Leukocyte beta-receptor alterations in hypertensive subjects.

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Research Article

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Leukocyte β-Receptor Alterations in Hypertensive Subjects

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Abstract. It has been suggested that β-adrenergic responsiveness is reduced in hypertension. To evaluate a possible alteration in human β-receptors that might account for diminished β-adrenergic responsiveness, we studied leukocytes from hypertensive and normotensive subjects after an overnight rest supine, and then after being ambulatory, a maneuver that increases plasma catecholamines approximately twofold. In supine samples, β-receptor affinity for the agonist isoproterenol was significantly reduced in hypertensives and was associated with a reduction in the proportion of β-receptors binding agonist with a high affinity from 42±6% in normotensive subjects to 25±2% in hypertensives ($P < 0.05$). Alterations in β-adrenergic-mediated adenylate cyclase activity paralleled the differences seen in the β-receptor affinity for agonist. In normotensive subjects, β-receptor density and the proportion of receptors binding agonist with high affinity were reciprocally correlated with plasma catecholamines. However, in the hypertensive subjects these correlations were not evident. Thus, our data suggest an alteration in leukocyte β-receptor interactions in hypertensive subjects, and may represent a generalized defect in β-receptor function in hypertension.

Introduction

In the hypertensive state, the basic hemodynamic abnormality is increased peripheral resistance. Two major factors that may underlie this abnormality are a defect in vascular smooth muscle (1) and/or increased sympathetic tone (2).

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Increased sympathetic tone could lead to enhanced peripheral resistance by (*a*) increasing renin release and thereby causing sodium retention, (*b*) increasing cardiac output, or (*c*) increasing arteriolar constriction, perhaps the most important mechanism for enhanced peripheral resistance.

In arteriolar vascular smooth muscle, the postsynaptic adrenergic receptor population is mainly comprised of α-receptors mediating vasoconstriction and β_2-receptors that mediate vasodilation. An increase in α-receptor responsiveness to catecholamines and/or a decrease in β_2-receptor responsiveness could result in an increase in arteriolar constriction and peripheral resistance. Some investigators have found increased α-adrenergic responsiveness in the hypertensive state (3, 4) although this has not always been observed (5). It has also been suggested that β-adrenergic responsiveness is reduced in the hypertensive state. Studies using several animal models of hypertension have demonstrated reduced responsiveness to β-receptor agonists in vitro (6–8) and in vivo (9). In man, several investigators have found reduced isoproterenol-stimulated cardiac chronotropic response in hypertensive subjects (10, 11), although this finding has not been universal (12).

The molecular basis for a reduction in β-adrenergic responsiveness in hypertension has been investigated in animal models. Studies have focused on changes in the β-receptor per se and in the β-receptor-stimulated adenylate cyclase system, which mediates β-adrenergic response through the synthesis of cyclic AMP (cAMP). Investigators have found a reduction in myocardial β-receptor density in renal hypertensive, deoxycorticosterone-salt hypertensive, as well as spontaneous hypertensive rats (SHR)[1] compared with β-receptor density of controls (13–15). In SHR, myocardial β-receptor affinity for β-adrenergic agonists has also been reported to be reduced (15). Similarly, β-adrenergic-stimulated adenylate cyclase activity has been examined in aortic and cardiac tissue from SHR by several investigators, with the majority of laboratories reporting reduced catecholamine-stimulated catalytic activity (15–18). Thus, using different experimental approaches, many investigators have

1. *Abbreviations used in this paper:* K_H and K_L, dissociation constants of high and low affinity states, respectively; G, guanine nucleotide regulatory protein; Gpp(NH)p, 5′-guanylylimidodiphosphate; IHYP, [^{125}I]iodohydroxybenzylpindolol; R_H and R_L, receptor populations in high and low affinity states, respectively; SHR, spontaneous hypertensive rats.

suggested that a reduction in responsiveness to β-adrenergic agonists occurs in hypertension, and that this reduction may be related to changes in the β-receptor-adenylate cyclase complex.

The β-receptor-adenylate cyclase complex consists of at least three components: the receptor; the catalytic moiety, which catalyzes cAMP synthesis; and the guanine nucleotide regulatory protein (G), which couples hormone occupancy of the receptor to stimulation of catalytic activity (19, 20). Analysis of radioligand binding to the β-receptor has characterized the formation of a high affinity complex between the β-receptor and β-adrenergic agonists (21). The high affinity state has been identified as the ternary complex, agonist-receptor-G (22). Formation of this complex appears to be required for β-adrenergic activation of adenylate cyclase, and reduction in the ability to form the high affinity complex is associated with reduced β-adrenergic responsiveness (23). In vitro studies of the phenomenon of desensitization of the β-receptor in animal model systems (23) have demonstrated a reduction in the number of high affinity complexes that can be detected subsequent to catecholamine exposure.

In man, lymphocytes have been used to monitor β-receptor properties ex vivo, since it has been suggested that changes in lymphocyte β-receptors may reflect alterations in β-receptors on less accessible tissues (e.g., heart and lung) (24–26). In normal subjects we have recently demonstrated that acute elevations of catecholamines within the physiological range result in decreased lymphocyte β-receptor function (27). Using the stimulus of upright posture to increase catecholamines we detected a reduction in the number of high affinity complexes and reduced β-adrenergic-stimulated adenylate cyclase activity. To investigate possible alterations in the β-receptor system in the hypertensive state, we have studied β-receptors from leukocytes of hypertensive subjects before and after acute physiological increases in plasma catecholamines mediated by postural change, and compared them to normotensive control subjects.

Methods

Eight subjects with borderline or mild hypertension (defined as a diastolic blood pressure of 90–105 mmHg measured on at least three occasions in the out-patient clinic) were studied. These subjects, aged 28–44, had uncomplicated essential hypertension without cardiovascular or renal abnormalities and otherwise normal history and physical examinations. The subjects had not taken any antihypertensive drugs for at least 6 wk preceding the study.

The eight normotensive control subjects (age 25–40 yr) had no prior history of hypertension and had casual blood pressures below 130/90 mmHg, as measured on at least six occasions, and had received no medication for at least 2 wk before study. All subjects received a diet containing 150 meq of sodium for at least 3 d before study.

After remaining in bed overnight in the Vanderbilt University Clinical Research Center and before the subject arose, a blood sample was drawn by venipuncture while the subject remained supine in the darkened hospital room. A second blood sample was drawn after the subject had been ambulatory for 3 h, a maneuver which approximately doubles plasma catecholamine levels (28). Samples were analyzed for β-receptor binding and plasma catecholamines. Adenylate cyclase activity was also measured in five normotensive and four hypertensive subjects.

Lymphocytes were isolated from fresh citrated blood according to the method of Boyum (29) and membrane lysates were prepared using the techniques of Aarons et al. (30) as previously described (27).

Radioligand binding studies were performed using [^{125}I]iodohydroxybenzylpindolol (IHYP; Amersham Corp., Arlington Heights, IL). For saturation binding curves, seven concentrations of IHYP were used in each assay. The incubations were as previously described (27). Receptor density (B_{max}) and dissociation constant (K_d) for IHYP were determined from saturation curves of specific IHYP binding that were analyzed by the method of Scatchard (31). Protein concentration was determined by the method of Lowry (32) using bovine serum albumin as a standard.

Receptor affinity for agonist was derived from isoproterenol competition for IHYP binding at 14 concentrations of isoproterenol from 100 μM to 1 nM. To determine the effects of guanine nucleotides, acting through the guanine nucleotide regulatory protein, (G), on β-receptor-agonist interactions, Gpp(NH)p, the hydrolysis-resistant analogue of guanosine triphosphate, was added to half of the incubations containing isoproterenol. IC_{50} for isoproterenol in the absence and presence of Gpp(NH)p was determined from logit transformations of competition curves. For each curve, binding was analyzed by a nonlinear curve fitting procedure using a generalized model for complex ligand-receptor systems (33). For the two affinity state model, estimates were derived for the proportions of the receptor population in the high (R_H) and low (R_L) affinity states and the dissociation constant for the high affinity state (K_H). The dissociation constant of the low affinity state (K_L) was determined experimentally as the inhibition constant (K_i) for isoproterenol competition for IHYP binding in the presence of 0.1 mM Gpp(NH)p. Adenylate cyclase activity was measured as described previously (27) with ~30 μg of lymphocyte protein in a total volume of 50 μl. cAMP was isolated by sequential Dowex and alumina chromatography as described by Salomon et al. (34) and corrected for recovery using [^{3}H]cAMP as the internal standard. Plasma epinephrine and norepinephrine were determined by radioenzymatic assay (35).

The statistical significance of differences was determined by the use of t test for paired and unpaired data as appropriate.

Results

Representative agonist competition binding curves from both hypertensive and normotensive subjects upright and supine are shown in Fig. 1. In normotensive subjects the competition curves were shifted to the right and steepened after assumption of the upright posture. In hypertensives, however, such a change was not seen with posture. In samples taken while supine, the receptor-agonist affinity was lower in hypertensives, producing a significantly higher IC_{50} for isoproterenol competition for IHYP binding in hypertensive subjects (IC_{50} = 272±22 nM) compared with normotensive controls (IC_{50} = 184±24 nM; $P < 0.05$). Additionally the decrease in β-receptor affinity for agonist was associated with a significant decrease in the proportion of receptors binding agonist with a high affinity (%R_H), from 42±6% in normotensive subjects to 25±2% in hypertensive subjects ($P < 0.05$) (Table I, Fig. 2).

The maneuver of upright posture resulted in a greater than twofold increase in plasma epinephrine and norepinephrine lev-

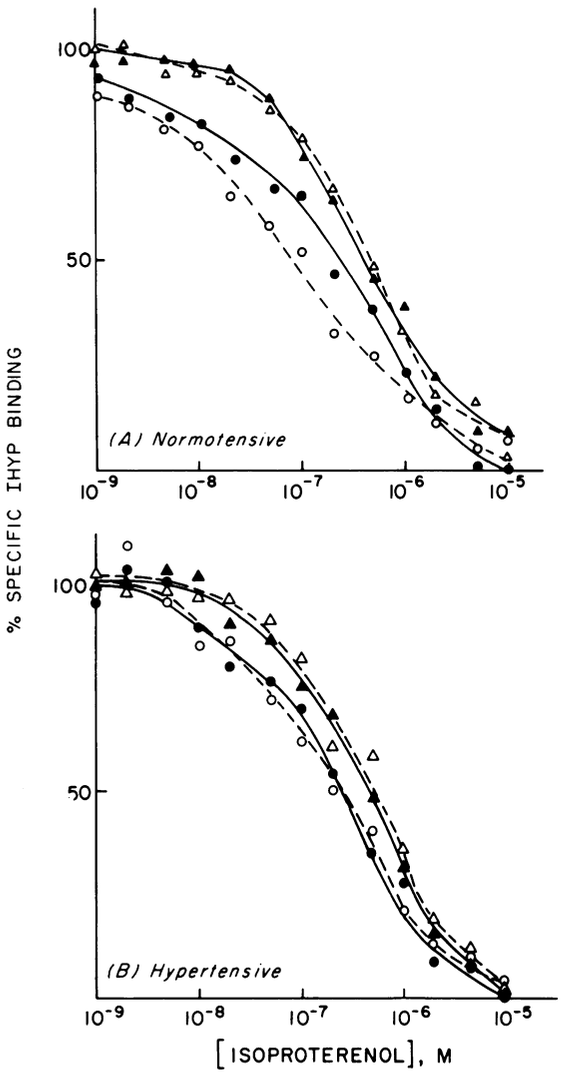

Figure 1. Effect of posture on β-receptor affinity for agonist. In normal subjects (*A*) the isoproterenol competition curve after 3 h of upright posture (●) is shifted to the right compared with the supine sample (○). Addition of Gpp(NH)p to competition curves from both supine (△) and upright (▲) samples resulted in identical curves. In hypertensive subjects (*B*) competition curves from supine samples were steeper and rightward shifted and were not altered in upright samples.

els in both hypertensive and normotensive groups. There were no significant differences in the catecholamine levels between the normotensive and hypertensive groups when the subjects were supine or upright (Fig. 3).

In normal subjects, we have previously demonstrated that the increase in plasma catecholamines induced by the stimulus of upright posture is associated with a concomitant reduction in both β-receptor affinity for agonists (i.e., an increase in IC_{50} for isoproterenol), and in $\%R_H$ (27). In the normotensive subjects

Table I. K_H and K_L and Proportion of Receptors in R_H in Normotensive and Hypertensive Subjects

	Normotensive		Hypertensive	
	Supine	Upright	Supine	Upright
$\%R_H$	42±6	24±3*	25±2‡	28±5
K_H (*nM*)	17±6	12±4	16±5	19±10
K_L (*nM*)	256±25	268±25	205±17	239±27

* $P < 0.05$ compared with supine.
‡ $P < 0.05$ compared with normotensive supine.

studied here, IC_{50} increased from 184±24 to 328±53 nM after 3 h of upright posture ($P < 0.05$). Associated with the decrease in β-receptor affinity for agonist there was also a reduction in $\%R_H$ in normotensive subjects from 42±6% (supine) to 24±3% (upright; $P < 0.05$) (Table I, Fig. 2). It was also of interest that $\%R_H$ was inversely correlated with log plasma norepinephrine ($r = -0.73$, $P < 0.001$) and log plasma epinephrine ($r = -0.57$, $P < 0.05$). Thus, increased plasma catecholamines were associated with decreased β-receptor affinity for agonists and $\%R_H$ in normotensive subjects.

In the hypertensive subjects, however, changes in β-receptor affinity for agonist with upright posture were not evident. No alterations in either IC_{50} for isoproterenol (272±22 nM: supine, to 299±42 nM: upright $P > 0.3$) nor $\%R_H$ (25±2%: supine, to 28±5: upright $P > 0.3$, Table I, Fig. 2) were seen with the same stimulus of upright posture and a virtually identical increase in plasma catecholamines to that observed in normotensive subjects (Fig. 3). K_H and K_L of the receptor for agonist were unaltered

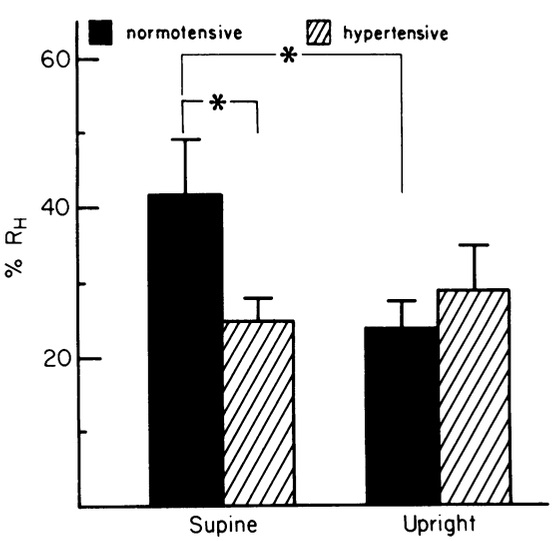

Figure 2. Alteration in R_H with posture in normotensive and hypertensive subjects. *, $P < 0.05$ compared with supine normotensive samples.

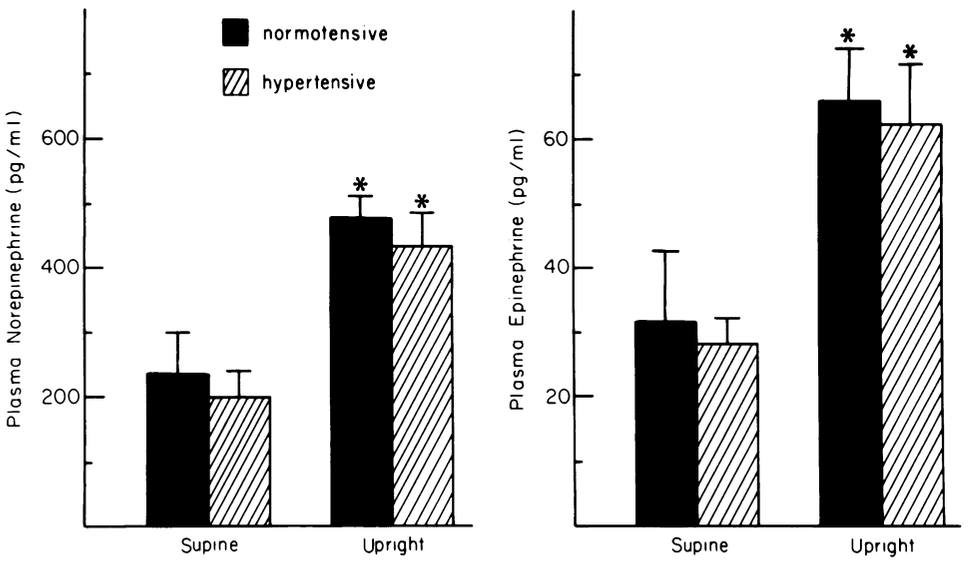

Figure 3. Effect of upright posture on plasma norepinephrine (*left*) and plasma epinephrine (*right*) in normotensive and hypertensive subjects. Values shown are mean±SEM. *, $P < 0.05$ compared with supine samples.

with posture, and did not differ between normotensive and hypertensive subjects (Table I).

The alterations in $\%R_H$ in normotensive and hypertensive subjects with posture were parallelled by the changes seen in β-adrenergic stimulated adenylate cyclase activity (Table II). In the normotensive subjects 0.1 mM isoproterenol-stimulated adenylate cyclase activity decreased >50% with the assumption of upright posture (4.6±1.3 pmol cAMP/min per mg protein: supine to 2.0±1.0 pmol/min per mg protein: upright $P < 0.05$). In contrast, isoproterenol-stimulated adenylate cyclase activity in samples taken from hypertensive subjects when supine were significantly decreased compared with normotensive subjects (0.6±0.4 pmol/min per mg protein; $P < 0.05$) and was not significantly altered with upright posture (1.3±0.6 pmol/min per mg protein). Basal activity did not differ between normotensive and hypertensive subjects nor was it altered with upright posture in either group (Table II).

In contrast to the marked differences in β-receptor-agonist interactions between normotensive and hypertensive groups, β-receptor-antagonist interactions were only subtly altered. No significant differences in B_{max} nor K_D values for the radiolabeled antagonist IHYP were detected between groups (Table III). However, the relationship between plasma catecholamines and B_{max} was altered in the hypertensive subjects. In normal subjects β-receptor density from supine samples was significantly correlated with supine plasma norepinephrine ($r = -0.77$, $P < 0.05$) (Fig. 4 *A*) confirming our previous findings (25), whereas this inverse relationship between supine plasma norepinephrine and B_{max} was not seen in the hypertensive subjects (Fig. 4 *B*).

Table II. Increase in Adenylate Cyclase Activity Over Basal Produced by Isoproterenol and Gpp(NH)p

	Normotensive		Hypertensive	
	Supine	Upright	Supine	Upright
0.1 mM Isoproterenol	4.6±1.3	2.0±1.0*	0.6±0.4*	1.3±0.6
0.1 mM Gpp (NH)p	29.7±6.0	26.9±8.2	19.7±2.6	14.9±5.7
Basal	13.9±2.7	15.4±4.0	9.3±1.8	10.0±3.3

Data are given in picomoles cAMP per milligram protein per minute.
* $P < 0.05$ compared with supine normotensives.

Discussion

As we have previously reported (27), the maneuver of upright posture acutely increases plasma catecholamines and is associated with a reduction of β-receptor affinity for the agonist isoproterenol in normal subjects. This reduction in receptor affinity has been interpreted as representing a functional "uncoupling" of the β-receptor from the guanine nucleotide binding protein of the adenylate cyclase system, thus resulting in an impaired ability to form the "high affinity state" of the receptor for agonist (R_H), the ternary complex, agonist-receptor-G. This reduction in $\%R_H$ has been shown to be associated with reduced β-receptor responsiveness (23, 27). In normal subjects the reduction in receptor affinity for agonist with upright posture is associated with an almost 50% reduction in the proportion of receptors in the high affinity state, $\%R_H$ (Fig. 2) and a comparable reduction in β-adrenergic-mediated adenylate cyclase activity. Also, $\%R_H$ is inversely correlated with plasma norepinephrine concentrations, thus suggesting that physiological increases in plasma catecholamines may be responsible for the reduced ability to form the high affinity receptor complex that is required for β-adrenergic activation of adenylate cyclase.

Our studies indicate that the pattern of β-receptor-agonist interactions is altered in hypertensive subjects. In the hyper-

Table III. β-Receptor-Antagonist Binding in Normotensive and Hyperensive Subjects

	B_{max}		K_d IHYP	
	Supine	Upright	Supine	Upright
	fmol/mg protein	*fmol/mg protein*	*pM*	*pM*
Normotensive	15±1	14±1	49±8	41±6
Hypertensive	18±2	17±2	57±8	52±9

B_{max} and K_d were not different in hypertensive compared with normotensive subjects and were not altered with upright posture.

tensive subjects, under supine conditions, lymphocyte β-receptor affinity for agonist, $\%R_H$, and isoproterenol-stimulated adenylate cyclase activity were significantly reduced compared with normotensives. With the stimulus of upright posture there was no further reduction in receptor affinity for agonist nor in $\%R_H$ (Fig. 2), nor adenylate cyclase activity. This altered pattern in hypertensive subjects may reflect a failure of the dynamic regulation of β-receptor-agonist interaction by catecholamines. Alternatively, it might be that β-receptor affinity for agonist is maximally attenuated under "basal" conditions in hypertensives and thus is not further altered by subsequent stimuli. If this latter possibility is the case, the stimulus for this maximal attenuation of β-receptor affinity under basal conditions is not obvious. In normal subjects we have related increased circulating levels of catecholamines to reduction in both receptor affinity for agonist and $\%R_H$. However the supine plasma catecholamine level was not elevated in the hypertensive subjects and thus could not account for our findings of reduced receptor affinity for agonist and decreased $\%R_H$ in hypertensive individuals. The possibility still exists that blunted β-receptor affinity for agonist in hypertensives might be due to either subtle increases in plasma catecholamines not detectable by single plasma determinations or mediated by other hormones interacting with the β-receptor.

The hypothesis that auto-regulation of the lymphocyte β-receptor is abnormal in hypertensives is further strengthened by our antagonist binding data. This study has confirmed our previous finding (25) that in normal subjects β-receptor density is inversely related to supine plasma catecholamines (Fig. 4 *A*). However, in the hypertensive group, this relationship is not evident (Fig. 4 *B*), again suggesting the possibility that either the regulation of the β-receptor by plasma catecholamines is altered in hypertension or that in hypertensive subjects there is some subtle change in catecholamine levels not reflected by the supine morning catecholamine level.

If our findings in circulating leukocytes reflect a generalized defect in β_2-receptor function in the hypertensive state, then reduced β_2-receptor responsiveness in the vasculature without concurrent reduction in α-receptor sensitivity might well result in abnormally elevated vascular tone. This blunted β-receptor vasodilator responsiveness coupled with normal or elevated

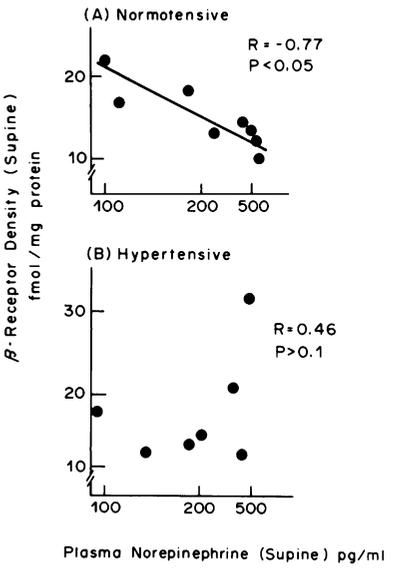

Figure 4. Relationship between β-receptor density and plasma catecholamines in normotensive (*A*) and hypertensive subjects (*B*).

α-receptor vasoconstrictor responsiveness might contribute to increased peripheral resistance and thus to the pathogenesis and maintenance of the hypertensive state. Further studies are required both to define the locus of the abnormality and also to determine its generality.

Acknowledgments

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References

1. Webb, R. C., and D. F. Bohr. 1981. Recent advances in the pathogenesis of hypertension. Consideration of structural, functional, and metabolic vascular abnormalities resulting in elevated arterial resistance. *Am. Heart J.* 102:251–264.

2. Abboud, F. 1982. The sympathetic system in hypertension. *Hypertension.* 4(Suppl. II):II-208–II-225.

3. Mendlowitz, M. 1973. Vascular reactivity in systemic arterial hypertension. *Am. Heart J.* 85:252–259.

4. Vlachakis, N. 1979. Blood pressure response to norepinephrine infusion in relationship to plasma catecholamines and renin activity in man. *J. Clin. Pharmacol.* 19:654–661.

5. Strecker, R. B., W. C. Hubbard, and A. M. Michelakis. 1975. Dissociation constant of the norepinephrine-receptor in normotensive and hypertensive rats. *Circ. Res.* 37:658–663.

6. Kunos, G., B. Robertson, W. H. Kan, H. Preiksaitis, and L. Mucci. 1978. Adrenergic reactivity of the myocardium in hypertension. *Life Sci.* 22:847–854.

7. Cohen, M. L., and A. Berkowitz. 1976. Decreased vascular relaxation in hypertension. *J. Pharmacol. Exp. Ther.* 196:396–406.

8. Godfraind, T., and D. Dieu. 1978. Influence of aging on the

isoprenaline relaxation of aortae from normal and hypertensive rats. *Arch. Int. Pharmacodyn. Ther.* 236:300–302.

9. Katovich, M. J., M. J. Fregly, and C. C. Barney. 1978. Reduced responsiveness to β-adrenergic stimulation in renal hypertensive rats. *Proc. Soc. Exp. Biol. Med.* 158:363–369.

10. McAllister, R. G., D. W. Love, G. P. Guthrie, J. A. Dominic, and T. A. Kotchen. 1979. Peripheral β-receptor responsiveness in patients with essential hypertension. *Arch. Int. Med.* 139:879–881.

11. Bertel, O., F. R. Buhler, W. Klowski, and B. E. Lutold. 1980. Decreased beta-adrenoreceptor responsiveness as related to age, blood pressure and plasma catecholamines in patients with essential hypertension. *Hypertension.* 2:130–138.

12. Leenan, F. H. H., P. Boer, and E. J. D. Mees. 1981. Peripheral β-adrenoreceptor responsiveness in young normotensive and hypertensive subjects. *Clin. Exp. Hyp.* 3:539–553.

13. Woodcock, E., J. Funder, and C. I. Johnston. 1979. Decreased cardiac β-adrenergic receptors in deoxycortisone-salt and renal hypertensive rats. *Circulation.* 45:560–565.

14. Yamada, S., H. I. Yamamura, and W. R. Roeske. 1980. Alterations in central and peripheral adrenergic receptors in deoxycorticosterone/salt hypertensive rats. *Life Sci.* 27:2405–2416.

15. Robberecht, P., J. Winand, P. Chatelain, P. Poloczek, J.-C. Camus, P. DeNeef, and J. Christophe. 1981. Comparison of β-adrenergic receptors and the adenylate cyclase system with muscarinic receptors and guanylate cyclase in the heart of spontaneously hypertensive rats. *Biochem. Pharmacol.* 30:385–387.

16. Bhalla, R. C., R. V. Sharma, and S. Ramanathan. 1980. Ontogenetic development of isoproterenol subsensitivity of myocardial adenylate cyclase and β-adrenergic receptors in spontaneously hypertensive rats. *Biochim. Biophys. Acta.* 632:497–506.

17. Bhalla, R. C., and R. V. Sharma. 1982. Characteristics of hormone-stimulated adenylate cyclase in vascular smooth muscle: altered activity in spontaneously hypertensive rat. *Blood Vessels.* 19:109–116.

18. Hamet, P., D. J. Franks, S. Adnot, and J. F. Coquil. 1980. Cyclic nucleotides in hypertension. *In* Advances in Cyclic Nucleotide Research. P. Hamet and H. Sands, editors. Raven Press, New York. 12:11–24.

19. Ross, E. M., and A. G. Gilman. 1980. Biochemical properties of hormone-sensitive adenylate cyclase. *Annu. Rev. Biochem.* 49:533–564.

20. Limbird, L. E. 1981. Activation and attenuation of adenylate cyclase. *Biochem. J.* 195:1–13.

21. DeLean, A., J. Stadel, and R. J. Lefkowitz. 1980. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J. Biol. Chem.* 255:7108–7117.

22. Limbird, L., D. M. Gill, and R. J. Lefkowitz. 1980. Agonist-promoted coupling of the β-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. *Proc. Natl. Acad. Sci. USA.* 77:775–779.

23. Kent, R. S., A. DeLean, and R. J. Lefkowitz. 1980. A quantitative analysis of beta adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modelling of ligand binding data. *Mol. Pharmacol.* 17:14–23.

24. Colucci, W. S., R. W. Alexander, G. H. Williams, R. E. Rucie, B. L. Holman, M. A. Konstam, J. Wynn, G. H. Mudge, E. Braunwald. 1982. Decreased lymphocyte beta-adrenergic-receptor density in patients with heart failure and tolerance to the beta adrenergic agonist-pirbuterol. *N. Engl. J. Med.* 305:185–190.

25. Fraser, J., J. Nadeau, D. Robertson, and A. J. J. Wood. 1981. Regulation of human leukocyte beta receptors by endogenous catecholamines. *J. Clin. Invest.* 67:1777–1784.

26. Aarons, R. D., and P. B. Molinoff. 1982. Changes in the density of beta adrenergic receptors in rat lymphocytes, heart and lung after chronic treatment with propranolol. *J. Pharmacol. Exp. Ther.* 221:439–443.

27. Feldman, R., L. E. Limbird, J. Nadeau, G. A. FitzGerald, D. Robertson, and A. J. J. Wood. 1983. Dynamic regulation of leukocyte beta adrenergic receptor-agonist interactions by physiological changes in circulating catecholamines. *J. Clin. Invest.* 72:164–170.

28. Robertson, D., G. A. Johnson, R. M. Robertson, A. S. Nies, D. G. Shand, and J. A. Oates. 1979. Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation.* 59:637–643.

29. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* 21(Suppl. 97):77–89.

30. Aarons, R. D., A. S. Nies, J. Gal, and L. Hegstrand. 1980. Elevation of β-adrenergic receptor density in human lymphocytes after propranolol administration. *J. Clin. Invest.* 65:949–957.

31. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. NY Acad Sci.* 51:660–672.

32. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

33. Hancock, A. A., A. L. DeLean, and R. J. Lefkowitz. 1979. Quantitative resolution of beta-adrenergic receptor subtypes by selective ligand binding: application of a computerized model fitting technique. *Mol. Pharmacol.* 16:1–9.

34. Salomon, Y. C., C. Landos, and M. Rodbell. 1973. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* 51:618–631.

35. Passon, P. G., and J. D. Peuler. 1973. A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem.* 51:618–631.