Platelet Alpha$_2$ Adrenoreceptors in Chronic Congestive Heart Failure

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Patients with chronic congestive heart failure (CHF) are known to have elevated plasma concentrations of norepinephrine. Although this elevation of catecholamines in plasma may facilitate myocardial contractility, it may also be toxic to the myocardium in the long term. The alpha$_2$ adrenoreceptor located on noradrenergic nerve terminals regulates neuronal norepinephrine release by feedback inhibition. This receptor is also located on human blood platelets. This study determines the status of platelet alpha$_2$ adrenoreceptors in 16 patients with CHF (class I and II in 7 and class III and IV in 9) and in 26 normal volunteers. Specific high-affinity binding of the alpha$_2$ agonist ^{3}H-clonidine and the alpha$_2$ antagonist ^{3}H-yohimbine was used to determine the number (B_{max}) of alpha$_2$ receptors and the dissociation constant (K_D) for the 2 ligands. In the control population, the B_{max} (in fmol/mg protein) for ^{3}H-clonidine was 33 ± 2 and for ^{3}H-yohimbine was 165 ± 12. There was a 25% difference in the maximum number of specific binding sites for ^{3}H-clonidine in the class III/IV group (B_{max} 24 ± 2, $p < 0.05$) and a 43% difference in the maximum number of specific binding sites for ^{3}H-yohimbine (B_{max} 94 ± 9; $p < 0.005$). There was a smaller but nonsignificant difference in the number of receptors on platelets from patients in the class I and II group. The K_D's were similar in all 3 groups. These differences correlated well with the increases in plasma norepinephrine levels between the normal group (273.8 ± 44.1 pg/ml) and the class III/IV group (1333.5 ± 244.9, $p < 0.0005$). This study supports the hypothesis that increased levels of circulating norepinephrine in CHF lead to a decrease in platelet alpha$_2$ adrenoreceptors. Further studies should be performed to determine whether pharmacologic stimulation of these receptors might lead to a decrease in the neuronal release of that norepinephrine which might be toxic to the myocardium. Monitoring of platelet alpha$_2$ adrenoreceptor number may provide a guide to therapy of CHF.

Patients with chronic congestive heart failure (CHF) are known to have elevated concentrations of circulating norepinephrine. The degree of elevation is related to the degree of left ventricular dysfunction.[1–3] Although the circulating norepinephrine may increase myocardial contractility, persistently elevated levels have been shown to be toxic to the myocardium in experimental animals[4–6] and in humans.[7,8]

The alpha$_2$ adrenoreceptor is located presynaptically on noradrenergic nerve terminals and has been shown to exert a negative feedback inhibition upon norepinephrine release when stimulated.[9–11] If neurogenically released norepinephrine has a detrimental effect on the myocardium, alterations in alpha$_2$ adrenoreceptor function might be of potential importance in CHF. Human blood platelets have been suggested as a model for the indirect study of changes in nerve cell function.[12] Studies which use receptor binding techniques and which measure the relative order of potencies of various adrenergic agonists and antagonists in displacing bound radioligand have shown that human platelets have alpha$_2$ adrenoreceptors similar to those present on noradrenergic nerve terminals.[13,14] Changes in the number and affinity of alpha$_2$ adrenoreceptor binding sites in the rat brain after various interventions have been well correlated with similar changes on human blood platelets.[14–17] These have also been correlated with changes in norepinephrine release and tension developed in field stimulation experiments upon isolated rat atrial strips.[18]

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The hypothesis of the present study was that alpha$_2$ adrenoreceptors on human blood platelets would be decreased by the increased circulating levels of norepinephrine found in severe chronic CHF and as such mirror changes found in the presynaptic site.

Methods

Patient population: Blood was obtained by venipuncture from healthy male and female volunteers (mean age 40 ± 3 years, n = 26). These control subjects were compared with patients with chronic CHF who were selected and classified by history, physical examination, and invasive hemodynamic monitoring findings. The patients were divided into standard New York Heart Association[19] class I and II (Group 1: mean age 62 ± 2 years, n = 7) and class III and IV groups (Group 2: mean age 68 ± 4 years, n = 9). The cause of the CHF was valvular (Group 1 = 3, Group 2 = 2), ischemic (Group 1 = 3, Group 2 = 5), and idiopathic (Group 1 = 1, Group 2 = 2). The 2 groups did not differ in the distribution of causes. Previous work[16] has shown the lack of age or sex dependency of either the total number or the affinity of platelet alpha$_2$ adrenoreceptors. None of the patients were taking alpha-adrenergic agonists or antagonists, indirectly acting adrenergic drugs, or inhibitors of norepinephrine uptake for at least 1 month before the study.

Written informed consent was obtained from all patients. This study was approved by the University of Michigan's Institutional Review Board. All assays were done without the knowledge of the patient's clinical characteristics.

Isolation of platelet membranes and radioligand binding assay: Platelet membranes were obtained by the method described by Garcia-Sevilla et al.[14] Briefly, 50 ml of blood was collected in polyethylene tubes which contained acid-citrate dextrose (ACD, 8:1, volume/volume). The blood was centrifuged at 160 *g* for 10 minutes (25°C), and the platelet-rich plasma was titrated to pH 6.5 with the ACD solution. This was then recentrifuged at 5,100 *g* for 15 minutes (25°C) to obtain a platelet pellet. The pellet was washed twice with 5 ml of Tyrode's buffer (m*M* concentrations: sodium chloride 137, potassium chloride 2.7, sodium biphosphate 0.36, magnesium chloride 0.10, sodium bicarbonate 12.0, dextrose 0.56, pH 8.0) and recentrifuged for 15 minutes at 5,100 *g*. The pellet was lysed by homogenization in 2 ml of ice-cold hypotonic buffer (Tris-EDTA, 5 m*M*, pH 7.5). The platelet membranes were obtained by centrifugation at 39,000 *g* for 10 minutes and then resuspended in the Tris incubation buffer (m*M*: Tris-hydrochloric acid 50, magnesium chloride 10, pH 7.5) used in the binding assay.

Total binding of ^{3}H-clonidine, an alpha$_2$-adrenoreceptor agonist, and of ^{3}H-yohimbine, an alpha$_2$-adrenoreceptor antagonist (New England Nuclear, Boston, Massachusetts) was measured in 1 ml aliquots of the fresh platelet membranes (0.254 ± 0.054 mg protein) which were incubated in duplicate at 25°C for 20 minutes with the radioligand. Nonspecific binding was determined by adding unlabelled clonidine or yohimbine (10^{-5} *M*), in addition to the respective tritiated ligand, to a second pair of incubates. Specific binding was

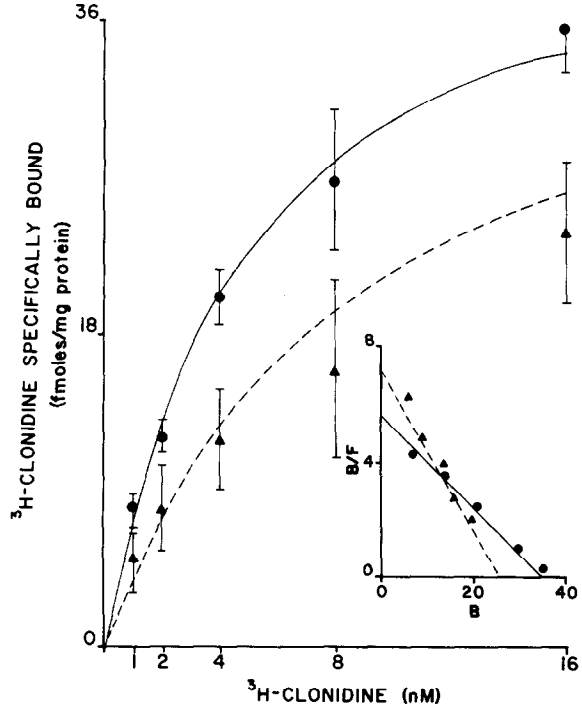

FIGURE 1. Specific binding of ^{3}H-clonidine to platelet membranes from normal subjects (**solid line**) and from patients with class III and IV congestive heart failure (**dashed line**) as a function of increasing concentrations (1 to 64 n*M*) of the ligand. **Ordinate,** ^{3}H-clonidine specifically bound (fmol/mg protein). **Abscissa,** concentration of ^{3}H-clonidine (n*M*). **Inset,** Scatchard plot showing the change in number of the high affinity binding site (normal subjects: B_{max} = 33 ± 2 fmol/mg protein, K_D = 5.5 ± 0.6 n*M* versus class III and IV: B_{max} = 24 ± 2, K_D = 3.4 ± 0.6 n*M*; $p < 0.05$). Results are expressed as the mean ± standard error of the mean. B = specifically bound ligand; B/F = specifically bound ligand/free ligand.

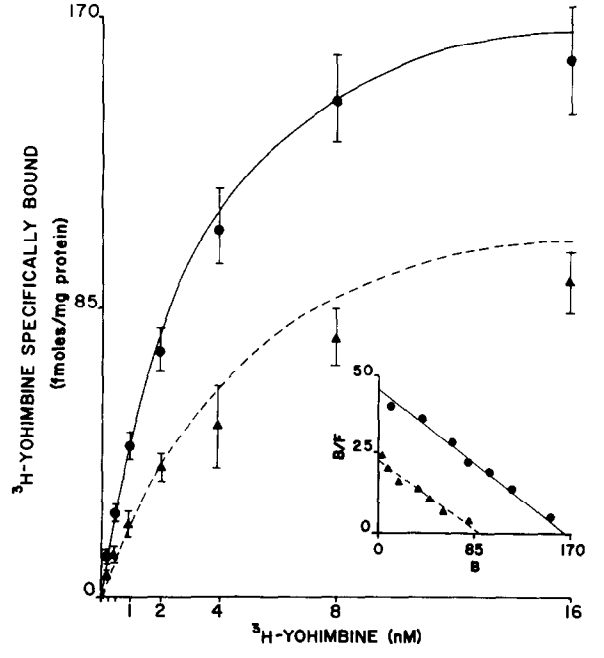

FIGURE 2. Specific binding of ^{3}H-yohimbine to platelet membranes from normal subjects (**solid line**) and from patients with class III and IV congestive heart failure (**dashed line**) as a function of increasing concentrations (0.25 to 16 n*M*) of the ligand. **Ordinate,** ^{3}H-yohimbine specifically bound (fmol/mg protein). **Abscissa,** concentration of ^{3}H-yohimbine (n*M*). **Inset,** Scatchard plot showing the change in number of the single binding site (normal subjects: B_{max} = 165 ± 12 fmol/mg protein, K_D = 4.0 ± 0.5 n*M* versus class III and IV: B_{max} = 94 ± 9, K_D = 4.4 ± 0.4; $p < 0.005$). Results are expressed as the mean ± standard error of the mean. B = specifically bound ligand; B/F = specifically bound ligand/free ligand.

defined as the difference between total and nonspecific binding. Incubations were terminated by adding 5 ml of the Tris incubation buffer to the sample. The membrane-bound tritiated ligand was recovered by rapid filtration of the diluted sample under vacuum through Whatman GF/C glass fiber filters. The filters were washed with two 10 ml aliquots of Tris incubation buffer (25°C). The filters were air dried and counted for radioactivity as described by Smith et al.[20] Proteins were determined by the method of Lowry et al.[21]

Catecholamine determination: Catecholamine determinations were performed by the radioenzymatic assay of Passon and Peuler.[22] The rat liver catechol-O-methyltransferase (COMT) was prepared according to the method of Axelrod and Tomchick.[23] All blood samples were collected as described above with the patient recumbent for at least 30 minutes. The samples were immediately centrifuged and the serum was frozen at −70°C.

Statistical analysis: Student's *t* test was used to test for the significance of differences. The level of significance was $p < 0.05$. Correlation coefficients for the binding isotherms were obtained by linear regression analysis which uses the method of least squares.

Results

Binding data: The specific binding of both ^{3}H-clonidine and ^{3}H-yohimbine to platelet membranes from both normal subjects and patients with chronic CHF was both saturable and of high affinity (Fig. 1 and 2). Scatchard analysis of the saturation isotherms again confirmed previous observations[15] that there was no correlation between age or sex and the total number of binding sites (B_{max}) or the affinity (K_D) of the radioligand for the site. The individual data for the control population for ^{3}H-clonidine (n = 26) was B_{max} = 33 ± 2 and K_D = 5.5 ± 0.6, and for ^{3}H-yohimbine (n = 16) was B_{max} = 165 ± 12, and K_D = 4.0 ± 0.5. There was no difference in the means for either ligand when the over 60-year-old subgroup was compared with the under 60-year-old group.

Patients with the more severe CHF, class III and IV (n = 9, Table I) had a 25% decrease in the maximum number of ^{3}H-clonidine binding sites compared with control subjects (B_{max} = 24 ± 2, $p < 0.05$, Fig. 1) with no change in affinity (K_D = 3.4 ± 0.6, difference not significant). There was also a marked decrease in the B_{max} for ^{3}H-yohimbine (43%, B_{max} = 94 ± 9, $p < 0.005$) but no change in the affinity of the ligand for the receptor (K_D = 4.4 ± 0.4, Fig. 2). Patients with class I and II chronic CHF (n = 7, Table II) had binding data with ^{3}H-yohimbine that suggested a trend towards a decrease in B_{max} as compared with normal subjects (B_{max} = 133 ± 16). This was not statistically significant. In this group, there were not enough studies done with ^{3}H-clonidine to analyze.

Plasma catecholamines: Plasma norepinephrine, epinephrine, and dopamine levels were measured in all control subjects and patients. In normal subjects, the norepinephrine value was 273.8 ± 44.1 pg/ml (range 54 to 503). There was a statistically significant increase in norepinephrine levels in patients with class I and II CHF (628.7 ± 97.6 pg/ml, range 338 to 1079; $p < 0.005$) with a far more significant increase in patients with class III and IV CHF (1333.5 ± 244.9 pg/ml, range 642 to 3142; $p < 0.0005$).

There was no significant difference in plasma epinephrine concentration between normal subjects and class I and II patients (normals 57.0 ± 17.4 pg/ml, class I and II 95.0 ± 48.7). Plasma epinephrine level was elevated significantly in patients with class III and IV CHF (117.1 ± 32.0, $p < 0.05$). Although there was no significant difference in plasma dopamine concentration between the normal group (12.5 ± 9.9 pg/ml) and the class I and II CHF group (38.5 ± 19.6), there also was a statistically significant increase in the class III and IV group (94.1 ± 31.1, $p < 0.01$).

There was a striking relationship for each group between an elevated level of plasma norepinephrine and a decrease in the total number of binding sites for the tritiated ligand (Fig. 3).

Discussion

The ^{3}H-clonidine binding site on human platelets has been characterized as an alpha$_2$-adrenoreceptor site similar to that located presynaptically.[13,14] Stimulation of alpha$_2$ presynaptic receptors[24,25] inhibits neurotransmitter release during nerve stimulation, whereas inhibition of the site increases the overflow of norepinephrine after nerve stimulation.

TABLE I ^{3}H-Clonidine and ^{3}H-Yohimbine Binding to Platelet Membranes of Patients With Congestive Heart Failure (Class III and IV)

Patient	Age (yr) & Sex	Plasma Norepinephrine (pg/ml)	^{3}H-Yohimbine Binding K_D (n*M*)	^{3}H-Yohimbine Binding B_{max} (fmol/mg protein)	^{3}H-Clonidine Binding K_D (n*M*)	^{3}H-Clonidine Binding B_{max} (fmol/mg protein)
K14	76M	1,133	4.6	108	...	...
K19	70F	1,122	3.6	98	...	...
K21	60M	3,143	2.5	41	...	...
C1	85M	643	4.0	120	...	...
C22	57M	813	6.1	62	1.8	17
C23	76F	1,397	4.2	109	2.4	28
C28	64M	1,329	6.3	93	3.2	26
C31	76M	913	4.1	106	4.3	26
C34	40F	1,510	3.9	108	5.3	24
Mean ±SEM	68.1 ±3.8	1,334 ±245	4.4 ±0.5	94 ±9	3.4 ±0.6	24 ±2

B_{max} = maximum number of binding site (fmol/mg protein); K_D = dissociation constant (n*M*); SEM = standard error of the mean.

TABLE II ^{3}H-Clonidine and ^{3}H-Yohimbine Binding to Platelet Membranes of Patients With Congestive Heart Failure (Class I and II)

Patient	Age (yr) & Sex	Plasma Norepinephrine (pg/ml)	^{3}H-Yohimbine Binding K_D (n*M*)	^{3}H-Yohimbine Binding B_{max} (fmol/mg protein)	^{3}H-Clonidine Binding K_D (n*M*)	^{3}H-Clonidine Binding B_{max} (fmol/mg protein)
K12	70F	845	5.6	144	...	...
K13	59F	1,079	6.0	157	...	...
K18	56M	626	3.2	111	...	...
K20	59F	604	2.5	192	...	...
C2	72F	499	3.0	109	...	...
C3	62M	338	6.2	113	...	...
C32	65M	409	3.2	84	2.8	24.0
Mean ±SEM	61.8 ±2.0	629 ±98	4.2 ±0.6	133 ±16	...	...

B_{max} = maximum number of binding site (fmol/mg of protein); K_D = dissociation constant (n*M*); SEM = standard error of the mean.

Although a recent review[26] suggested that the number of alpha$_2$ adrenoreceptors on human platelets as determined by ^{3}H-yohimbine binding is not subject to down-regulation, data presented here suggest this is not the case. The total number of binding sites, B_{max}, as defined by ^{3}H-yohimbine binding, was 43% less in the class III and IV CHF patients and 19% less in the class I and II patients as compared with normal subjects. This was statistically significant for the class III and IV patients ($p < 0.0005$). Changes of a similar magnitude were also noted using ^{3}H-clonidine as the radioligand (class III and IV 25%, $p < 0.05$). Other work from our laboratory[14] has shown significant increases in the number of platelet alpha$_2$ adrenoreceptors in other settings, such as a group of depressed patients versus a normal control population. Treatment with tricyclic antidepressants[14] or with electroconvulsive shock therapy[16] led to a significant decrease in the number of these binding sites. These changes are mirrored by changes in the presynaptic alpha$_2$ adrenoreceptor in the rat brain after identical interventions.[17] It has been suggested that changes in receptor number may not be of physiologic importance.[26] Again, work in our laboratory has correlated decreases in alpha$_2$-adrenoreceptor number in rat brain after long-term tricyclic antidepressant drug treatment[18] with increased norepinephrine release from adrenergic neurons in the isolated rat left atrium.[18]

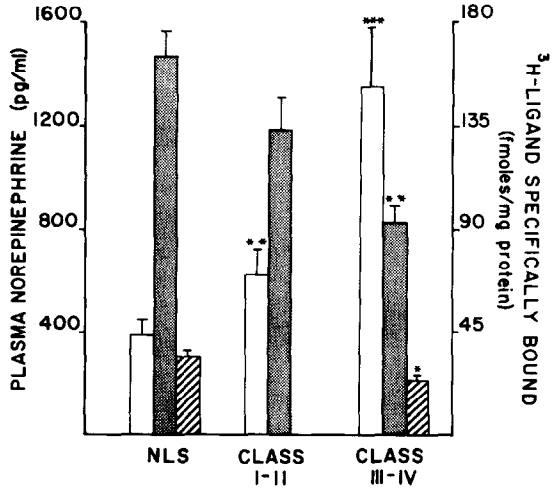

FIGURE 3. Relationship of plasma norepinephrine levels (**open bars**) and the maximum number of ^{3}H-yohimbine (**shaded bars**) and ^{3}H-clonidine (**cross-hatched bars**) binding sites. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ (between control group and group with class III/IV failure). NLS = normal subjects.

Further evidence of the physiologic importance of this decrease in the number of presynaptic receptors in patients with severe congestive failure may be found in the recent work by Swedberg et al.[27] They defined a correlation between myocardial norepinephrine release and the stroke work index. As left ventricular function decreased, there was an increase in norepinephrine released, as measured by the arterial-coronary sinus difference. In our study, a decrease in left ventricular function was associated with fewer alpha$_2$ adrenoreceptors, a condition which would permit increased neuronal norepinephrine release.

Older work by Covell et al,[28] however, was interpreted as not suggesting increased neuronal release of norepinephrine in CHF. They showed that stimulation of the right cardio-accelerans nerve in the dog produced sharply diminished increases in heart rate and right ventricular contractile force in animals with right heart failure versus normal control animals. In addition, they showed that the myocardial response to exogenous norepinephrine was unchanged from normal values, which suggested to them that the quantity of neurotransmitter released per nerve impulse was reduced in their experimental model of heart failure. That the cardiac norepinephrine depletion found in chronic CHF did not affect the contractile function of cardiac muscle was shown with cat papillary muscle isolated from chronically denervated heart[29] and with isolated rat hearts.[30] One would have, perhaps, expected increased release of norepinephrine with the decrease in number of platelet alpha$_2$ adrenoreceptors. This inconsistency may possibly be explained by a decrease in cardiac stores of norepinephrine in CHF,[31-33] a defect in binding or synthesis of catecholamines in congestive failure,[34] or by a decrease in tyrosine hydroxylase activity.[35] Although it is believed that the platelet alpha$_2$ adrenoreceptor "down-regulates" in response to the high plasma concentrations of norepinephrine, it is possible that any similar change in the neuronal receptors in the

myocardium may occur in order to permit an increase in norepinephrine release. The increased release of catecholamine would permit increased inotropic support of the failing myocardium. That different tissues may respond differently in CHF has been suggested by data which showed decreased myocardial norepinephrine stores in light of normal renal stores.[32]

In our study, there was a significant increase in plasma norepinephrine levels in patients with class III and IV CHF versus those of normal volunteers ($p < 0.0005$) and also in those with only class I or II CHF ($p < 0.005$). Although the blood samples were obtained by venipuncture and not through an indwelling catheter, previous work suggests this does not affect the plasma norepinephrine concentration.[36] These values are consistent with previously reported studies[1,4,5] which correlated an elevated plasma norepinephrine level with the degree of left ventricular dysfunction. There were no significant differences in the levels of epinephrine and dopamine in the normal subjects and the class I/II group, although there was a difference in the levels of epinephrine ($p < 0.05$) and dopamine ($p < 0.01$) in the normal groups and the class III/IV group.

This study suggests that in CHF, human platelet $alpha_2$ adrenoreceptors may serve as a marker of elevated levels of circulating norepinephrine. Changes in the platelet receptor number may be a method of monitoring the therapy of congestive failure. Identification and characterization of these receptors suggest the possibility of using $alpha_2$-agonist agents such as clonidine or alpha methyldopa to stimulate these inhibitory sites and thus decrease norepinephrine release. This may, in turn, protect the myocardium from the excessively high levels of circulating norepinephrine as has been done with beta-adrenergic blockade. Further studies in humans are needed to ascertain whether successful therapy in patients with CHF restores the receptor number to normal. These changes should be correlated in animal models of heart failure with neuronal release of norepinephrine after nerve stimulation. Such changes may prove important in developing new therapeutic strategies in the treatment of CHF.

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References

1. **Thomas JA, Marks BH.** Plasma norepinephrine in congestive heart failure. Am J Cardiol 1978;41:233–243.
2. **Cohn JN, Levine BT, Frances GS, Goldsmith S.** Neurohumoral control mechanisms in congestive heart failure. Am Heart J 1981;102:509–514.
3. **Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN.** Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. Am J Cardiol 1982;49:1654–1666.
4. **Eliot RS, Todd GL, Clayton FC, Pelper GM.** Experimental catecholamine-induced acute myocardial necrosis. Adv Cardiol 1978;25:107–118.
5. **Schenk EA, Moss AJ.** Cardiovascular effects of sustained norepinephrine infusions. II. Morphology. Circ Res 1966;18:605–615.
6. **Szakaes JE, Cannon A.** L-norepinephrine myocarditis. Am J Clin Pathol 1958;30:425–434.
7. **Garcia R, Jennings JM.** Pheochromocytoma masquerading as a cardiomyopathy. Am J Cardiol 1972;29:568–571.
8. **Kline JK.** Myocardial alterations associated with pheochromocytomas. Am J Pathol 1961;38:539–551.
9. **Langer SZ.** Presynaptic regulation of the release of catecholamines. Pharmacol Rev 1981;32:337–362.
10. **Starke K, Docherty JR.** Recent developments in alpha-adrenoreceptor research. J Cardiovasc Pharmacol 1980;2(suppl 3):S269–S286.
11. **Dixon WR, Mosimann WF, Weiner N.** The role of presynaptic feedback mechanisms in regulation of norepinephrine release by nerve stimulation. J Pharmacol Exp Ther 1979;209:196–204.
12. **Stahl SM.** The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. Arch Gen Psychiatry 1977;34;509–516.
13. **Shattil SJ, McDonough M, Turnbull J, Insel PA.** Characterization of alpha-adrenergic receptors in human platelets using [^{3}H] clonidine. Mol Pharmacol 1981;19:179–183.
14. **Garcia-Sevilla JA, Hollingsworth PJ, Smith CB.** $Alpha_2$ adrenoreceptors on human platelets: selective labelling by [^{3}H] clonidine and [^{3}H] yohimbine and competitive inhibition by antidepressant drugs. Eur J Pharmacol 1981;74:329–341.
15. **Garcia-Sevilla JA, Zis AP, Hollingsworth PJ, Greden JF, Smith CB.** Platelet $alpha_2$ adrenergic receptors in major depressive disorder. Arch Gen Psychiatry 1981;38:1327–1333.
16. **Smith CB, Zis AP, Garcia-Sevilla JA, Hollingsworth PJ.** Electroconvulsive therapy decreases $alpha_2$ adrenoreceptors on human platelet membrane (abstr). Fed Proc 1982;41:1633.
17. **Smith CB, Garcia-Sevilla JA, Hollingsworth PJ.** $Alpha_2$ adrenoreceptors in rat brain are decreased after long-term tricyclic anti-depressant drug treatment. Brain Res 1981;210:413–418.
18. **Crews FT, Smith CB.** Presynaptic alpha-receptor subsensitivity after long-term antidepressant treatment. Science 1978;202:332–334.
19. Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 7th ed. Boston: Little, Brown, 1973:286.
20. **Smith CB, Sheldon MI, Bednarczyk JH, Villareal JE.** Morphine-induced increases in the incorporation of ^{14}C-tyrosine into ^{14}C-dopamine and ^{14}C-norepinephrine in the mouse brain: antagonism by naloxone and tolerance. J Pharmacol Exp Ther 1972;180:547–557.
21. **Lowry OH, Rosebrough NF, Farr AG, Randall RJ.** Protein measurements with the Folin phenol reagent. J Biol Chem 1951;193:265–275.
22. **Passon PG, Peuler JO.** A simplified radiometric assay for plasma norepinephrine and epinephrine. Anal Biochem 1973;51:618–631.
23. **Axelrod J, Tomchick R.** Enzymatic O-methylation of epinephrine and other catechols. J Biol Chem 1958;233:702–705.
24. **Starke K.** Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. Naunyn Schmiedebergs Arch Pharmacol 1972;274:18–45.
25. **Langer SZ, Dubocovich ML, Celush SM.** Prejunctional regulatory mechanisms for noradrenaline release elicited by nerve stimulation. In: Almgren C, Carlsson A, Engel J, eds. Chemical Tools in Catecholamine Research II. North Holland, New York: Elsevier, 1975:183–191.
26. **Motulsky HJ, Insel PA.** Adrenergic receptors in man. Direct identification, physiologic regulation and clinical alterations. N Engl J Med 1982;307:18–29.
27. **Swedberg K, Chatterjee K, Rolzen M, Ports T, Parmley W.** Myocardial norepinephrine release in congestive heart failure (abstr). Circulation 1982;66:Suppl II:II–23.
28. **Covell JW, Childsey CA, Braunwald E.** Reduction in the cardiac response to postsynaptic nerve stimulation in experimental heart failure. Circ Res 1966;19:51–56.
29. **Spann JF Jr, Sonnenblick EH, Cooper T, Childsey CA, Willamn VL, Braunwald E.** Cardiac norepinephrine stores and the contractile state of heart muscle. Circ Res 1966;19:317–325.
30. **Dhalla NS, Naides KJ, Bhagat B, Cristensen K.** Biochemical basis of heart function. 1. Relation of catecholamine stores and contractile force in an isolated rat heart. Cardiovasc Res 1971;5:376–382.
31. **Childsey CA, Braunwald E, Morrow AG.** Catecholamine excretion and cardiac stores of norepinephrine in congestive heart failure. Am J Med 1965;39:442–451.
32. **Spann JF Jr, Childsey CA, Pool PE, Braunwald E.** Mechanism of norepinephrine depletion in experimental heart failure produced by aortic constriction in the guinea-pig.Circ Res 1965;17:312–321.
33. **Childsey CA, Sonnenblick EH, Morrow AG, Braunwald E.** Norepinephrine stores and contractile force of papillary muscle from the falling human heart. Circulation 1966;33:43–51.
34. **Rutenberg HL, Spann JF Jr.** Alterations of cardiac neurotransmitter activity in congestive heart failure. Am J Cardiol 1973;32:472–480.
35. **Pool PE, Covell JW, Levitt M, Gibb J, Braunwald E.** Reduction of cardiac tyrosine hydroxylase activity in experimental congestive heart failure. Circ Res 1967;20:349–353.
36. **Robertson D, Johnson GA, Robertson RM, Nies AS, Shard DB, Oates JA.** Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. Circulation 1979;59:637–643.