Congestive heart failure (CHF) is an increasingly prevalent condition that bears a high morbidity and mortality rate. An essential process in its pathophysiology is the activation of a variety of neural and hormonal systems. These include the sympathetic nervous system, the renin-angiotensin-aldosterone axis and the endothelin system. Initially, these responses are adaptive and act to enhance inotropy and maintain systemic pressure by increasing peripheral vascular resistance. Eventually, however, they lead to a worsening of cardiovascular function and cardiac decompensation (1). Sympathetic overactivity is characterized by elevated norepinephrine (NE) spillover to plasma, especially from the renal and cardiac beds (2), as measured by the isotope-dilution technique (3). Sympathetic nervous activation has been demonstrated to be associated with survival in CHF (4,5), and elevated cardiac sympathetic activity has also been associated with sudden death (6).

The recognition of the importance of neurohormonal activation in CHF has resulted in a major change in our approach to its management, from the use of symptomatic treatment with diuretics and digitalis alone, to the utilization of specific means to counteract the neurohormonal derangement (1). Thus, angiotensin-converting enzyme inhibitors and, more recently, beta-blockers, have become crucial in the current management of CHF (7). The use of beta-blockers has been shown to enhance systolic function and to improve prognosis (7). Beta-adrenergic receptor blockade, however, does not entirely negate the detrimental effects of the exaggerated sympathetic outflow observed in heart failure. It cannot attenuate the effects of vasoactive sympathetic co-transmitters, such as dopamine and neuropeptide Y (8). Further, the high renal sympathetic tone in CHF is left unchecked (2).

The next logical step in therapy of heart failure might be to investigate the value of reducing neurotransmitter release. Our group has previously shown that there is increased activity of central noradrenergic neurons in heart failure, as reflected by the increased rates of central monoamine spillover (9). This finding has renewed interest in the possibility of central sympathetic inhibition. While it is known that there are sympathoinhibitory alpha-2 adrenergic receptors in the brainstem (10), they also exist presynaptically on peripheral sympathetic nerves (11). However, the role of these latter receptors in regulating NE release...
remains controversial. Human studies designed to study these presynaptic receptors, using alpha-2 adrenergic agonists, such as clonidine (12), and antagonists, such as phentolamine (13), have been difficult to interpret because of their dependence on imprecise measures of sympathetic function, such as the venous-arterial concentration gradient for plasma NE. The radio-tracer method that we have previously developed and employed, however, is of greater precision in that it recognizes and adjusts for the fact that all organs release NE into and remove NE from the circulation simultaneously (3).

Clonidine is a potent sympatholytic drug that has been shown to have beneficial hemodynamic effects after acute intravenous (IV) administration in heart failure (14). The sympathetic inhibition achieved by clonidine has been largely attributed to activation of alpha-2 adrenergic receptors in the central nervous system (CNS) (15). Our objective was to clarify whether peripheral alpha-2 adrenergic receptors contributed to this sympathoinhibitory action.

METHODS

Patient characteristics. The study group comprised a consecutive series of 10 patients with heart failure (age 58.1 ± 5.8 years) and 15 healthy volunteers (45.9 ± 8.5 years) (age ± SD). All the study subjects were male, except for one patient with heart failure. The patients with heart failure were all in New York Heart Association functional class II or III. Their left ventricular ejection fraction (LVEF) was 23.8 ± 4.4% (mean ± SD). Of these patients, six had an ischemic and four had a nonischemic cardiomyopathy. No patient had diabetes or a diagnosed peripheral neuropathy. All patients continued taking their normal medications. No patient was taking a beta-blocker, alpha-2 adrenergic agonist or antidepressant medication. Medications consisted of angiotensin-converting enzyme inhibitor, digoxin and diuretics. Both the patients’ clinical condition and their medications had been stable for at least one month. The healthy control subjects were recruited by advertisement in the general community. The study was performed with the approval of the Alfred Hospital Ethics Review Committee and all the subjects gave written informed consent.

Experimental procedures. All experiments were performed in the morning after a light breakfast. All subjects had refrained from smoking and consuming caffeinated beverages over the 12 h before the procedure. Forearm volume was measured by water displacement. Under a local anesthetic, the brachial artery of the nondominant arm was cannulated (3F, 5cm, Cook, Brisbane, Australia) for arterial pressure monitoring, blood sampling and drug administration. In the same arm, the antecubital vein was cannulated for deep venous (skeletal muscle drainage) blood sampling (SF Hoffman sheath, Cook). This cannula was advanced retrogradely into the forearm so that its tip could no longer be palpable, or for a distance of 10 cm. Forearm blood flow (FBF) was measured by strain-gauge venous occlusion plethysmography (16). For each determination, four to five measurements were performed, and the results were averaged. Hand blood flow was excluded by the use of a cuff at the wrist that was inflated to suprasystolic levels.

Study protocol. The NE isotope-dilution technique, as previously developed by this laboratory (3,17), was employed to provide a biochemical index of global and forearm sympathetic nerve activity. In brief, tritiated levo-(7-3H)-norepinephrine (New England Nuclear, Boston, Massachusetts) was infused at a rate of 0.5 to 1 μCi/min through a peripheral vein for 45 min to achieve steady-state plasma concentrations. Once steady-state had been achieved, baseline FBF was measured. Arterial and deep venous samples were obtained for calculation of forearm and global NE spillover. Then, clonidine (Boehringer-Ingleheim, Ingelheim, Germany) was infused into the brachial artery at 0.05 μg/100 ml forearm/5 min. The FBF measurements and blood sampling were again undertaken. Immediately after this, clonidine was administered intra-arterially (I/A) at 0.48 μg/100 ml forearm/5 min (12). All measurements were then repeated.

After an interval of >30 min, IV clonidine was successively administered at two doses, 1 and 2 μg/kg, each infused over 10 min. At the end of each infusion, FBF and blood sampling for catecholamine spillover was again performed.

Analysis of plasma catecholamines. Blood samples were collected into ice-chilled tubes containing an anticoagulant, ethyleneglycol-bis (beta-amino-ethyl ether) N,N’-tetraacetic acid (EGTA) and reduced glutathione to prevent oxidation. After centrifugation at 4°C, plasma samples were stored at −70°C until assayed. Norepinephrine plasma concentrations were determined by high performance liquid chromatography with electrochemical detection as previously described (18). The plasma tritiated NE concentration was determined by liquid scintillation spectroscopy after collection of the eluant from the electrochemical detector cell using a fraction collector.

Norepinephrine spillover measurements. Total body NE spillover rate was calculated as follows:

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**Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>CHF</td>
<td>congestive heart failure</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>FBF</td>
<td>forearm blood flow</td>
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<tr>
<td>FSO</td>
<td>forearm spillover</td>
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<tr>
<td>I/A</td>
<td>intra-arterial</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>NS</td>
<td>not significant</td>
</tr>
<tr>
<td>PAR</td>
<td>plasma appearance rate</td>
</tr>
<tr>
<td>PF</td>
<td>plasma flow</td>
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Intra-arterial clonidine caused a decrease in FBF in both groups (Fig. 1A and 1B). At the low dose, there was a small, nonsignificant fall in FBF: 21.3 ± 3.8 to 19.4 ± 2.7 ml/min in the control group and 24.7 ± 7.35 to 23.2 ± 6.4 ml/min in the patient group. With the high dose, the changes in FBF were: 21.3 ± 3.8 to 15.4 ± 2.0 ml/min (p = 0.01) and 24.7 ± 7.35 to 14.8 ± 3.5 ml/min (p = 0.015), respectively. At the higher dose, this represented a decrease in FBF in the control and patient groups of 28% and 40%, respectively (p = NS for between groups comparison).

In the control group, FSO of NE in the control and patient groups of 28% and 40%, respectively. At the higher dose, this represented a decrease in FBF across the forearm.

Recognizing that regional spillover can be influenced by blood flow, we also calculated the plasma appearance rate (PAR) of NE, as described by Chang et al. (16):

\[
\text{PAR} = \frac{\text{FSO of NE}}{1 - \left[\text{NEE}\right]} 
\]

Statistical analysis. Data are presented as mean value ± SEM, unless otherwise stated. Statistical analysis was performed using statistical software (SigmaStat, version 2.03, Chicago, Illinois). Within-group analysis was performed using one-way repeated measures analysis of variance (ANOVA) and the Tukey Test was employed in post-hoc analysis. Group differences were obtained using two-way repeated measures ANOVA with the Bonferroni multiple comparison test. A p value of <0.05 was considered statistically significant.

RESULTS

The baseline comparisons between the two groups are given in Table 1. There was no significant difference in baseline FBF and FSO of NE. However, the patient group had a much higher total body spillover of NE than the healthy control subjects.

Effects of I/A clonidine. As expected, I/A clonidine at the two doses did not result in any significant changes in global NE spillover (control 1.86 ± 0.33 to 1.87 ± 0.27, 1.86 ± 0.28 nmol/min and CHF 5.26 ± 1.39 to 4.44 ± 1.1, 4.33 ± 1.2 nmol/min).

Intra-arterial clonidine caused a decrease in FBF in both groups (Fig. 1A and 1B). At the low dose, there was a small, nonsignificant fall in FBF: 21.3 ± 3.8 to 19.4 ± 2.7 ml/min in the control group and 24.7 ± 7.35 to 23.2 ± 6.4 ml/min in the patient group. With the high dose, the changes in FBF were: 21.3 ± 3.8 to 15.4 ± 2.0 ml/min (p = 0.01) and 24.7 ± 7.35 to 14.8 ± 3.5 ml/min (p = 0.015), respectively. At the higher dose, this represented a decrease in FBF in the control and patient groups of 28% and 40%, respectively (p = NS for between groups comparison).

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Heart Failure</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>FBF, ml/min</td>
<td>21.3 ± 3.8</td>
<td>24.7 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>FSO, pmol/min</td>
<td>12.1 ± 1.4</td>
<td>8.97 ± 2.65</td>
<td>NS</td>
</tr>
<tr>
<td>TBS, nmol/min</td>
<td>1.86 ± 0.33</td>
<td>5.26 ± 1.39</td>
<td>0.01</td>
</tr>
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</table>

FBF = forearm blood flow; FSO = forearm spillover of norepinephrine; NS = nonsignificant; TBS = total body spillover of norepinephrine.

With low dose I/A clonidine, FSO of NE in the control group decreased from 12.1 ± 1.4 to 8.96 ± 1.18 pmol/min (p = 0.03). In the patient group, a nonsignificant increase in FSO was seen from baseline of 8.97 ± 2.65 to 14.3 ± 6.23 pmol/min. High dose I/A clonidine resulted in a change from 12.1 ± 1.4 to 6.19 ± 1.01 pmol/min (p < 0.001) and 8.97 ± 2.65 to 11.88 ± 5.72 pmol/min (p = NS), respectively (Fig. 2A and 2B). The difference in the response to I/A clonidine between the two groups (decrease by 49% and increase by 32%) was significant (p = 0.004).

Since regional NE spillover is to some extent dependent on blood flow (19), we also calculated the PAR of NE in the forearm, as described by Chang et al. (16). In the control group, PAR (in pmol/min) decreased from 30.3 ± 7.4 to 18.1 ± 3.4 (p < 0.05) and to 14.7 ± 3.6 (p < 0.001) with the two doses of I/A clonidine. In the patient group, the change in PAR (in pmol/min) was from 9.1 ± 2.4 to 13.9 ± 7.2 (p = NS) and to 13.5 ± 3.3 (p = NS). Again, as with FSO, the difference in response to I/A clonidine between the groups was significant (p = 0.005).

Effects of IV clonidine. Intravenous clonidine resulted in a reduction in the systolic blood pressure: in the control group, from 134 ± 5.8 to 127 ± 4.4 and 114.3 ± 4.0 mm Hg, p = 0.02 and p < 0.001, respectively and in the patient group, from 125.8 ± 9.8 to 116.4 ± 9.2 and 100.2 ± 6.2 mm Hg, p = NS and p = 0.01, respectively. There was no significant change in FBF in either group with intravenous administration.
DISCUSSION

Clonidine has been shown to decrease cardiac and global NE spillover in heart failure when administered parenterally (14,20). It is highly lipophilic and its major site of action has been presumed to be at the central alpha-2 adrenoceptors on sympathetic inhibitory neurons in the brain stem (15). A deterrent to its long-term use is sedation and depression, as a result of activation of these receptors. In the periphery, there are known to be alpha-2 adrenergic receptors located presynaptically and postsynaptically at sympathetic nerve terminals (11). The role of these receptors in modulating peripheral neurotransmitter release in heart failure has not been elucidated. If these receptors are physiologically active, then future drug therapy could be targeted at them. In so doing, direct sympathetic attenuation could be achieved without the side effects resulting from central alpha-2 adrenoceptor activation.

There is some evidence that, in health, neuronal alpha-2 adrenoceptors are functionally important in reducing NE release (21). However, it is not known whether in heart failure, in the presence of chronic overexposure to neurotransmitter, changes in receptor number or function or both take place. The strongest evidence for such a phenomenon is in the down-regulation of beta-1 adrenoceptors that occurs in the failing heart (22). Previous investigators have demonstrated that the number of platelet alpha-2 adrenoceptors is decreased in heart failure, having established that human platelets have an alpha-2 adrenoceptor density similar to those present on the nerve terminals (23). In the absence of sympathetic innervation of platelets, the relevance of this finding is uncertain. In another study, there was a decreased sensitivity to IV clonidine in patients with CHF (24), when compared with control subjects. However, systemic administration cannot selectively study the role of the peripheral alpha-2 adrenoceptors.

Effects of I/A clonidine on regional blood flow and NE release. The novel finding of this study is that NE spillover in the healthy forearm is substantially reduced by clonidine given I/A, but that this effect is lost in the patients with heart failure. This indicates a down-regulation of the peripheral alpha-2 adrenoceptor in heart failure.

As others have previously found, I/A clonidine does result in a reduction in FBF (12), and in our study, this effect was preserved in CHF. Most of the postsynaptic alpha-adrenergic receptors located within the nerve junction are the alpha-1 type (11). Clonidine has a high, though not absolute, selectivity for the alpha-2 type adrenoceptor (12), so that the demonstrated reduction in FBF in patients with CHF may be due to vasoconstriction caused by activation of the postsynaptic alpha-1 adrenoceptors on vascular smooth muscle. Alternatively, in heart failure, down-regulation of neuronal presynaptic alpha-2 adrenoceptors may occur in the absence of down-regulation of the vascular alpha-2 adrenoceptors, which remain capable of eliciting unpaired vasoconstriction.

It is appreciated that regional spillover of NE is altered by changes in blood flow, decreasing with declining flow, probably due to increased NE extraction locally (19). However, the reduction in forearm NE spillover in the control group observed in this study is likely to have resulted from true inhibition of NE release, rather than from a reduction in flow. Firstly, on existing evidence, a reduction in regional NE release of 50% with I/A clonidine is too profound to have resulted alone from a flow reduction of 28% (3,17). Secondly, the plasma NE appearance rate, which has been proposed to be a largely flow independent measure (16), also showed a similar dose-dependent reduction in forearm NE spillover with clonidine. Finally, and
perhaps most importantly, no reduction in regional NE release was seen with local alpha-2 adrenoceptor stimulation in the heart failure group, despite a 40% decrease in FBF, a reduction that was similar in magnitude to that observed in the control group.

**Effects of systemic clonidine on regional NE spillover.** The well-recognized sympathoinhibitory actions of IV clonidine have been explained pharmacologically in the context of two possible sites of action: in the periphery on sympathetic nerves, and the brain centers controlling sympathetic outflow. In the present study, control subjects demonstrated a dramatic reduction in regional (74%) and global NE (46%) spillover. In the patients with heart failure, IV clonidine resulted in a 54% reduction in global NE release with the high dose; however, only a trend to reduction in regional NE spillover was seen. An explanation for these disparate effects of IV clonidine on forearm spillover of NE in CHF could be that the major determinant of forearm NE spillover is local, rather than central alpha-2 adrenoceptor activity, and that these autoinhibitory peripheral receptors in heart failure are downregulated, with preservation of the central alpha-2 adrenoceptors. Alternatively, it is also possible that, in heart failure, there is a global down-regulation of the alpha-2 adrenoceptor (24), and the inhibitory effects on whole body NE spillover are mediated through central imidazoline receptors (25).

**Clinical implications.** Moxonidine, an imidazoline ligand acting on the CNS receptors to decrease sympathetic activation, has been demonstrated to result in a dramatic reduction in global NE release in patients with heart failure (26). It should be noted that the MOXCON study, designed to examine the effect of moxonidine in heart failure, was recently terminated because of excess mortality in the treatment group. Moxonidine has a powerful sympatholytic action (26), and it is unclear why this favorable effect should be associated with excess mortality in heart failure. A detailed report of the study is yet to be published.

**CONCLUSIONS**

The present study aimed to clarify the importance of presynaptic alpha-2 adrenoceptors in the regulation of NE release in health and in heart failure. When compared with control subjects, we found evidence of a substantial downregulation of peripheral alpha-2 adrenoceptors functionality in CHF. These data, therefore, mitigate against a likely clinical role for agents that specifically target these receptors. As a further conclusion, the findings suggest that the lack of sympathoinhibitory activity demonstrated by these receptors in heart failure may possibly contribute to the higher NE release in CHF, due to a failure of the autoinhibitory feedback mechanism in the synaptic cleft (27).

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**REFERENCES**