Comparison of the Effects of ORG 30029, Dobutamine and High Perfusate Calcium on Function and Metabolism in Rat Heart

Donald J. Grandis, Guy A. MacGowan and Alan P. Koretsky

1Pittsburgh NMR Center for Biomedical Research, 2University of Pittsburgh Medical Center and 3Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213, USA

Received 30 April 1998, accepted in revised form 4 September 1998

D. J. Grandis, G. A. MacGowan and A. P. Koretsky. Comparison of the Effects of ORG 30029, Dobutamine and High Perfusate Calcium on Function and Metabolism in Rat Heart. Journal of Molecular and Cellular Cardiology (1998) 30, 2605–2612. Cardiac contractility may be enhanced via multiple cellular mechanisms resulting in varied effects on cardiac energetics. The mechanisms that account for the varied energetic responses are not well understood. The purpose of this investigation was to compare the effects of the calcium sensitizing agent ORG 30029 (N-hydroxy-5,6-dimethoxy-benzo[b]thiophene-2-carboximidamide hydrochloride, a calcium sensitizing agent which increases contractility without increasing calcium transients significantly), dobutamine and high perfusate calcium on contractility and energetics. Langendorff-perfused rat hearts were stimulated with ORG 30029, dobutamine and high perfusate calcium in graduated concentrations while myocardial oxygen consumption (MVO₂) and force-time integral were measured. ORG 30029, dobutamine and high perfusate calcium increased contractility in a dose-dependent manner. Despite an increase of 50% in systolic pressure and a 17% increase in force-time integral from control, ORG 30029 had no significant effect on MVO₂ at the lower concentrations (n = 6). However, dobutamine (n = 4) and high perfusate calcium (n = 4) caused a 65% increase in systolic pressure and a 17% increase in force-time integral and a 50% and 41% increase in MVO₂ respectively (P<0.05). High energy phosphates (by 31P NMR), and lactate production were unaltered by these agents, suggesting that metabolism was steady state. Basal metabolism tended to increase slightly with dobutamine but not with ORG 30029 or high perfusate calcium. ORG 30029, dobutamine, and high perfusate calcium increase contractility in perfused rat hearts with disparate effects on energetics. These differences may be accounted for, in part, by differences in energy expenditure for calcium handling.

© 1998 Academic Press

Key Words: 31P NMR; Myocardial Oxygen consumption; Calcium sensitizer; Inotropic agents.

Introduction

Cardiac contractility may be enhanced via multiple cellular mechanisms with varied effects on cardiac energetics (Alpert et al., 1992). When contractile force is increased by an elevation in the calcium transient (the rise in intracellular calcium concentration during depolarization) there is a proportionate rise in myocardial oxygen consumption (MVO₂) (Wu et al., 1992). Contractility may also be increased by enhancing the “sensitivity” of the contractile proteins to calcium (Lee and Allen, 1997). Studies of the effects of calcium sensitizing agents on cardiac energetics have yielded various results. Some studies have shown that increasing calcium sensitivity increases contractility in a more energetically efficient manner than inotropic agents which increase the calcium transient amplitude (Aoyagi et al., 1991, Groß et al., 1993). However, other studies have not found any difference in efficiency (deTombe et al., 1992, Hata et al., 1992).

We previously compared the effects of the unique calcium sensitizing agent EMD 57033, which does not affect calcium transients (Gambassi et al., 1993,
Solaro et al., 1993), to dobutamine in isolated rat hearts. In that study, despite a 40% increase in cardiac work, dobutamine increased MVO$_2$ 47% while EMD 57033 did not cause any significant increase in MVO$_2$ (Grandis et al., 1995). Dobutamine has $\beta_1$ agonist properties and may decrease calcium sensitivity (Ruffolo, 1987; Endoh and Blinks, 1989). Thus, the different effects of the two agents on calcium sensitivity could have caused the different energetic responses. Conversely, the energetic differences may be accounted for by differences in the energetic costs of calcium handling. Finally, because dobutamine shortened duration of contraction significantly and EMD 57033 did not, it was impossible to compare the effects on energetics of dobutamine and EMD 57033 at similar increases in force-time integral.

ORG 30029 (N-hydroxy-5,6-dimethoxy-benz(o)b-thiophene-2-carboximidamide hydrochloride) is a cardiotonic agent which increases contractility both by inhibition of phosphodiesterase activity as well as via calcium sensitizing (Cottney et al., 1990). ORG 30029 has been demonstrated to increase contractility several-fold with minimal effects on calcium transients (Kawabata and Endoh, 1993). The purpose of this investigation was to compare the energetic effects of increasing contractility via graded concentrations of ORG 30029 to that of dobutamine and high perfusate calcium, both of which increase calcium transients significantly. High perfusate calcium concentration was employed because it is able to increase contractility without $\beta_1$ agonism or shortened duration of contraction, like dobutamine (deTombe et al., 1992). At lower concentrations, ORG 30029 caused significantly less of an increase in MVO$_2$ than did dobutamine and high perfusate calcium. To probe possible mechanisms for this difference in MVO$_2$ at these concentrations, lactate production, phosphate metabolite concentrations (by $^{31}$P NMR), and basal metabolism were determined with each agent.

Materials and Methods

Heart perfusion

Male Sprague–Dawley rats (Taconic Farms, Germantown, N.Y.) weighing 325–350 g were fasted overnight. Animals were anesthetized with 50 mg pentobarbital and anticoagulated with 1000 U heparin intraperitoneally. Hearts were excised and perfused within 15 s in the Langendorff manner (Neely et al., 1967). Hearts were perfused at a constant perfusion pressure of 80 mmHg. Coronary perfusion pressure was measured via an in-line pressure transducer. Perfusate was not recirculated. To measure left ventricular pressure, a water-filled latex balloon attached to 3 cm non-compliant tubing was placed through the mitral valve into the left ventricle. Left ventricular pressure was determined using a pressure transducer (Gould, Cleveland, OH).

To assure a uniform heart rate in all studies, hearts were placed at 5 Hz using a stimulator and non-metallic pacing leads (KCl-agar) attached to the right ventricular apex. To eliminate autonomous beating, the atria was removed and the AV node was crushed. Left ventricular diastolic pressure was set at 8 mmHg pressure during the initial equilibration period by adjusting the intraventricular balloon volume. The mean volume of the balloon was 0.1 ± 0.04 ml. If the left ventricular systolic pressure did not reach 80 mmHg the heart was not used.

The perfusate was a modified Krebs–Henseleit buffer containing the following constituents: 115 mM NaCl, 4.5 mM KCl, 26 mM NaHCO$_3$, 1.8 mM MgSO$_4$, 2.0 mM CaCl$_2$, 0.1 mM NaEDTA, 10 mM pyruvate and 11 mM glucose (Neely et al., 1967). KH$_2$PO$_4$ was excluded from the buffer to enable accurate measurements of intracellular inorganic phosphate ($P_i$) from $^{31}$P NMR. Pyruvate was added to the buffer to maximize the redox state of the heart mitochondria and minimize the production of ATP by glycolysis (Kingsley-Hickman et al., 1986). Perfusate osmolarity was determined (Wescor vapor pressure osmometer) to be between 285 and 295 mOsM. To eliminate the effects of catecholamine release by pacing, 0.1 µM esmolol (Dupont, Wilmington, DE) was added to the buffer. Perfusate temperature was maintained at 37°C via an in-line thermistor and heat exchanger. The perfusate was oxygenated by vigorous bubbling of the solution with a mixture of 95% O$_2$ and 5% CO$_2$. The pH of the perfusate was 7.40. At the end of each experiment, the hearts were removed from the cannula and weighed. Hearts were air dried for 2 days and weighed again for the dry weight.

Oxygen consumption

Myocardial oxygen consumption (MVO$_2$) was determined by the product of perfusate flow rate and the difference between afferent and efferent perfusate oxygen content. To avoid exchange between the effluent perfusate and ambient air, the heart was suspended in a small, closed glass chamber. Effluent dripped off the heart but hearts were
suspended in effluent. Samples were obtained at the site of exit of the effluent from the glass chamber. Oxygen content was measured with either a blood gas analyzer (ABL-30, Copenhagen, Denmark) or oxygen electrode (YSI, Yellow Springs, OH). Oxygen consumption was calculated as \( \mu \text{mol/min/g dry weight of heart.} \)

\[ \text{[ADP]} = \text{[ATP]} - \text{[total creatine-PCr]} / [\text{PCr}] \cdot K_{\text{app}}; \]

where [ATP] and [ADP] represent the sum of all possible ionic species.

Two to three \( ^{31} \text{P} \) NMR spectra were obtained for each heart during the control steady-state period and inotropic state. The values for [PCr], [ADP], [P], pH and [Mg\(^{2+}\)] from spectra during each period were averaged for the control period and each inotropic agent. [ATP] and [TOTAL CREATINE] were determined by HPLC as previously described.

Lactate production

Lactate production was determined by measuring the difference in lactate concentrations between the perfusate entering and exiting the heart. Lactate concentration was determined using a standard commercial assay (Sigma Chemical Co., Kit #735, St Louis, MO). Three measurements were made during control period and during the infusion of the agents.

Experimental protocol

Throughout the entire experiment, left ventricular pressure was continuously measured while MVO\(_2\) lactate production, and \( ^{31} \text{P} \) NMR spectra were obtained every 8–10 min. A control steady-state was established when three consecutive measurements of the \( ^{31} \text{P} \) NMR spectra, MVO\(_2\), and pressure did not vary by more than 10%. This period was approximately 35 min in duration. In three separate experiments, no inotropic agents were infused and demonstrated that cardiac function, MVO\(_2\) and \( ^{31} \text{P} \) NMR spectra were stable in this model for over 120 min (data not shown). After a control steady-state was established, the perfusate was changed to the same perfusate with an inotropic agent added at a particular concentration. Each experiment used only one agent at one concentration per study. ORG 30029 was kindly donated by Organon Pharmaceuticals. It is relatively insoluble in water and thus was dissolved in DMSO (Fisher, Pittsburgh, PA). The DMSO solution was added to the perfusion buffer to yield a final DMSO to water concentration of 1:1800. In a separate set of experiments, DMSO alone was added to buffer at a concentration of up to 1:1000 and had no effect on cardiac metabolism or function (data not shown). Dobutamine (Eli Lilly, Indianapolis, IN) and calcium were added directly to
Table 1  Functional and Energetic Effects of ORG 30029, dobutamine and perfusate calcium

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Number</th>
<th>Systolic pressure (mmHg)</th>
<th>Diastolic pressure (mmHg)</th>
<th>Force–Time Integral (kdyne·sec)</th>
<th>Coronary Flow (ml/min)</th>
<th>MVO₂ (µmol/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26</td>
<td>90 ± 8</td>
<td>6 ± 3</td>
<td>17 ± 3</td>
<td>22 ± 6</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>ORG 30029 0.25 mM</td>
<td>6</td>
<td>136 ± 13*</td>
<td>4 ± 4</td>
<td>24 ± 3*</td>
<td>20 ± 3</td>
<td>34 ± 8</td>
</tr>
<tr>
<td>ORG 30029 0.375 mM</td>
<td>6</td>
<td>152 ± 12*</td>
<td>3 ± 4</td>
<td>29 ± 4*</td>
<td>23 ± 8</td>
<td>45 ± 4*</td>
</tr>
<tr>
<td>Dobutamine 60 µg</td>
<td>4</td>
<td>157 ± 18*†</td>
<td>3 ± 3</td>
<td>21 ± 5$</td>
<td>27 ± 7</td>
<td>52 ± 12*†</td>
</tr>
<tr>
<td>Dobutamine 75 µg</td>
<td>3</td>
<td>183 ± 8*$§</td>
<td>0 ± 0</td>
<td>24 ± 7</td>
<td>26 ± 5</td>
<td>57 ± 4*$§</td>
</tr>
<tr>
<td>Dobutamine 6 µM</td>
<td>3</td>
<td>151 ± 20*$¶</td>
<td>1 ± 2</td>
<td>26 ± 1*</td>
<td>27 ± 1</td>
<td>48 ± 4*</td>
</tr>
<tr>
<td>[Ca²⁺] 4 mM</td>
<td>3</td>
<td>167 ± 8*$##</td>
<td>1 ± 2</td>
<td>33 ± 3*$$$</td>
<td>35 ± 5*$###</td>
<td>64 ± 9*$$$</td>
</tr>
</tbody>
</table>

Data presented as means ± standard deviation. P<0.05; * vs control, † vs 0.25 mM ORG 30029, § vs 0.375 mM ORG 30029, # vs 60 µg dobutamine, ¶ vs 90 µg dobutamine.

Results

ORG 30029, dobutamine, and high perfusate calcium each increased systolic pressure and force–time integral in a concentration-dependent manner (Table 1). Each caused a downward trend in the diastolic pressure. However, despite a 50% increase in systolic pressure and a 17% increase in force–time integral compared to control, ORG 30029 had no significant effect on MVO₂ at the lower concentrations. This effect on MVO₂ differed from that of dobutamine and high perfusate calcium where a 65% increase in systolic pressure and a 17% increase in force–time integral compared to control resulted in a 50% and 41% increase in MVO₂, respectively. Developed pressure was effected in a similar manner to force–time integral by the three agents (Fig. 1). The different effects on MVO₂ between the agents was less significant at higher concentrations. Coronary flow tended to increase (Suga, 1990). The force–time integral was derived by the formula: mmHg/seconds/surface area of the balloon·1329 dyne/cm². To calculate balloon surface area, it was assumed that the balloon was spherical. The force–time integral was expressed in kilodynes-seconds (kdyne·secs).

The functional, metabolic, and ³¹P NMR data obtained in the control steady-state period from all experiments were pooled. Analysis of variance for repeated measures were used to determine differences within study populations and a post hoc Bonferroni/Dunn “t” test was used to test for significant differences between groups. Data are expressed as means ± standard deviation. The number of experiments are shown in the Tables.
Inotropic and Energetic Effects of ORG 30029, Dobutamine and Calcium

Figure 1 Relationship between changes in developed pressure and myocardial oxygen consumption (top), and force–time integral and myocardial oxygen consumption (bottom) for dobutamine, high perfusate calcium, and ORG 30029 in rat hearts. Dobutamine caused a greater increase in myocardial oxygen consumption for a similar change in force-time integral than did calcium. Both agents caused a greater rise in myocardial oxygen consumption than did ORG 30029.

with 6 mM perfusate calcium but not 4 mM perfusate calcium, dobutamine, or ORG 30029.

To elucidate possible mechanisms for the differences in contractile economy between the inotropic agents, high energy phosphate concentrations, lactate production, and basal metabolism were determined in hearts perfused at the concentrations that caused the largest differences in contractile economy. High energy phosphate concentrations in hearts were determined in a subset of the experiments presented in Table 1 with 31P NMR. As shown in Table 2, there were no significant differences in the phosphate metabolite concentrations; indicating that the production and utilization of high energy phosphates were in a steady-state. Likewise, lactate production was unaffected by perfusion by each agent. Basal metabolism, as measured in the KCl arrested heart, was not significantly altered by each inotropic agent (Table 3) though there was a trend towards an
Table 2 Effects of ORG 30029, dobutamine and calcium on high energy phosphate concentrations, pH, [Mg$^{2+}$], and lactate production

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ORG 30029</th>
<th>Dobutamine</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td></td>
<td>0.375 mM</td>
<td>60 µM</td>
<td>6 mM</td>
</tr>
<tr>
<td>Number</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>[PCr] (µmol/g DW)</td>
<td>54 ± 7</td>
<td>51 ± 8</td>
<td>51 ± 7</td>
<td>46</td>
</tr>
<tr>
<td>[ADP] (µmol/g DW)</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>[Pi] (µmol/g DW)</td>
<td>15 ± 13</td>
<td>13 ± 11</td>
<td>22 ± 15</td>
<td>17</td>
</tr>
<tr>
<td>pH_i</td>
<td>7.02 ± 0.10</td>
<td>6.94 ± 0.13</td>
<td>6.92 ± 0.15</td>
<td>7.00</td>
</tr>
<tr>
<td>[Mg$^{2+}$] (mmol/l)</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>0.08 ± 0.05</td>
<td>0.05 ± 0.05</td>
<td>0.8 ± 0.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Data presented as means ± standard deviation. There were no statistically significant differences between the agents with respect to the parameters measured. DW, dry weight.

Table 3 Effects of ORG 30029, dobutamine and calcium on basal metabolism in the KCl-arrested rat heart (mean values)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline developed MVO$_2$</th>
<th>Baseline MVO$_2$</th>
<th>KCl arrest pressure</th>
<th>KCl arrest MVO$_2$</th>
<th>Agent + KCl pressure</th>
<th>Agent + KCl MVO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORG 30029</td>
<td>2</td>
<td>82</td>
<td>36</td>
<td>14</td>
<td>17</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>2</td>
<td>87</td>
<td>40</td>
<td>19</td>
<td>19</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Calcium</td>
<td>2</td>
<td>80</td>
<td>36</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>14</td>
</tr>
</tbody>
</table>

Discussion

The present investigation demonstrated significant differences in energetic responses to increased contractility in perfused rat hearts between the calcium sensitizing agent ORG 30029, the β$_1$ agonist dobutamine, and high perfusate calcium. These differences resulted in divergent effects on cardiac energetics. The effect of dobutamine and high perfusate calcium on cardiac function, metabolism, and phosphate metabolites by ³¹P NMR have been well described previously (Kingsley-Hickman et al., 1992). The responses seen in this study are very similar to previous reports. To our knowledge, the simultaneous effects of ORG 30029 on MVO$_2$, phosphate metabolites, and function have not been investigated.

The energetic effects of ORG 30029 seen in the current investigation are consistent with those seen in other trials with other calcium sensitizing agents. Gross et al., demonstrated a similar difference between the calcium sensitizing agent EMD 57033 and dihydroouabain in guinea pig hearts (Gross et al., 1993). This result was consistent with our comparison of EMD 57033 and dobutamine in rat hearts (Grandis et al., 1995). However, other studies have not found a difference in efficiency between calcium sensitizing agents and conventional inotropic agents. A study comparing the effects of pimobendan and dobutamine on MVO$_2$ in dog hearts found no significant difference in the efficiency of contraction between these two agents, though economy increased 14% with pimobendan (Hata et al., 1992). Another study in dog hearts demonstrated no energetic differences between EMD 53998 (another calcium sensitizing agent) and calcium infusion (de Tombe et al., 1992). The conflicting results between these studies may be due to differences in species used, pharmacological actions of the agents, or experimental methods employed. The energetics of the isovolumic heart differ from that of the ejecting heart (Alpert et al., 1992., Suga, 1990). Thus, it is uncertain whether the marked differences in cardiac energetics observed between agents in this investigation would be found in the ejecting heart as well.

What are possible mechanisms for the disparate responses in MVO$_2$ between the different inotropic agents employed in the current investigation? Neither lactate production or high energy phosphate concentrations were altered in hearts stimulated with these agents. Thus, alterations in these parameters cannot account for the different effects on MVO$_2$ by these inotropic agents. Though basal metabolism tended to increase slightly with dobutamine, the 25% increase in MVO$_2$ with
Inotropic and Energetic Effects of ORG 30029, Dobutamine and Calcium
dobutamine in the KCl-arrested heart cannot fully account for the 50% increase in MVO₂ in the beating heart. Furthermore, high perfusate calcium did not affect MVO₂ in the KCl-arrested heart but did increase MVO₂ in the beating heart by 41%. It is possible that these agents altered the P:O ratio in mitochondria. However, this ratio has been demonstrated to be unaffected in rat hearts stimulated with dobutamine (Kingsley-Hickman et al., 1992).

Some investigators have proposed that calcium sensitizing agents like ORG 30029 enhance the myosin–actin interaction during contraction by increasing the rate of cross-cycle cycling (Lee and Allen, 1997). It is conceivable that this enhancement may create an improvement in the force generated per mole ATP hydrolyzed. β₁-Adrenoreceptor agonists such as dobutamine have been demonstrated to decrease calcium sensitivity in mammalian hearts (Endoh and Blinks, 1989). High perfusate calcium may also diminish calcium sensitivity by calmodulin-mediated inositol 1,4,5-triphosphate suppression of calcium sensitivity (Demaille and Pechère, 1983). Thus, ORG 30029 may have increased contractile economy as compared to control, dobutamine, or high calcium via energetic enhancement at the myosin–actin level. Similarly, dobutamine may have decreased contractile economy as compared to control via β₁ adrenoreceptor agonism.

Alternatively, different effects on calcium transients by these agents may also account for the differences seen in energetics. At the lower concentrations (like those employed in the current investigation) ORG 30029 has been demonstrated to increase cardiac contractility with minimal effects on calcium transients (Kawabata and Endoh, 1993). At higher concentrations, however, ORG 30029 increases phosphodiesterase activity and increases calcium transient amplitude (Cottney et al., 1990, Kawabata and Endoh, 1993). β₁-Adrenoreceptor agonists such as dobutamine and high perfusate calcium increase calcium transients several-fold (Endoh and Blinks, 1989, Kawabata and Endoh, 1993). Because an increase in calcium transient amplitude would increase energy expenditure for calcium handling, it is also tenable that ORG 30029 would cause less of an increase in MVO₂ than dobutamine and high perfusate calcium for a similar increase in contractility. It has been estimated that calcium handling utilizes 25–45% of the energy consumed by the beating mammalian heart (Suga, 1990). The data in the current study are consistent with this estimation.

In summary, ORG 30029 increased MVO₂ less than dobutamine and high perfusate calcium for similar increase in force–time integral. These changes occurred without affecting high energy phosphate concentrations, lactate production, or basal metabolism. The energetic differences may be accounted for by differences in calcium handling or effects on the myosin–actin interaction.

Acknowledgments

This work was supported by a National Institutes of Health Facilities Grant RR-03631 to the Pittsburgh NMR Center for Biomedical Research, an NIH Award (HL-40354) and RDCA (HL-02847) to A.P.K. and an NIH training grant #ST32 DK07458-09, Grant-in-Aid from the Pennsylvania Affiliate of the American Heart Association to D.J.G. We thank the Richard King Mellon Foundation, the Lucille Markey Charitable Trust, the Ralph M. Parsons Foundation, and the Ben Franklin Partnership Program of the Commonwealth of Pennsylvania for providing financial support for establishing the NMR Center for Biomedical Research. Org 30029 was generously donated by Organon International.

References


GAMBASSI G, CAPAGROSSI MC, KLOCKOW M, LAKATTA EG, 1993. Enantiomeric dissection of the effects of the...


