density cannot be attributed to differences in ambient levels of circulating catecholamines. Tissue levels of norepinephrine in the left ventricle were significantly depressed in the group with chronic pernephritic hypertension. This depletion in LV norepinephrine levels is similar to that seen in pressure overload hypertrophy secondary to aortic banding16 and may indicate that the normal adrenergic mechanisms are being exhausted or that in the hypertrophic process the adrenergic innervation has not increased proportionally to the increase in cell volume. It is conceivable that the increased density of β-adrenergic receptors is the reverse of the desensitization process whereby a downregulation of β-adrenergic recep-
tors is found with long-term exposure to high levels of catecholamines.22

Another major difference of the present investigation from previous work in this field was the finding of a decreased affinity for the β-adrenergic receptor; the Kᵢ for 'H-DHA rose from 5 0 ± 0 7 to 10 4 ± 1 2 nM. No other study on hypertension found a substantial change in affinity; however, a previous study from our laboratory that investigated the effects of LV hypertrophy induced by long-term aortic banding observed a marked increase in the Kᵢ for 'H-DHA.16 Because the hypertrophy was more severe in that study, we compared the data from a subgroup of dogs with aortic banding with a similar level of LV hypertrophy to the dogs with perinephritic hypertrophy-induced LV hypertrophy (see Table 1). It was interesting to note that almost identical increases in β-adrenergic receptor number and Kᵢ were observed in both groups of dogs when equivalent levels of hypertrophy were compared.

These data suggest that pressure overload hypertrophy in dogs, whether induced by mechanical obstruction (aortic banding) or perinephritic hypertrophy, results in similar increases in β-adrenergic receptor density and decrease in affinity. It is important to keep in mind that if plasma levels of catecholamines had been elevated, an increase in β-adrenergic receptor density might not have been observed. Although the change in receptor number could be due to the reduction in levels of LV norepinephrine, the change in affinity cannot be explained by decreased catecholamine levels in the heart. Another recent study from our laboratory investigated the effects of chronic cardiac denervation and found severe reductions (98%) in LV norepinephrine levels and increased β-adrenergic receptor density but no change in affinity.24 It is speculative, but conceivable, that the hypertrophic process is associated with expression of a different form of the β-adrenergic receptor. This type of receptor microheterogeneity might explain the altered affinity in the hypertrophied heart.

In contrast to what was observed for the β-adrenergic receptor, muscarinic, cholinergic receptor density decreased from control values with no change in the Kᵢ for 'H-QNB. The decrease in muscarinic, cholinergic receptor number could reflect a decrease in cholinergic innervation, a loss of parasympathetic modulation of sympathetic activity, or a decrease in membrane sarcoplasmic content. In support of the last possibility are the measurements of Na-K-ATPase, which were found to be slightly depressed in the hearts from the dogs with chronic hypertension. In this connection, Lee et al. found depressed levels of Na-K-ATPase in hearts from spontaneously hypertensive rats. The decreased cholinergic receptor density could merely reflect a dilutional effect in response to the increase in myocardial cell size and cellular protein without a concomitant increase in cell membrane receptor content. Alternatively, the sarcoplasmic preparation could be less pure because of contamination with non-myocyte elements. The only other study on muscarinic receptors in hypertension is that by Yamada et al., who found no change with deoxycorticosterone acetate-induced hypertension in rats. In that study the degree of LV hypertrophy (26%) was less than that observed in the present study (49%) in dogs with perinephritic hypertension. Thus, the differences between these two studies could be due to species, model of hypertension, or degree of hypertrophy of the heart.

In conclusion, the increase in β-adrenergic receptor number and decrease in affinity in dogs with perinephritic hypertension may reflect a generalized response to pressure overload hypertrophy and depletion of myocardial catecholamines, rather than other metabolic and hormonal aberrations associated with the hypertensive process.

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