Decreased Beta-Adrenergic Receptor Density in Rat Myocardium during Hemorrhagic Shock

Katsuko MIZUMACHI, Mayumi YAHAGI, Hiroaki KAWABATA, Shinkichi TEZUKA, Tomoko HONDA and Kazuo OKADA

We investigated alterations in the number and affinity of cardiac beta-adrenergic receptors during hemorrhagic shock. Forty male Wistar rats were divided into two groups: (1) a shock group (n = 20), in which mean arterial blood pressure was decreased to 40–50 mmHg by bleeding and kept constant for 6 h; and (2) a control group (n = 20), which underwent a sham operation. We used (−)[^{3}H]dihydroalprenolol for the determination of the number and affinity of beta-adrenergic receptors in myocardial membranes. An additional 25 rats were used for determination of plasma epinephrine and norepinephrine concentrations. Scatchard analysis showed a 20% reduction ($P < 0.05$) in beta-adrenergic receptor density in the shock group (70.3 ± 3.5 fmol·mg^{-1} protein) compared to the control group (90.0 ± 4.8 fmol·mg^{-1} protein) but no significant change in the affinity (2.52 ± 0.06 vs. 2.31 ± 0.09 nmol·l^{-1}, control vs. shock). Plasma catecholamine concentrations were increased significantly at 1, 2, 4 and 6 h after the start of hypotension. These data suggest that increased levels of plasma catecholamines in hemorrhagic shock may be correlated a significant loss of beta-adrenergic receptors in rat myocardium. (Key words: myocardium, beta-adrenergic receptor, down-regulation, plasma catecholamine)

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Desensitization of the beta-adrenergic receptors in response to agonist exposure has been reported in several tissues and in isolated cell systems[1,2]. This phenomenon is particularly important in cardiac receptors in relation to systemic circulation. Karliner et al.[3] reported a 54% reduction in beta-receptor number in myocardial cells when these cells were incubated with isoprenaline for 4 hrs. Chang et al.[4] reported a reduced number of beta-receptors in cardiac membrane from rats receiving chronic isoproterenol. In contrast to these results for exposure to exogenous catecholamines, several investigators have reported desensitization of beta-adrenergic receptors in pathophysiological states, such as endotoxicosis and congestive heart failure, in which plasma concentrations of endogenous catecholamines were increased. Romano et al.[5] reported decreased affinity to beta-adrenergic agonist, but not antagonist binding in rat myocardial membranes at the terminal stages of endotoxic shock. Shepherd et al. reported a decrease in adrenergic responsiveness and beta-receptor density (number) in crude

Department of Anesthesiology, Teikyo University School of Medicine, Tokyo, Japan

Address reprint requests to Dr. Katsuko Mizumachi: Department of Anesthesiology, Teikyo University School of Medicine, 2-11-1 Kaga Itabashi-Ku, Tokyo, 173 Japan

membrane preparations[6] and isolated myocytes from rats after a lethal dose of endotoxin administration[7]. Thomas et al.[8] have proposed a correlation between the plasma norepinephrine concentration and the degree of left ventricular dysfunction in patients with congestive heart failure. However, alterations in beta-adrenergic receptor numbers during hemorrhagic shock have not yet been investigated. It is important to examine whether beta-adrenergic receptors are influenced by the elevated endogenous plasma catecholamines.

To provide one of the possible explanations for the regulations of cardiac responsiveness to endogenous catecholamines during hemorrhagic shock, we evaluated the number and affinity of beta-adrenergic receptors in rat myocardial membranes after a 6 h of hemorrhagic shock in which the state of irreversible cardiac dysfunction may be induced[9].

Materials and Methods

Animal preparation

Sixty-five male nonfasted Wistar rats (6–8 weeks old) were used. In the first series of experiments, 40 rats were divided into two groups: a shock group (n = 20), and a control group (n = 20). The rats were anesthetized with ether, underwent tracheotomy and allowed to breath spontaneously. The anesthesia was maintained during catheterization and the bleeding. Catheters filled with heparinized saline (200 units$\cdot$ml^{-1}) were then placed in the carotid and femoral arteries. The carotid arterial line was used for bleeding and the femoral arterial line was attached to a pressure transducer (P23ID; Gould-Statham Instruments, USA) which was used for continuos blood pressure monitoring. Blood pressure was recorded with a 361 Polygraph (NEC San-ei Co., Tokyo, Japan). After 30 min, the rats were hemorrhaged into a plastic reservoir containing 50 units heparin sodium, and the mean arterial pressure was maintained at 40–50 mmHg for 6 h by movement of blood to or from the reservoir. Control rats were subjected to the same procedure except bleeding. After 6 h of hypotension, the hearts were quickly excised whole, washed in ice-cold saline, and "freeze-clamped" using a Wollenberger clamp precooled in liquid nitrogen. The frozen heart was stored at −80°C, and subjected to cardiac membrane preparation 1 to 7 days later. In the second series of experiments, catecholamine determination was carried out. The remaining 25 rats were subjected to the same experimental protocol as for the hemorrhagic shock group. About 4 ml of blood was collected in heparinized tubes (20 units heparin sodium) from five rats each at 0, 1, 2, 4 and 6 h after the hypotensive events. Blood samples were immediately cooled on ice and centrifuged at 3,000 rpm for 10 min at 4°C. After addition of 5 mM sodium metabisulphite (0.2 ml$\cdot$ml^{-1} plasma) to prevent oxidation. The plasma was stored at −80°C until analysis.

Cardiac membrane preparation

Myocardial cell membranes were prepared according to the procedure of Mukherjee et al.[10] with slight modification by Mukherjee et al.[11]. In brief, 5 hearts (3–4 g) were minced in four volumes of ice-cold buffer (0.25 M sucrose, 5 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4), and homogenized with a Polytron PT10 (setting at 5) for three 5-second bursts with cooling. The homogenate was centrifuged at 1,000 g for 10 min at 4°C. The pellet was discarded and the supernatant was centrifuged at 10,000 g for 10 min at 4°C. The resultant supernatant fluid was carefully decanted from the pellet and then centrifuged at 39,000 g for 10 min at 4°C. The pellet was washed twice with ice-cold incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5) and finally suspended in buffer containing 75 mM Tris-HCl, pH 7.5, and 25 mM $MgCl_2$ at a concentration of 3–4 mg protein$\cdot$ml^{-1}. The yields of membrane protein per gram of heart wt were about 3.0 mg$\cdot$g^{-1} wet wt. Membranes were immediately frozen, and stored at −80°C until radioligand binding assays were performed within 3 months later. Protein concentration was measured by the method of Lowry et al.[12] using bovine serum albumin (Sigma

Chemical, St. Louis, MO) as a standard.

Radioligand binding assay

The radioligand binding assay was performed as described by Mukherjee et al.[10]. Approximately 300–400 μg of membrane protein was incubated with 0.2–11 nM (−)[^{3}H]dihydroalprenolol ([^{3}H]DHA) (99.9 Ci·mmol^{-1}; New England Nuclear, Boston, MA) at 37°C for 10 min in incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5). The total incubation volume was 150 μl. Nonspecific binding was evaluated in the presence of 10 μM (±)propranolol. Binding was terminated by addition of 2 ml of ice-cold incubation buffer followed by rapid vacuum filtration through Whatman GF/C filters (Whatman, Clifton, NJ). The filters were washed immediately with 20 ml of ice-cold incubation buffer, dried at 40°C for 60 min, and placed in a scintillation vial containing 5 ml of scintillation cocktail (Aquasol 2; New England Nuclear, Boston, MA). Radioactivity was counted in a liquid scintillation spectrometer (LSC-703; Aloka, Tokyo, Japan) at an efficiency of 52%. Specific binding was determined by subtracting nonspecific from total binding. The equilibrium dissociation constant (K_D) and the maximum number of binding sites (Bmax) for [^{3}H]DHA were determined from Scatchard analysis[13] of the saturation binding isotherm.

Catecholamine determination

Plasma epinephrine and norepinephrine concentrations were determined by high performance liquid chromatography (HPLC) with a fluorometric detector (FP-540D; Japan Spectro-scopic Co., Ltd.). Initially, 5 ng of carrier catecholamines was added to 1 ml of plasma sample, and the sample was vortexed with 50 mg of alumina for 30 min in 2 ml of Tris buffer (2 M Tris, 20 mg·ml^{-1} EDTA-2 Na, pH 8.6). After centrifugation at 3,000 rpm for 5 min, the alumina was washed twice with deionized distilled water and transferred to a microvial. Catecholamines were extracted from the alumina by vortex mixing in 150 μl of 0.4 N perchloric acid for 30 min. Then, 10 to 50 μl of extract were subjected to HPLC (655; Hitachi, Ltd., Tokyo, Japan) with a silica-ODS column (4.6 × 250 mm). The mobile phase (0.1 M phosphate buffer, pH 2.5, 50 mg·l^{-1} EDTA-2 Na, 120 mg·ml^{-1} 1-octane sulfonic acid sodium salt, 37 ml·l^{-1} acetonitrile) was pumped at a flow rate of 0.7 ml·min^{-1}. The excitation and emission wavelengths were 280 and 315 nm, respectively. These procedures were carried out as previously described[14,15].

Statistical analysis

Student's t test was used for statistical analysis of Bmax and K_D, and one-way analysis of variance was used for the plasma catecholamine concentrations.

Results

Figure 1 shows the mean arterial blood pressure and bleeding volume during hemorrhagic hypotension. The numbers of rats surviving this hemorrhagic shock protocol are given in parentheses. The decrease in bleeding volume after 1 h of the hypotensive period was due to infusion of shed blood.

Figure 2 shows the binding of [^{3}H]DHA to the myocardial membranes prepared from the hemorrhagic shock and control rats. Nonspecific binding varied linearly with radioactive ligand concentration. Specific binding (7.7 ± 0.4–56.1 ± 5.8 fmol·mg^{-1} protein in hemorrhagic shock, 8.3 ± 0.4–76.0 ± 3.9 fmol·mg^{-1} protein in control) was saturable with increasing ligand concentration, and was usually between 84% and 96% of total binding. Figure 3 shows a representative Scatchard plot of the specific binding. Data from 20 rats in the control and the shock groups are summarized in table 1. A linear regression coefficient for the Scatchard plot was always within 0.84–0.99. The number of [^{3}H]DHA binding sites (Bmax) was significantly decreased by 20% ($P < 0.05$) without a change in the affinity.

Figure 4 shows plasma catecholamine concentrations during hemorrhagic hypotension. In both epinephrine and norepinephrine values, a significant increase was observed at 1 h after the start of hypotension and the 2, 4,

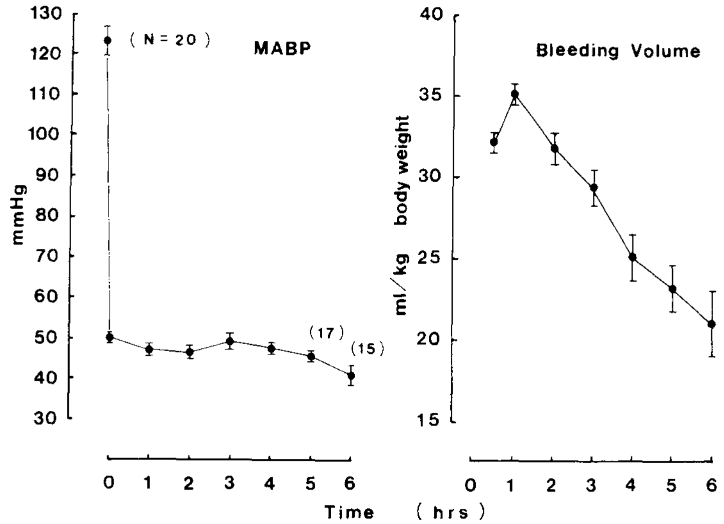

Fig. 1. Mean arterial blood pressure (MABP) and bleeding volume during hemorrhagic hypotension. Rats were bled from the carotid artery, and MABP was held at 40–50 mmHg for 6 h. During the decompensatory phase of hemorrhagic hypotension, the shed blood was reinfused to maintain blood pressure. Values represent means ± SEM. The number of rats that survived the hemorrhagic shock protocol are given in parentheses.

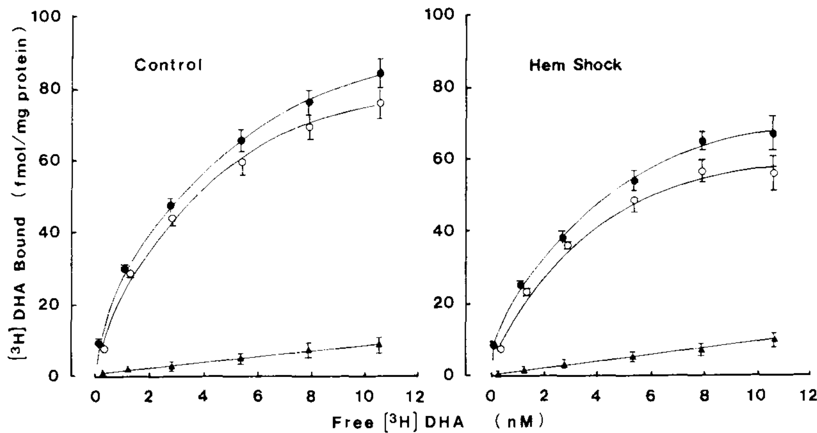

Fig. 2. Binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA) to rat myocardial membranes in the control and hemorrhagic shock group. Specific binding (—○—) was determined by subtracting nonspecific binding (—▲—) from total binding (—●—) . Total binding was determined by measuring the amount of radioactivity retained on filters. Nonspecific binding was determined by measuring the radioactivity when incubations were performed in the presence of 10 μM unlabeled d,l-propranolol. Data shown are mean ± SEM of duplicate determinations using 4 membrane preparations each from hemorrhagic shock and control hearts.

and 6 h values remained elevated.

Discussion

Mechanisms involved in the development of the irreversible state in late hemorrhagic shock have been proposed from various aspects. One of the mechanisms suggested is due to failure of periferal circulation by decresed vasomotor tone and a resultant fall in venous return which in turn may lead to a decrease in coronary flow[16–18]. Other mechanisms are related to the deterioration of cardiac function. Wiggers[19] first suggested that myocardial failure played

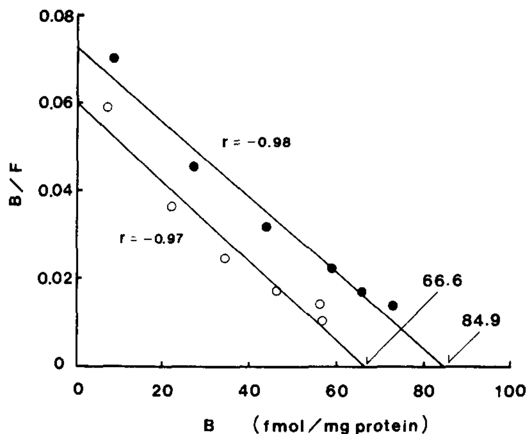

Fig. 3. Representative Scatchard plot of [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to rat myocardial membranes (control, —●—; shock, —○—; B, bound [^{3}H]DHA; F, free [^{3}H]DHA). The lines were drawn by linear regression analysis and the correlation coefficients were as indicated. The abscissa intercept indicates the maximum number of binding sites (Bmax).

Table 1. Maximum Number of [^{3}H]-DHA Binding Sites (Bmax) and Dissociation Constant (K_D) in Cardiac Membranes from Hemorrhagic Shock and Control Rats

	Bmax	K_D	
	fmol·mg^{-1} protein	nmol · l^{-1}	
Control	90.0 ± 4.8	2.52 ± 0.06	r = −0.98 ± 0.01
Hem shock	70.3 ± 3.5*	2.31 ± 0.09	r = −0.94 ± 0.07

^{3}H-DHA: [^{3}H]dihydroalprenolol
Values are mean ± SEM.
*$P < 0.05$ versus control.

an important role in the advanced stages of hemorrhagic shock. His conclusion were based on observation of discrepancy between the increasing the venous pressure and the decreasing cardiac output or arterial pressure in late hemorrhagic shock. Sarnoff et al.[20] reported that insufficient coronary flow and myocardial failure played a complicated role in late hemorrhagic shock. Other studies proposed to explain the impaired cardiac function in hemorrhagic shock include the presence of myocardial depressant factors[21], sympathetic exhaustions[22], uncoupling of excitation-contraction system[23], and myocardial zonal lesions[24].

In clinical point of cardiac dysfunction, desensitization of cardiac adrenergic receptors has been reported in congestive heart failure[8,25]. Receptor regulations that contribute to the desensitization is uncoupling of the receptor from adenylate cyclase and down-regulation (a decrease in receptor number) associated with an agonist-promoted internalization of the receptors. Alterations in total beta-adrenergic receptor number are known to occur later in the sequence of desensitization[26]. Desensitization may provide one of the mechanisms which lead to cardiac dysfunction during hemorrhagic shock. However, available information is few on this point and some of it is controversial[5–7]. Therefore, we evaluated the number and affinity of total beta-adrenergic receptors from cardiac membranes in the advanced stage of hemorrhagic shock.

In our experiments 6 h of hemorrhagic hypotension resulted in a 20% decrease in rat myocardial beta-adrenergic receptor number with no significant changes in affinity for [^{3}H]DHA. Plasma epinephrine and norepinephrine in the present studies were significantly increased at 1, 2, 4 and 6 h after the start of hypotension compared to the prebleeding value (fig. 4). Our findings support the studies of Shepherd et al.[7] in which they reported a significant increase in plasma catecholamines and a 25% reduction in surface beta-receptors on rat isolated myocytes 4 h

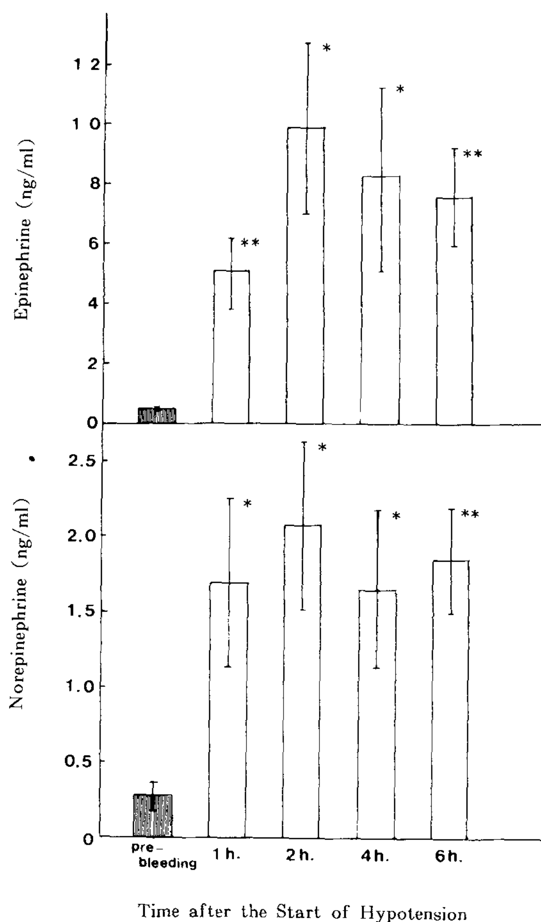

Fig. 4. Plasma epinephrine and norepinephrine concentrations in rats during hemorrhagic hypotension. Each data point represents mean ± SEM of duplicate determinations from five experiments. (*$P < 0.05$, **$P < 0.01$, compared to the prebleeding value.)

after a lethal dose of endotoxin (1 $mg \cdot kg^{-1}$) administration. Farnebo et al.[27] measured catecholamines in plasma and myocardial tissues during 4 h of hemorrhagic shock in both awake and anesthetized rats. They indicated that an increase of plasma catecholamines in the awake rats was greater than in the anesthetized rats at the onset of bleeding, and the tissue content of norepinephrine was not significantly decreased in the heart while a substantial increase of epinephrine was seen after bleeding for 4 h. Their studies indicated that anesthesia depressed the initial sympatho-adrenal response to bleeding, and tissue content of catecholamines has not been depleted in late hemorrhagic shock. These data and our findings suggest that cardiac beta-adrenergic receptors may be down-regulated by endogenous catecholamines during hemorrhagic shock.

Down-regulation of cardiac beta-adrenergic receptors has also been noted in acidosis[28]. In contrast to down-regulation, increase in number of beta-adrenergic receptors (up-regulation) has been demonstrated in ischemic heart[11,29]. Mukherjee et al.[11] produced experimental myocardial ischemia, and showed that significant increase in beta-adrenergic receptor density in ischemic myocardial tissue as compared to nonischemic regions after 1, 3 and 8 h of coronary artery ligation. Maisel et al.[29] demonstrated that beta-receptors were externalized from the intracellular light vesicles to the sarcolemmal fraction in myocardial ischemia. We did not measure physiological responses or metabolites and hence it is not known whether the heart in our experimental animal acutually developed ischemia or acidosis. Shömig et al.[30] have speculated that different phases of norepinephrine release depends on the situation of heterogenous reduction in myocardial blood flows, subsequent condition of heterogenous myocardial ischemia. Norepinephrine release in our experiments showed the pattern of two phases, the early phase (up to 4 h) and the late phase (4–6 h) after the start of hypotension. This suggested that heterogenous myocardial ischemia might be occured during hemorrhagic hypotension in our experiments. Therefore, it is probable that the results we observed appeared to represent an integrated response under dynamic control.

Recent biochemical studies[31,32] have suggested the mechanism involved in receptor recovery after down-regulation and given some evidence about the translocation of receptors between two subcellular compartments. Maisel et al.[31] have shown that 1 h of ischemia promoted externalization of beta-adrenergic receptors whereas 1.5 h after injection of epinephrine (0.25 $mg \cdot kg^{-1}$, ip) promoted internalization of beta-adrenergic

receptors in guinea pig hearts.

In conclusion, decrease in total beta-adrenergic receptors, down-regulation which resulted from an increase of endogenous catecholamines, would be one of the potential mechanisms for cardiac dysfunction in the terminal stages of hemorrhagaic shock.

To understand cardiac function during hemorrhagic shock, further precise studies on mechanism of receptor regulation by measuring surface and internalized receptors in relation to the temporal endogenous catecholamine release in prallel with physiological response are needed.

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References

1. Stiles GL, Caron MG, Lefkowitz RJ: β-Adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol Rev 64:661–743, 1984
2. Harden TK: Agonist-induced desensitization of the β-Adrenergic receptor-linked adenylate cyclase. Pharmacol Rev 35:5–32, 1983
3. Karliner JS, Simpson PC, Honbo N, Woloszyn W: Mechanisms and time course of $beta_1$ adrenoceptor desensitisation in mammalian cardiac myocytes. Cardiovasc Res 20:221–228, 1986
4. Chang HY, Klein RM, Kunos G: Selective desensitization of cardiac beta adrenoceptors by prolonged *in vivo* infusion of catecholamines in rats. J Pharmacol Exp Ther 221:784–789, 1982
5. Romano FD, Jones SB: Characteristics of myocardial β-adrenergic receptors during endotoxicosis in the rat. Am J Physiol 251:R359–R364, 1986
6. Shepherd RE, McDonough KH, Burns AH: Mechanism of cardiac dysfunction in hearts from endotoxin-treated rats. Circ shock 19:371–384, 1986
7. Shepherd RE, Lang CH, McDonough KH: Myocardial adrenergic responsiveness after lethal and nonlethal doses of endotoxin. Am J Physiol 252:H410–H416, 1987
8. Thomas JA, Marks BH: Plasma norepinephrine in congestive heart failure. Am J Cardiol 41:233–243, 1978
9. Wiggers CJ: The physiology of shock. New York: Commonwealth Fund, 1950
10. Mukherjee A, Wong TM, Buja LM, Lefkowitz RJ, Willerson JT: Beta adrenergic and muscarinic cholinergic receptors in canine myocardium: effects of ischemia. J Clin Invest 64:1423–1428, 1979
11. Mukherjee A, Bush LR, McCoy KE, Duke RJ, Hagler H, Buja LM, Willerson JT: Relationship between β-adrenergic receptor numbers and physiological responses during experimental canine myocardial ischemia. Circ Res 50:735–741, 1982
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275, 1951
13. Scatchard G: The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660–672, 1949
14. Ingebretsen OC, Flatmark T: Active and passive transport of dopamine in chromaffin granule ghosts isolated from bovine adrenal medulla. J Biol Chem 254:3833–3839, 1979
15. Jackman GP, Carson VJ, Bobik A, Skews H: Simple and sensitive procedure for the assay of serotonin and catecholamines in brain by high-performance liquid chromatography using fluorescence detection. J Chromatogr 182:277–284, 1980
16. Rothe CF, Selkurt EE: Cardiac and peripheral failure in hemorrhagic shock in the dog. Am J Physiol 207:203–214, 1964
17. Zweifach BW, Fronek A: The interplay of central and peripheral factors in irreversible hemorrhagic shock. Prog Cardiovasc Dis 18:147–180, 1975
18. Koyama S, Aibiki M, Kanai K, Fujita T, Miyakawa K: Role of central nervous system in renal nerve activity during prolonged hemorrhagic shock in dogs. Am J Physiol 254:R761–R769, 1988
19. Wiggers CJ: Present status of the shock problem. Physiol Rev 22:74–123, 1942
20. Sarnoff SJ, Case RB, Waithe PE, Isaacs JP: Insufficient coronary flow and myocardial failure as a complicating factor in late hemorrhagic shock. Am J Physiol 176:439–444, 1954
21. Brand ED, Lefer AM: Myocardial depressant factor in plasma from cats in irreversible post-oligemic shock. Proc Soc Exp Biol Med 122:200–203, 1966
22. Glaviano VV, Klouda MA: Myocardial catecholamines and stimulation of the stellate ganglion in hemorrhagic shock. Am J Phys-

iol 209:751–756, 1965
23. Warner M, Smith JM, Eaton R, Robbins A, Hess ML: The excitation-contraction coupling system of the myocardium in canine hemorrhagic shock. Circ Shock 8:563–572, 1981
24. Hackel DB, Ratliff NB, Mikat E: The heart in shock. Circ Res 35:805–811, 1974
25. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB: Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. N Engl J Med 307:205–211, 1982
26. Lefkowitz RJ, Caron MG: Regulation of adrenergic receptor function by phosphorylation. Curr Top Cell Regul 28:209–231, 1986
27. Farnebo LO, Hallman H, Hamberger B, Jonsson G: Catecholamines and hemorrhagic shock in awake and anesthetized rats. Circ Shock 6:109–118, 1979
28. Marsh JD, Margolis TI, Kim D: Mechanism of diminished contractile response to catecholamines during acidosis. Am J Physiol 254:H20–H27, 1988
29. Maisel AS, Motulsky HJ, Insel PA: Externalization of β-adrenergic receptors promoted by myocardial ischemia. Science 230:183–186, 1985
30. Schömig A, Dart AM, Dietz R, Mayer E, Kübler W: Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: Locally mediated release. Circ Res 55:689–701, 1984
31. Maisel AS, Motulsky HJ, Ziegler MG, Insel PA: Ischemia- and agonist-induced changes in α- and β-adrenergic receptor traffic in guinea pig hearts. Am J Physiol 253:H1159–H1166, 1987
32. Limas CJ, Limas C: Rapid recovery of cardiac β-adrenergic receptors after isoproterenol-induced "down"-regulation. Circ Res 55:524–531, 1984