Dobutamine Potentiates Amrinone’s Beneficial Effects in Moderate but Not in Advanced Heart Failure

31P-MRS in Isolated Hamster Hearts

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There is controversy as to whether potent inotropic agents are beneficial or detrimental in moderate to severe heart failure. Accordingly, we studied the effects of amrinone, amrinone plus dobutamine, and dobutamine alone on mechanical performance, myocardial oxygen consumption, and high energy phosphate metabolism in different stages of congestive heart failure in the cardiomyopathic Syrian hamster. In hearts with moderate heart failure, administration of amrinone, amrinone plus dobutamine, and dobutamine alone increased developed pressure significantly, whereas the phosphorylation potential increased significantly only with amrinone and amrinone plus dobutamine. In hearts with advanced heart failure, administration of amrinone and amrinone plus dobutamine increased developed pressure significantly, whereas dobutamine alone had no effect. The phosphorylation potential improved significantly only with amrinone. Thus, amrinone improved mechanical performance and mitochondrial activity in both heart failure states. Dobutamine potentiated amrinone’s beneficial effects in moderate heart failure, but negated the positive inotropic effect of amrinone in advanced heart failure. Therefore, hearts responded differently to potent inotropic agents depending on the severity of heart failure. (Circulation Research 1990;66:747-753)

A augmentation of cardiac contractility represents a mainstay in the treatment of congestive heart failure associated with impaired systolic ventricular function. Because of the well-known paucity of energy reserves in the myocardium and the further jeopardized balance between energy production and utilization in the chronically over-loaded heart, treatment with any powerful positive inotropic agent may exhaust the limited energy supply in the depressed myocardium.1,2

Amrinone, a selective phosphodiesterase III inhibitor,3 has been shown to exert concentration-dependent vasodilatory and positive inotropic effects in a variety of species in vivo and in vitro.4 Although positive inotropic drugs usually tend to increase myocardial oxygen consumption, administration of amrinone in patients with congestive cardiomyopathy and dogs with ischemic heart failure has resulted in improved hemodynamic performance associated with little change in myocardial oxygen consumption.5,6 However, the relative contributions of vasodilation and increased contractility to the improved hemodynamic state with amrinone are controversial,4 since some investigators have found no evidence for a positive inotropic effect in patients with heart failure and have ascribed amrinone’s hemodynamic effects solely to the vasodilatory action of the drug.7-9

Dobutamine, a synthetic catecholamine, exhibits a potent positive inotropic effect on the myocardium with little effect on peripheral vascular tone.10 However, dobutamine treatment of patients with severe heart failure has shown disparate results. Some investigators have shown that short-term administration of dobutamine resulted in long-term functional improvement,11 while others showed only partial
improvement of symptoms of severe congestive heart failure over a longer treatment period. Recent results suggested that in patients with severe heart failure the addition of amrinone to dobutamine may improve left ventricular performance compared with the effect of either drug alone. This improvement might be due to synergistic effects on cyclic AMP.

Magnetic resonance spectroscopy (MRS) provides a unique opportunity for assessment of the dynamic aspects of high energy phosphates in the intact, beating heart during drug treatment. Since any drug that increases myocardial work can be expected to increase the rate of energy expenditure, changes in high-energy metabolism of cardiomyopathic hearts with different stages of heart failure and already compromised energy reserve are of special interest. Therefore, the purpose of this study was assessment of the effects of 1) amrinone alone, 2) dobutamine alone, and 3) the combined administration of amrinone and dobutamine on myocardial performance, phosphorylation potential, metabolism, and myocardial oxygen consumption in cardiomyopathic hamster hearts in both moderate and advanced heart failure.

**Materials and Methods**

Syrian hamsters of the UM-X7.1 strain, a derivative of the Bio 14.6 strain, were used as experimental animals. The first group consisted of animals between 180 and 200 days of age. At this age the peak necrotic stage is terminated and there is evidence of moderate heart failure. In the second group, animals were older than 220 days. At this age the hamsters are in severe advanced heart failure, and at 250 days nearly all animals have died because of myocardial pump failure or arrhythmias. Healthy age-matched hamsters from the same strain served as controls. All hamsters were maintained under identical conditions with free access to laboratory chow and water.

**Perfused Heart Preparation and Experimental Protocol**

Hamsters were anesthetized with ether before midline sternotomy. The hearts were rapidly excised and perfused by the Langendorff method, as previously described, by use of a modified Krebs-Henseleit solution containing (mM) NaCl 117, KCl 4.3, MgCl₂ 2.4, KH₂PO₄ 0.1, NaHCO₃ 25, CaCl₂ 2.4, EDTA 0.5, and glucose 15, and insulin 10 units/l. Perfusate temperature was maintained constant at 35°C by use of countercurrent heat exchangers and a thermostat-regulated circulating water bath. Maintenance of a constant heart rate, pacing leads were inserted into the right ventricular base and connected to a Model 5320 pulse generator (Medtronic, Minneapolis, Minnesota). Hearts were paced at a rate between 220 and 230 beats/min.

Measurement of left ventricular pressure was obtained from a cannula inserted through the left atrium and mitral valve into the left ventricular cavity. The cannula was sutured firmly into place at the level of the atrial appendage to provide a tight seal and then connected to a pressure transducer. Pressures were recorded on a four-channel dynograph (Beckman Instruments, Fullerton, California). Coronary flow was measured by continuous siphoning of the effluent from the right ventricular outflow tract. Aortic perfusate (arterial) and right ventricular outflow tract (venous) samples were obtained before and after the heart was placed in the magnet and immediately measured for partial pressure of oxygen with a gas analyzer (Corning, Medfield, Massachusetts). Myocardial oxygen consumption was calculated as the product of coronary flow and the arteriovenous oxygen difference across the heart.

The hearts were perfused with Krebs-Henseleit buffer for 15–20 minutes before data collection. This procedure allowed the heart preparation to reach a steady state. Hemodynamic and ³¹P-MRS spectra were then taken. Then 5×10⁻⁴ M amrinone or 10⁻⁶ M dobutamine was added to the Krebs-Henseleit buffer. After a 20-minute reequilibration period, hemodynamic measurements and ³¹P-magnetic resonance spectroscopy (MRS) spectra were collected. During a third phase, the hearts were perfused with Krebs-Henseleit medium containing both 5×10⁻⁴ M amrinone and 10⁻⁶ M dobutamine, and all measurements were collected after reequilibration.

**High-Pressure Liquid Chromatography**

End-point values of high energy phosphate compounds were collected by use of high-pressure liquid chromatography (HPLC) of freeze-clamped myocardial tissue after termination of the metabolic processes at a predetermined phase of the cardiac cycle. Fifty percent of the hearts were freeze clamped at systole and 50% at diastole for correlation of energy metabolites with NMR data since the latter were averaged over the heart cycle. Pneumatic cylinders were driven at 75 psi, allowing the temperature in the center of the heart to drop to −80°C within 5 msec. Preparation of acid extracts and analysis of high energy phosphate compounds by HPLC has been described earlier.

**³¹P-MRS**

³¹P-MRS spectra of the beating isolated perfused heart were obtained on a 5.6-T vertical 76-mm-bore magnet as described earlier. In the hamster hearts, 472 transients were accumulated over 10 minutes. Correction for spin-lattice relaxation effects was performed as described previously. A phosphate percent was calculated for inorganic phosphate (Pᵢ), phosphocreatine (PCr), and ATP as previously described. Absolute values were derived from HPLC measurements (n=6). The total mean creatine value of the isolated hearts was 24 mmol for normal hamster hearts (n=10) and 22 mmol for cardiomyopathic hamster hearts (n=10). The PCr/Pᵢ ratio and the phosphorylation potential according to Brand and Lehninger were derived from these measurements, based on the calculation of free ADP as
ADP\textsubscript{free} = (Cr×ATP)/(K\text{eq}×H^+×PCr)

where the creatine kinase equilibrium ($K_{eq}$) was adjusted for pH and taken as 2.36 at a pH of 7.0.\textsuperscript{22} The phosphorylation potential was expressed as \(\ln[\text{(ATP)/(ADP)(P_i)}]\) based on the natural log and molar values. Intracellular pH was estimated from the chemical shift of the pH-dependent P\textsubscript{i} peak relative to PCr peak.\textsuperscript{23}

**Statistical Analysis**

Data are reported as mean±SEM unless otherwise noted. Scheffe's test for multiple contrasts was applied for detection of significant differences based on an analysis of variance.\textsuperscript{24} The null hypothesis was rejected at the 95% confidence level, $p<0.05$ was considered significant.

**Results**

**Characterization of Hearts With Moderate and Advanced Heart Failure**

Hearts of cardiomyopathic hamsters beyond 220 days of age exhibited extensive dystrophic calcification of the myocardium, dilated left atria filled with thrombotic material, and thrombi in all cardiac cavities. Dry heart weight and coronary flow were significantly lower ($p<0.005$) compared with cardiomyopathic hamster hearts less than 200 days of age (Table 1).

**TABLE 1. Characteristics of Normal Hamsters and Cardiomyopathic Hamsters With Moderate and Advanced Heart Failure**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>CF (ml/min)</th>
<th>DHW (g)</th>
<th>DHW/BW (g/kg)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ($n=32$)</td>
<td>&lt;300</td>
<td>10.8±0.8</td>
<td>0.105±0.002</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Moderate heart failure ($n=22$)</td>
<td>&lt;200</td>
<td>8.3±0.4</td>
<td>0.104±0.002</td>
<td>0.78±0.02</td>
</tr>
<tr>
<td>Advanced heart failure ($n=31$)</td>
<td>&gt;220</td>
<td>6.4±0.7</td>
<td>0.086±0.002</td>
<td>0.65±0.02</td>
</tr>
</tbody>
</table>

All values are mean±SEM. CF, coronary flow; DHW, dry heart weight; BW, body weight.

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**FIGURE 1. Hemodynamic data for normal hamster hearts and cardiomyopathic hamster hearts with moderate and advanced heart failure.** Hearts from three groups of hamsters, healthy hamsters (NORMAL), cardiomyopathic hamsters in moderate heart failure (MOD HF) (180–200 days of age), and hamsters in advanced heart failure (ADV HF) (>220 days of age) were perfused, as described in “Materials and Methods,” with baseline conditions (CTR) ($n=16$), with amrinone (AM) ($n=8$), or with dobutamine (DO) ($n=8$), followed by AM+DOB ($n=16$). (The $n$ values were the same for each of the three groups of hamster hearts, i.e., NORMAL, MOD HF, and ADV HF.) The mean values and the standard error of the mean is shown for developed pressure (panel a), rate-pressure-product (panel b), left ventricular end-diastolic pressure (panel c) and oxygen consumption (panel d).
Effect of Amrinone, Amrinone Plus Dobutamine, and Dobutamine on Normal Hamster Hearts

During administration of amrinone, amrinone plus dobutamine, and dobutamine alone, heart rate (p<0.03), left ventricular peak systolic pressure (p<0.001), left ventricular developed pressure (p<0.001, Figure 1a), and rate pressure product (p<0.001, Figure 1b) increased significantly. Coronary flow increased from 10.8±0.8 ml/min during control state to 14.5±0.5 ml/min (p<0.001) during administration of amrinone plus dobutamine and to 11.7±0.6 with dobutamine alone. Myocardial oxygen consumption increased significantly (p<0.001) during treatment with dobutamine and amrinone plus dobutamine (Figure 1d). Mechanical performance and myocardial oxygen consumption increased with amrinone plus dobutamine and dobutamine alone, but the phosphorylation potential ln[(ATP)/(ADP) (Pi)] decreased significantly (p<0.05) during dobutamine treatment. The phosphorylation potential was calculated from standardized NMR values. The free ADP was calculated from standardized NMR values as described earlier.20

Effect of Amrinone, Amrinone Plus Dobutamine, and Dobutamine on Cardiomyopathic Hearts With Moderate Failure

During administration of amrinone and amrinone plus dobutamine, heart rate (p<0.005), left ventricular peak systolic pressure (p<0.04 and p<0.001, respectively), left ventricular developed pressure (p<0.005, Figure 1a), and rate pressure product (p<0.001, Figure 1b) increased significantly. Left ventricular end-diastolic pressure decreased (p<0.004, Figure 1c). Coronary flow increased from 8.8±0.4 ml/min during control state to 11.4±0.3 ml/min (p<0.005) and 13.2±0.3 ml/min (p<0.005) during treatment with amrinone and amrinone plus dobutamine, respectively. Coronary flow was 10.3±0.9 with dobutamine alone. During administration of dobutamine, heart rate (p<0.04), left ventricular peak systolic pressure (p<0.001), left ventricular developed pressure (p<0.001), and rate pressure product (p<0.001) increased. Left ventricular end-diastolic pressure decreased (p<0.01), but significantly less (p<0.05) compared with amrinone and amrinone plus dobutamine. Amrinone plus dobutamine and dobutamine alone caused a significant increase (p<0.04) in myocardial oxygen consumption. Administration of amrinone caused a significant increase in PCr (p<0.001), PCr/Pi ratio (p<0.02), and the phosphorylation potential (p<0.03) (Table 2). A decrease in P, (p<0.02) and increases in PCr (p<0.001), PCr/Pi ratio (p<0.001), and the phosphorylation potential (p<0.001) were observed during treatment with amrinone plus dobutamine. There were no significant changes in these high energy phosphate compounds during dobutamine treatment.

Effect of Amrinone, Amrinone Plus Dobutamine, and Dobutamine on Cardiomyopathic Hearts With Advanced Heart Failure

During administration of amrinone, heart rate (p<0.001), left ventricular peak systolic pressure (p<0.001), left ventricular developed pressure (p<0.001, Figure 1a), rate pressure product (p<0.001, Figure 1b), and myocardial oxygen consumption (p<0.02, Figure 1d) increased. Left ventricular developed pressure and rate pressure product increased significantly (p<0.02) with amrinone plus dobutamine compared with control, but significantly less (p<0.001) compared with amrinone alone. Dobutamine alone caused no significant changes of left ventricular pressure and myocardial oxygen consumption. Coronary flow increased from

| Table 2. High Energy Phosphates in Normal Hamster Hearts and Cardiomyopathic Hamster Hearts With Moderate and Advanced Heart Failure During Control State and Administration of Amrinone, Amrinone Plus Dobutamine, and Dobutamine |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | P, (mM)         | PCr (mM)        | β-ATP (mM)      | pH              | PCr/Pi          |
| Normal          |                 |                 |                 |                 |                 |
| CTR (n=9)       | 3.8±1.1         | 12.2±0.3        | 10.3±0.7        | 7.17±0.03       | 5.5±1.8         | 9.2±0.3         |
| AM (n=6)        | 4.8±1.2         | 13.6±0.6        | 10.4±0.3        | 7.07±0.03       | 3.7±0.8         | 9.8±0.4         |
| DO (n=6)        | 10.6±1.2        | 9.1±0.7         | 7.8±0.8         | 7.14±0.03       | 0.9±0.1         | 8.7±0.1         |
| AM+DO (n=7)     | 3.5±1.1         | 13.5±0.8        | 10.8±0.4        | 7.08±0.02       | 7.3±1.8         | 9.3±0.6         |
| Moderate heart failure |                |                 |                 |                 |                 |
| CTR (n=13)      | 11.4±0.7        | 5.0±0.4         | 6.3±0.2         | 7.10±0.02       | 0.5±0.1         | 7.9±0.1         |
| AM (n=6)        | 10.9±0.5        | 8.1±0.5         | 6.7±0.2         | 7.14±0.04       | 0.8±0.1         | 8.5±0.1         |
| DO (n=6)        | 10.5±1.1        | 6.0±0.6         | 6.4±0.4         | 7.12±0.03       | 0.8±0.2         | 8.2±0.3         |
| AM+DO (n=13)    | 8.2±0.7         | 8.5±0.9         | 7.1±0.3         | 7.09±0.02       | 1.1±0.1         | 9.0±0.2         |
| Advanced heart failure |             |                 |                 |                 |                 |
| CTR (n=13)      | 11.5±1.3        | 7.3±1.0         | 6.5±0.4         | 6.97±0.06       | 0.8±0.2         | 8.3±0.3         |
| AM (n=6)        | 8.5±1.7         | 9.9±0.5         | 9.5±0.6         | 7.13±0.02       | 1.6±0.06        | 9.2±0.3         |
| DO (n=6)        | 15.3±1.5        | 4.6±0.8         | 5.8±0.7         | 6.98±0.04       | 0.4±0.1         | 7.3±0.5         |
| AM+DO (n=6)     | 9.7±1.7         | 10.6±0.7        | 8.6±0.6         | 7.09±0.02       | 1.4±0.3         | 9.1±0.3         |

P, inorganic phosphate; PCr, phosphocreatine; β-ATP, β-adenosine triphosphate; PP, phosphorylation potential; CTR, control state; AM, amrinone; DO, dobutamine.
6.4±0.7 during control state to 8.9±0.7 with amrinone, to 9.5±1.1 during dobutamine treatment, and to 8.5±0.6 with dobutamine plus amrinone. β-ATP increased (p<0.001) during administration of amrinone and amrinone plus dobutamine, and the phosphorylation potential improved (p<0.02) during amrinone and amrinone plus dobutamine treatment.

Discussion

Amrinone exhibits positive inotropic and vasodilatory effects in vivo and in vitro. Selective inhibition of cyclic nucleotide phosphodiesterase III may be the main mechanism of this drug action, thereby raising myocardial cyclic AMP and augmenting calcium delivery to the sarcoplasm. Administration of amrinone in patients with heart failure results in an improved hemodynamic state, but the relative contributions of vasodilation and increased contractility are controversial.

Dobutamine, a synthetic sympathomimetic amine, exerts potent positive inotropic action. Some recent studies reported long-term functional improvement in patients with severe heart failure after short-term administration of dobutamine, while others found disparate results. However, in patients with chronic congestive heart failure the effect of amrinone was found to be comparable with dobutamine, and supplementary administration of amrinone in patients pretreated with dobutamine resulted in an additive improvement of left ventricular performance. This presumably occurred because of synergistic effects of cyclic AMP.

Administration of inotropic drugs in chronic congestive heart failure may be potentially deleterious, because any drug that increases myocardial energy utilization might accelerate myocardial cell death in the already energy-starved failing heart. Amrinone causes little change in myocardial oxygen consumption in patients with severe heart failure and in dogs with acute left ventricular failure, presumably because of a reduction in ventricular size and the resultant diminished ventricular wall stress.

Therefore, the aims of the present study were assessment of the effects of amrinone, amrinone plus dobutamine, and dobutamine alone on mechanical performance, myocardial oxygen consumption, and the phosphorylation potential in cardiomyopathic hamster hearts with moderate and advanced heart failure. The phosphorylation potential ionic equation that gives the overall energy state of the heart at equilibrium.

In moderate heart failure, mechanical performance and the phosphorylation potential improved during administration of amrinone and amrinone plus dobutamine. During dobutamine treatment mechanical performance improved, while the phosphorylation potential did not change from baseline. In advanced heart failure, mechanical performance and the phosphorylation potential improved during treatment with amrinone, but did not change with dobutamine alone. Furthermore, dobutamine appeared to negate the effects of amrinone.

Amrinone plus dobutamine and dobutamine alone caused a more powerful positive inotropic response in normal hamster hearts and cardiomyopathic hamster hearts with moderate heart failure compared with amrinone alone. The positive inotropic effect, resulting in a significant decrease of the end-diastolic pressure in hearts with moderate heart failure, was more pronounced during administration of amrinone and amrinone plus dobutamine compared with dobutamine alone. In contrast, in hearts with advanced heart failure positive inotropic responses were significantly stronger during amrinone treatment compared with both amrinone plus dobutamine and dobutamine alone. The diminished positive inotropic response to dobutamine in hearts with advanced heart failure may be due to altered catecholamine metabolism and desensitization of myocardial β-adrenoceptors. In association, cyclic AMP is significantly decreased in the myocardium of cardiomyopathic hamsters compared with control hamsters. Any agent that increases cyclic AMP, such as dobutamine or amrinone, might be expected to improve relaxation, since cyclic AMP is believed to control the activity of phospholamban, the regulatory protein for calcium uptake to the sarcoplasmic reticulum. On the other hand, in cardiomyopathic hamster hearts with advanced heart failure, where intracellular calcium levels are markedly elevated, a further enhancement of cytosolic calcium influx causes calcium overload and alters the calmodulin-mediated increase of phosphodiesterase activity. Calcium overload is often associated with an inhibition of the sodium/calcium exchange and less pressure generation for a given calcium release, because the concentration curve for calcium versus calcium-binding proteins shifts toward higher calcium concentration. Calcium overload may be responsible for the attenuated positive inotropic response of amrinone plus dobutamine in cardiomyopathic hamster hearts with advanced heart failure.

The phosphorylation potential improved significantly in cardiomyopathic hamster hearts treated with amrinone or amrinone plus dobutamine irrespective of the severity of heart failure. At the same time, contractility and myocardial oxygen consumption were also significantly augmented except in hearts with advanced heart failure treated with amrinone plus dobutamine, and therefore, ATP utilization increased. Nevertheless, the improved phosphorylation potential may be due to an improvement of mitochondrial activity, which is known to be diminished in these hearts.

Concurrently with a substantially improved mechanical performance and an increased myocardial oxygen consumption, the phosphorylation potential increased during administration of amrinone in late heart failure. Dobutamine alone showed no significant change, and it negated the effects of amrinone when the two agents were given together in
late heart failure. There were short periods of arrhythmia and fibrillation when the failing hearts were first switched to perfusion with a positive inotropic agent; thus, the addition of antiarrhythmic agents may be beneficial with concurrent amrinone treatment in late heart failure.

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