Methionine-Induced Positive Inotropic Effect in Rat Heart: Possible Role of Phospholipid N-Methylation

Vincenzo Panagia, Mahesh P. Gupta, Pallab K. Ganguly, and Naranjan S. Dhalla

Perfusion of isolated rat heart with L-methionine produced a positive inotropic effect that was temporally preceded, as well as accompanied, by an increase of methyl group incorporation into N-methylated phospholipids of the myocardium. Maximal increase in contractile force development was associated with maximal methyl group incorporation. Both parameters showed a dose-related dependence on methionine and correlated positively (r = 0.965) upon regression analysis of the data. The presence of adenosine, L-homocysteine thiolactone and erythro-9-(2-hydroxy-3-nonyl) adenine in the perfusion medium inhibited the positive inotropic effect as well as the incorporation of methyl groups into phospholipids. Cycloleucine, an inhibitor of S-adenosylmethionine synthetase, also reduced the increase in contractility by methionine. Methionine-induced positive inotropic effect could be modulated by varying Ca²⁺ concentration in the perfusate and was inhibited by ryanodine, a blocker of sarcoplasmic reticular Ca²⁺ release. These observations indicate that L-methionine may serve as a powerful positive inotropic agent and suggest that phospholipid N-methylation plays an important role in functional activity of rat heart. (Circulation Research 1988;62:51-55)

L-Methionine, an essential amino acid, is increasingly used as a supplement in a wide variety of health foods. Methionine deficiency has been associated with disturbances in lipid metabolism and myocardial lesions.1,2 Since methionine is required for transmethylations, its deficiency was proposed to result in insufficient methylation and heart disease.3 In this regard, the S-adenosyl-L-methionine (AdoMet)-mediated methylation of phosphatidylethanolamine (PE) has been the subject of several studies.4-6 Although changes in phospholipid N-methylation activity have been identified in different experimental models of heart disease,7-10 the significance of phospholipid methylation in heart function is far from clear. Since AdoMet does not enter the cell efficiently,11,12 phospholipid methylation can be carried out via the intracellular formation of AdoMet by perfusing the heart with methionine. This report indicates that perfusion of rat heart with L-methionine was associated with both stimulation of phospholipid N-methylation and the force of cardiac contraction.

Materials and Methods

Healthy male Sprague Dawley rats (300-400 g) were decapitated and their hearts excised and placed in ice-cold, oxygenated Krebs-Henseleit solution. After trimming of the atra, extraneous fat, and connective tissue, the ventricles were arranged for coronary perfusion according to the procedure of Langendorff as described previously.13 Equilibration perfusion was carried out for 15 minutes with Krebs-Henseleit medium containing (in mM): NaCl 120, NaHCO₃ 25, KCl 4.8, KH₂PO₄ 1.2, MgSO₄ 1.25, CaCl₂ 1.25, and glucose 8.6 (pH 7.4). This solution was gassed continually with 95% O₂-5% CO₂ and maintained at 37°C. Coronary flow was maintained at 7.8 ml/min, and the hearts were driven electrically at 280 pulses/min. A resting tension of 2 g was applied to the heart upon starting the perfusion. Contractile force was monitored on a Gibson polygraph recorder by means of a Grass FT.03 force displacement transducer.

L-Methionine (75-600 µM) was added to the perfusion medium and inhibitors, when used, were added in the presence of methionine. In some experiments, incorporation of [³H]methyl groups into phospholipids of cardiac tissue was studied by perfusing hearts with L-[³H-methyl] methionine and, at the end of each experiment, phospholipids were extracted from the cardiac homogenate and fractionated as described earlier.7,14 In these experiments, phospholipid N-methylation was tested directly in cardiac homogenate because [³H]methyl group incorporation into phospholipids of various purified subcellular membranes (i.e., sarcolemma, sarcoplasmic reticulum, or mitochondria) upon [³H]methionine perfusion of the heart could not be assessed with sufficient precision, as it has been noted with other tissues upon similar experimental conditions.4 Other experimental details are indicated in figure legends.

L-[³H-Methyl] methionine (specific activity, 80.0 Ci/mmol) and L-[7-³H,N]norepinephrine (specific activity, 43.9 Ci/mmol) had a radiochemical purity greater than 98.0% and 99.7%, respectively, and were purchased from New England Nuclear, Dorval, Canada. L-Methionine, adenosine, L-homocysteine thio-
lactone, cycloleucine, l-norepinephrine • HCI, and phosphatidylcholine were purchased from Sigma Chemical Co., St. Louis, Mo. Phosphatidyl-N-monomethylethanolamine and phosphatidyl-N,N-dimethylethanolamine were obtained from Calbiochem Behring, San Diego, Calif. Erythro-9-(2-hydroxy-3-nonyl) adenine • HCI was purchased from Burroughs Wellcome Co., Research Triangle Park, N.C., and ryanodine from Progressive Agri-Systems Inc., Wind Gap, Penn. All other reagents were of analytical grade.

Results were expressed as the mean±SEM. Statistical analysis was performed by Student’s t test and a probability of <0.05 was considered statistically significant.

Results

Figure 1 (upper two curves) shows the typical effect of L-methionine (300 µM) on cardiac contractile force and rate of force development (dF/dt). L-Methionine began to increase the cardiac contractility within about 15 minutes of perfusion; the maximal increase in contractile force and dF/dt occurred within 25 to 30 minutes. The positive inotropic action was maintained for prolonged periods (60 to 75 minutes) by perfusing the hearts with methionine. This effect was reversible within 30 minutes upon perfusing the hearts with methionine-free medium. Figure 1 (lower two curves) also shows the effect of methionine in the presence of adenosine, L-homocysteine thiolactone (L-HCT), and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). The above mixture is known to increase the endogenous formation of S-adenosyl-l-homocysteine (AdoHcy), an inhibitor of AdoMet-mediated phospholipid N-methylation. The presence of adenosine, L-HCT, and EHNA inhibited about 80% of the L-methionine effect; however, in the absence of L-methionine these agents had no effect on the cardiac contractility.

Figure 2 shows that the L-methionine effect on contractile activity was dependent upon the concentration of L-methionine (75–600 µM) used in the perfusion medium. This action was drastically reduced not only by the mixture of adenosine, L-HCT, and EHNA but also by cycloleucine, an inhibitor of the synthesis of AdoMet from methionine. Moreover, methyl acetimidate, an amino group blocking agent and an inhibitor of phospholipid N-methylation, also abolished L-methionine–induced stimulation of cardiac developed tension (data not shown).

The incorporation of [3H]methyl groups from L-[3H-methyl] methionine into phospholipids during stimulation of cardiac contractility by methionine was examined in separate experiments. Time-course studies revealed a progressive increase of total methyl group incorporation into cardiac phospholipids (1.4, 12.1, 19.2, and 28.8 nmol [3H]methyl groups/g heart at 3, 10, 15, and 30 minutes of 300 µM [3H-methyl] methionine perfusion, respectively; n = 2), which preceded and then accompanied the enhancement in force development (Figure 1, upper panel). Dose response studies further indicated a close relation between [3H]methyl group incorporation and increase in contractile force upon perfusing the heart with various concentrations of labeled methionine (Figure 2). In fact, when methyl

![Figure 1](link)  
**Figure 1.** Cardiac contractile force and rate of force development (dF/dt) after perfusion with 300 µM L-methionine alone (upper two tracings) and in the presence of 100 µM adenosine (AD)+230 µM L-homocysteine thiolactone (HCT)+10 µM EHNA (lower two tracings). Hearts were initially equilibrated for 15 minutes in the absence of methionine to obtain basal values. L-Methionine and the inhibitors were added later in the perfusion medium as shown by arrows.

![Figure 2](link)  
**Figure 2.** Dose-response relations in phospholipid N-methylation and cardiac contractile force after 30 minutes' perfusion with L-methionine. Total [3H]methyl group incorporation into cardiac phospholipids (○, n = 3) was carried out as indicated in “Materials and Methods” by using [3H-methyl] methionine. Changes in developed force (△) with methionine alone (●) and in the presence of 100 µM adenosine+230 µM L-homocysteine thiolactone+10 µM EHNA (□) or 1 mM cycloleucine (▲) are expressed as percent of basal values (6.2 ± 0.8 g). Values are mean±SEM.
Table 1. Incorporation of [\textsuperscript{3}H]Methyl Groups into Cardiac Phospholipids After 30 Minutes' Perfusion With L-[\textsuperscript{3}H-Methyl] Methionine and Inhibitors of Phospholipid N-Methylation

<table>
<thead>
<tr>
<th>dF/dt (%) increase</th>
<th>[\textsuperscript{3}H]Methyl group incorporation (nmol/g heart wt/30 min)</th>
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<tbody>
<tr>
<td>Total</td>
<td>PMME</td>
</tr>
<tr>
<td>300 \mu M L-[\textsuperscript{3}H-Methyl] methionine</td>
<td>91.6±11.7</td>
</tr>
<tr>
<td>300 \mu M L-[\textsuperscript{3}H-Methyl] methionine + 100 \mu M adenosine, 10 \mu M EHNA, 230 \mu M L-homocysteine thiolactone</td>
<td>23.1±2.5*</td>
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</table>

dF/dt is expressed as percent increase over basal values (premethionine). PMME, phosphatidyl-A'-monomethyl-ethanolamine; PDME, phosphatidyl-N,N-dimethylethanolamine; PC, phosphatidylcholine.

\*p<0.05 vs. 300 \mu M L-[\textsuperscript{3}H-methyl] methionine.

Values are mean±SEM of 5 experiments.

group incorporation and force development data (both expressed as percent of maximal increase) were correlated, a positive linear relation was obtained and the linear correlation coefficient (r) was calculated to be 0.965 (method of least squares). Consistent with the results reported above, at the peak developed tension maximal amount of [\textsuperscript{3}H]methyl groups was incorporated into heart N-methylated phospholipids after 30 minutes perfusion with 300 \mu M methionine (Table 1).

This incorporation was inhibited when the hearts were perfused in the presence of labeled L-methionine, adenosine, l-HCT, and EHNA.

Experiments in Figure 3A show that at the steady state, methionine-induced positive inotropic effect was sensitive to the Ca\textsuperscript{2+} concentration of the perfusion medium. In fact, a progressive increase in developed force was achieved by 0.3–2 mM Ca\textsuperscript{2+} in a concentration-dependent manner. Since the inotropic properties of cardiac muscle are primarily dependent on the amount of Ca\textsuperscript{2+} released from the sarcoplasmic reticulum (SR) to the myofilaments,\textsuperscript{17} ryanodine, a natural plant alkaloid that has been shown to inhibit Ca\textsuperscript{2+} release\textsuperscript{18} without interfering with the transsarcolemmal routes of Ca\textsuperscript{2+} entry,\textsuperscript{18–20} was added in the perfusion medium. A relation exists between the concentrations of ryanodine and the degree of inhibition of methionine-induced effect, which was abolished almost completely by 20 \mu M ryanodine (Figure 3B).

Finally, to test whether or not a differential release of norepinephrine from noradrenergic nerve terminals upon methionine perfusion might contribute to the observed positive inotropic effect, hearts were preperfused with [\textsuperscript{3}H]norepinephrine, washed, and then perfused with or without methionine. Data in Figure 4.
indicate that the spontaneous \([^{3}H]\)norepinephrine efflux\(^{21}\) was unaltered by methionine.

**Discussion**

In the present investigation, we have shown that L-methionine produced a positive inotropic effect on heart which was temporally preceded, as well as accompanied, by an increase of methyl group incorporation into cardiac N-methylated phospholipids. Both phospholipid N-methylation and force development showed a dose-related dependence on methionine and correlated positively upon regression analysis of the data. Since the L-methionine action was inhibited by increasing the endogenous levels of AdoHcy, blocking amino group of intramembranal PE molecule or preventing the synthesis of AdoMet (the physiologic methyl donor for methyl transferase reactions), the incorporation of methyl groups into phospholipids can be seen to be involved in stimulating cardiac contractility. Because the inotropic effect of L-methionine, unlike catecholamine, was evident after a lag period of 15 minutes, it is likely that the site of action of L-methionine may be of intracellular origin. The finding that the rate constant of spontaneous \([^{3}H]\)norepinephrine efflux did not vary with methionine also seems to exclude a possible involvement of norepinephrine in the observed methionine effect. It may be noted that an increased number of cardiac \(\beta\)-receptors has been shown to be associated with an increase in phospholipid N-methylation\(^{2}\); therefore, an enhancement in \(\beta\)-receptor density by methionine-activated N-methylation is a possibility that needs to be examined in detail. The increment of cardiac contractility by methionine can be explained on the basis of mechanisms by which the action potential stimulus initiates the contractile process in heart muscle. Accordingly, the central role of Ca\(^{2+}\) in cardiac excitation-contraction coupling\(^{17}\) could be related to the methionine-induced stimulation of cardiac contraction. Active Ca\(^{2+}\) transport by cardiac SR is considered to be important in myocardial contraction-relaxation process,\(^{17}\) in that Ca\(^{2+}\) released from the SR induces contraction while relaxation is produced by the accumulation of Ca\(^{2+}\) in SR. In this regard, we have shown that methionine-induced positive inotropic effect can be modulated by varying Ca\(^{2+}\) concentration and can be inhibited by ryanodine, a well-known blocker of SR Ca\(^{2+}\) release.\(^{18}\) These results are interpreted to mean that methionine may act, at least partly, via the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism.\(^{22}\) On the other hand, the Ca\(^{2+}\) pump ATPase is the major component of the SR which serves as an energy transducer and translocator of Ca\(^{2+}\) across the SR membranes.\(^{17}\) We have recently reported\(^{23}\) that Ca\(^{2+}\) pump is activated upon AdoMet-dependent methylation of membrane phospholipids. Based on the assumption that the amount of Ca\(^{2+}\) released by SR upon excitation of the cardiac muscle may be directly proportional to the amount of Ca\(^{2+}\) uptake, one can speculate that perfusion of heart with L-methionine increases phospholipid N-methylation in SR membrane and thus may promote force of contraction. This view is further supported by the fact that Ca\(^{2+}\) pump activity of cardiac sarcolemma, which is responsible for the efflux of Ca\(^{2+}\),\(^{17}\) is also activated by AdoMet-induced methylation.\(^{24}\)

A clear understanding of the concept of changing myocardial contractility in which the mechanical performance of cardiac muscle can be altered by essential amino acid is important since nutritional factors are known to play a direct role in cardiovascular diseases.\(^{3}\) Exacerbation of the frequency of myocardial lesions by dietary insufficiency of L-methionine has been suggested earlier.\(^{2}\) Therefore, increased requirements for specific dietary factors would result from physiologic needs associated with a certain physical state of the membrane that is affected by the conversion of a lipid molecule into another. From the results presented in this study it is tempting to speculate about the possible role of L-methionine in the treatment of cardiac disease where contractile force generation is depressed. Nonetheless, the pronounced increase in cardiac contraction over a prolonged period by perfusing the heart with methionine suggests that this amino acid is a powerful positive inotropic agent.

**References**

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