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 alpha2 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 alpha2 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 alpha2 agonist 3H-clonidine and the alpha2 antagonist 3H-yohimbine was used to determine the number (Bmax) of alpha2 receptors and the dissociation constant (Kd) for the 2 ligands. In the control population, the Bmax (in fmol/mg protein) for 3H-clonidine was 33 ± 2 and for 3H-yohimbine was 165 ± 12. There was a 25% difference in the maximum number of specific binding sites for 3H-clonidine in the class III/IV group (Bmax 24 ± 2, p < 0.05) and a 43% difference in the maximum number of specific binding sites for 3H-yohimbine (Bmax 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 Kd’s were similar in all 3 groups. These differences correlated well with the increases in plasma norepinephrine levels between the normal group (273.6 ± 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 alpha2 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 alpha2 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. Although the circulating norepinephrine may increase myocardial contractility, persistently elevated levels have been shown to be toxic to the myocardium in experimental animals and in humans.

The alpha2 adrenoreceptor is located presynaptically on noradrenergic nerve terminals and has been shown to exert a negative feedback inhibition upon norepinephrine release when stimulated. If neurogenically released norepinephrine has a detrimental effect on the myocardium, alterations in alpha2 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. 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 alpha2 adrenoreceptors similar to those present on noradrenergic nerve terminals. Changes in the number and affinity of alpha2 adrenoreceptor binding sites in the rat brain after various interventions have been well correlated with similar changes on human blood platelets. These have also been correlated with changes in norepinephrine release and tension developed in field stimulation experiments upon isolated rat atrial strips.
The hypothesis of the present study was that α2 adrenoreceptors on human blood platelets would be decreased by the increased circulating levels of nor- epinephrine 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 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 has shown the lack of age or sex dependency of either the total number or the affinity of platelet α2 adrenoreceptors. None of the patients were taking α-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 García-Sevilla et al. Briefly, 50 ml of blood was collected in polyethylene tubes which contained acid-citrate dextrose (ACD) and 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 centrifuged at 5,100 g for 15 minutes at 25°C to obtain a platelet pellet. The pellet was washed twice with 5 ml of Tyrode's buffer (mM concentrations: sodium chloride 137, potassium chloride 2.7, 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, mM, 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 (mM: Tris-hydrochloric acid 50, magnesium chloride 10, pH 7.5) used in the binding assay.

Total binding of 3H-clonidine, an α2-adrenoreceptor agonist, and of 3H-yohimbine, an α2-adrenoreceptor antagonist (New England Nuclear, Boston, Massachusetts) was measured in 1 ml aliquots of the fresh platelet membranes (0.54 ± 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
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 3H-clonidine and 3H-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 observations24 that there was no correlation between age or sex and the total number of binding sites (Bmax) in either normal subjects or 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 3H-clonidine binding site on human platelets has been characterized as an alpha2-adrenoreceptor site similar to that located presynaptically.21,14 Stimulation of alpha2 presynaptic receptors24,25 inhibits neurotransmitter release during nerve stimulation, whereas inhibition of the site increases the overflow of norepinephrine after nerve stimulation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Plasma Norepinephrine (pg/ml)</th>
<th>Bmax (fmol/mg protein)</th>
<th>KD (nM)</th>
<th>Rmax (fmol/mg protein)</th>
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<tr>
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<tr>
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<td>4.4</td>
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</tbody>
</table>

\[ B_{\text{max}} = \text{maximum number of binding site (fmol/mg protein)}; \text{KD} = \text{dissociation constant (nM)}; \text{SEM} = \text{standard error of the mean.} \]
Although a recent review suggested that the number of alpha2 adrenergceptors on human platelets as determined by 3H-yohimbine binding is not subject to down-regulation, data presented here suggest this is not the case. The total number of binding sites, $B_{\text{max}}$, as defined by 3H-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 3H-clonidine as the radioligand (class III and IV 25%, $p < 0.05$). Other work from our laboratory has shown significant increases in the number of platelet alpha2 adrenergceptors in other settings, such as a group of depressed patients versus a normal control population. Treatment with tricyclic antidepressants or with electroconvulsive shock therapy led to a significant decrease in the number of these binding sites. These changes are mirrored by changes in the presynaptic alpha2 adrenergceptor in the rat brain after identical interventions. It has been suggested that changes in receptor number may not be of physiologic importance. Again, work in our laboratory has correlated decreases in alpha2-adrenergceptor number in rat brain after long-term tricyclic antidepressant drug treatment with increased norepinephrine release from adrenergic neurons in the isolated rat left atrium.

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. 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 alpha2 adrenergceptors, a condition which would permit increased neuronal norepinephrine release.

Older work by Cove et al., 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 and with isolated rat hearts. One would have, perhaps, expected increased release of norepinephrine with the decrease in number of platelet alpha2 adrenergceptors. This inconsistency may possibly be explained by a decrease in cardiac stores of norepinephrine in CHF, a defect in binding or synthesis of catecholamines in congestive failure, or by a decrease in tyrosine hydroxylase activity. Although it is believed that the platelet alpha2 adrenergceptor “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.

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 < 0.05). 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. These values are consistent with previously reported studies 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 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 alpha2-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