Am J Physiol Heart Circ Physiol 287: H812–H817, 2004.
First published April 8, 2004; 10.1152/ajpheart.01042.2003.

Acute negative inotropic effects of homocysteine are mediated via the endothelium

Richard H. Kennedy,[1,3] Richard Owings,[1] Nawal Shekhawat,[3] and Jacob Joseph[1,2]

Departments of [1]Pharmaceutical Sciences, [2]Internal Medicine, and [3]Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Submitted 5 November 2003; accepted in final form 2 April 2004

Kennedy, Richard H., Richard Owings, Nawal Shekhawat, and Jacob Joseph. Acute negative inotropic effects of homocysteine are mediated via the endothelium. *Am J Physiol Heart Circ Physiol* 287: H812–H817, 2004. First published April 8, 2004; 10.1152/ajpheart.01042.2003.—Previous studies have shown that chronic hyperhomocysteinemia is associated with an adverse cardiac remodeling and heart failure. This study, which utilized coronary-perfused hearts and superfused papillary muscle, was designed to determine whether homocysteine acts acutely to alter cardiac contractile function. Left ventricular developed pressure was used as a measure of systolic function in the Langendorff-perfused heart, whereas isometric developed tension was used in papillary muscle. All preparations were bathed in physiological buffer and paced electrically. Initial results showed that homocysteine elicits a relatively rapid onset (maximum effect observed within 5 min), concentration-dependent (10–300 μM), and moderate negative inotropic action (maximum decrease in tension was ~15% of control values) in Langendorff-perfused hearts but not in papillary muscle. In contrast, effluent from homocysteine-treated hearts decreased contractility in papillary muscle, and all inotropic actions were largely eliminated when brief Triton X-100 treatment was utilized to inactivate the coronary endothelium in the intact heart. The homocysteine-induced decrease in contractile function was not antagonized by N^{ω}-nitro-L-arginine, a nitric oxide synthase inhibitor, or the cyclooxygenase inhibitor indomethacin. Thus data suggest that pathophysiological concentrations of homocysteine elicit an acute negative inotropic effect on ventricular myocardium that is mediated by a coronary endothelium-derived agent other than nitric oxide or products of cyclooxygenase. Future studies are required to elucidate the mechanism by which homocysteine acts to elicit the release of the proposed endothelial mediator, the identity of the proposed paracrine agent, and the mechanism of its negative inotropic action.

contractility; Langendorff-perfused heart; papillary muscle; Triton X-100 treatment; rat

HYPERHOMOCYSTEINEMIA (Hhe) has been linked to cardiovascular disease in several studies; however, most of these reports have focused on its vascular and endothelial actions. For example, in 1969, McCully (16) reported that severe Hhe resulting from inborn errors of metabolism results in vascular disease, and other investigators (18) subsequently found that the prevalence of elevated plasma homocysteine increases from an estimated 5% in the general population to 13–47% in patients with symptomatic vascular disease. Retrospective and prospective studies (3, 17, 21) also showed an association between Hhe and atherothrombotic diseases, including myocardial infarction and stroke.

In addition to the accumulating evidence linking Hhe to vascular pathology and a prothrombotic state, recent studies have demonstrated that chronic Hhe elicits adverse effects on the myocardium. Blacher and coworkers (2) examined 75 patients with end-stage renal disease undergoing hemodialysis and showed a positive correlation between echocardiographically measured left ventricular mass index and plasma homocysteine, even after adjustment for age, gender, systolic blood pressure, and hematocrit. In addition, a recent Framingham study report (30) indicated that plasma homocysteine levels are an independent risk factor for congestive heart failure. Studies in our laboratory (11) using rat models showed that 10 wk of Hhe elicits an adverse cardiac remodeling and diastolic dysfunction in normotensive animals, and exacerbates the remodeling and diastolic dysfunction observed in the hypertensive heart (11). Thus, in addition to its well-described vascular actions, chronic Hhe acts in the absence of other stimuli to elicit direct adverse effects on cardiac structure and function, suggesting that it is an independent risk factor for cardiac remodeling and dysfunction.

This study was designed to determine whether homocysteine also has acute effects on cardiac systolic function, because such actions may complement the previously described chronic effects on cardiac structure and contribute to the associated cardiac dysfunction. Previous work by Tyagi et al. (27) in ex vivo cardiac rings of the rat right ventricle and left ventricle showed that homocysteine enhances diastolic tension and that this action is enhanced by removal of the endocardial endothelium. In addition, studies in various tissues demonstrated acute effects of homocysteine that could affect cardiac contractile function were they to occur in the heart. For example, homocysteine was shown to inhibit Na^+-K^+-ATPase activity in the hippocampus (24), decrease intracellular calcium concentration in arteriolar smooth muscle (28), and inhibit the transient outward current (I_{to}) in rat ventricular myocytes (22).

MATERIALS AND METHODS

Animals. All procedures in this study were approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences. Three-month-old male Sprague-Dawley rats (300–325 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN) and maintained in our institutional Division of Laboratory Animal Medicine on a 12:12-h light-dark cycle with free access to chow and water.

Langendorff-perfused hearts. Rats were anesthetized with a volatile anesthetic, and hearts were removed and immediately perfused via the aorta with an oxygenated Krebs-Henseleit (KH) solution (37°C) of the

Address for reprint requests and other correspondence: R. H. Kennedy, Dept. of Pharmaceutical Sciences, Mail Slot 522, Univ. of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205 (E-mail: kennedyrichardh@uams.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

following composition (in mM): 118.0 NaCl, 27.1 $NaHCO_3$, 3.7 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 1.0 KH_2PO_4, and 11.1 glucose. The flow rate was set at 7.0 ml·g heart^{-1}·min^{-1}, a value similar to that observed when flow is examined at a constant pressure of 70 mmHg; coronary pressure was monitored continuously by a Statham pressure transducer. Both atria were removed, and the ventricles were paced electrically at 250 beats/min by platinum contact electrodes positioned on the interventricular septum. A fluid-filled balloon catheter was placed in the left ventricle to measure intraventricular pressure, and the heart was enclosed in a humidified, temperature-controlled chamber. Contractile function was monitored by measuring developed pressure (peak systolic pressure − diastolic pressure) at the preload balloon volume that elicited 90–95% of maximal basal contractile function. In addition to a polygraph recording, all data were digitized and analyzed with the use of acquisition and analysis software (CODAS; DataQ Instruments; Akron, OH). After equilibration for 60 min, the inotropic effects of homocysteine were examined by the addition of the amino acid to the perfusing solution.

The coronary vascular endothelium was inactivated in some hearts by brief perfusion with Triton X-100. Langendorff-perfused preparations were set up as described above, and after 30-min equilibration an infusion of Triton X-100 (1:200 dilution) equivalent to 1% of the flow rate was delivered into the KH buffer immediately above the aorta for 1 s (14, 20). Subsequently, the hearts were perfused for 25 min with normal KH buffer before the inotropic actions of homocysteine were examined as described above. The effectiveness of the Triton treatment in inactivating the endothelium was verified by its ability to diminish nitric oxide (NO) release.

Papillary muscle. Papillary muscle (diameter <0.7 mm) was isolated from the rat heart, suspended in the KH solution described above (37°C), and paced via contact electrodes at 3.0 Hz. Resting tension (set at 90% L_{max}) and force of isometric contraction were monitored via force-displacement transducers. After a 60-min equilibration period, basal contractility was recorded, and the inotropic effects of homocysteine or the effluent collected from Langendorff-perfused hearts were examined. The papillary preparations were exposed to effluent within 1 min of its collection from the heart.

Measuring nitrate/nitrite. The nitrate/nitrite concentration, as measured by a modification of the Greiss technique (19), was used as an estimate of NO production. Equal concentrations of effluent and Greiss reagent (0.8% sulfanilamide in 0.5 N HCl-0.075% *N*-naphthylethylene diamine) were mixed and shaken gently for 15 min at room temperature. Absorbance was then measured at 543 nM, and concentration was determined with the use of a standard curve obtained with sodium nitrite.

Statistical analysis. Data were evaluated by ANOVA with a Student-Newman-Keuls post hoc test or by *t*-test as appropriate with the use of SigmaStat software (SPSS; Chicago, IL). The criterion for significance was a P value <0.05. Data are reported as means ± SE.

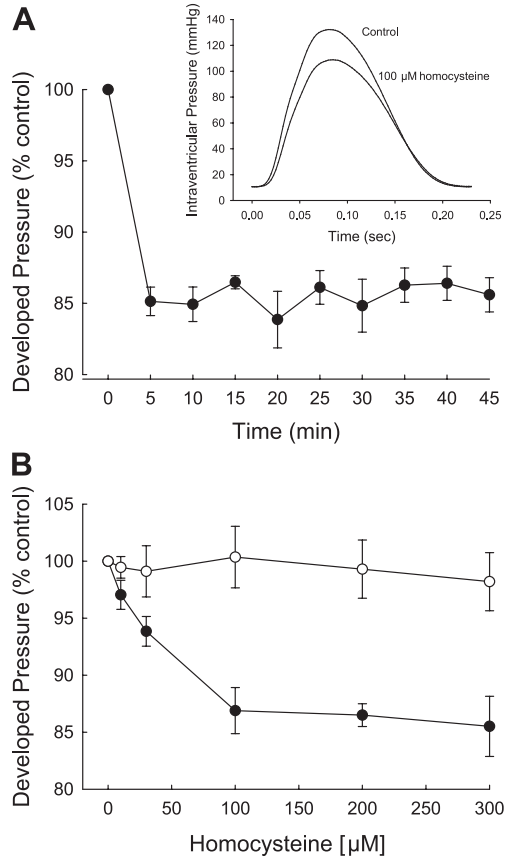

Fig. 1. Effects of homocysteine on developed pressure (peak systolic − diastolic pressure) in Langendorff-perfused rat hearts. Preparations were perfused with an oxygenated Krebs-Henseleit solution (37°C) at 7.0 ml·g heart^{-1}·min^{-1} and paced electrically at 250 beats/min by contact electrodes positioned on the interventricular septum. A fluid-filled balloon catheter was placed in the left ventricle with volume being set at the value that elicited 90–95% of maximal basal contractile function. *A*: time-dependent negative inotropic effects of 100 μM homocysteine. Data are presented as a percentage of prehomocysteine control values (95.7 ± 7.6 mmHg; $n = 5$). Homocysteine elicited a statistically significant ($P < 0.05$) decrease in developed pressure at all time points examined between 5 and 45 min. *Inset*, representative tracings that were recorded before exposure to 100 μM homocysteine and after a steady-state response was observed. *B*: concentration-dependent inotropic effects of homocysteine (●; $n = 5$) and cysteine (○; $n = 5$) as examined with the use of cumulative addition. Data are presented as a percentage of prehomocysteine (101.9 ± 2.3 mmHg) and precysteine (105.0 ± 6.9 mmHg) control values. Vertical bars represent means ± SE. Homocysteine elicited a concentration-dependent decrease in developed pressure that was significantly different ($P < 0.05$) from control values at concentrations of 30 μM and greater.

RESULTS

Effects of homocysteine on Langendorff-perfused heart. Initial experiments examined the acute effects of homocysteine on contractility in coronary-perfused myocardium by examining changes in left ventricular developed pressure in Langendorff-perfused preparations. As shown in Fig. 1*A*, 100 μM homocysteine elicited a negative inotropic effect that reached maximum levels of ~85% of control values within 5 min. The response remained stable for at least 45 min of continuous exposure, and contractile function returned to near control values (98 ± 2% of control; $n = 5$) within 15 min of washout. The concentration-response curve (Fig. 1*B*) showed that this negative inotropic action was detectable at concentrations between 10 and 300 μM, with the first statistically significant effect being observed at 30 μM and little change occurring between 100 and 300 μM. The amino acid had no effect on diastolic pressure (data not shown) but elicited a concentration-dependent reduction in coronary pressure (from a control value of 74.9 ± 6.5 to 69.7 ± 5.0 and 65.1 ± 5.6 mmHg at 30 and 300 μM, respectively; $n = 5$). In contrast, equivalent concentrations of cysteine had no effect on contractility or coronary pressure.

Effects of homocysteine on isolated papillary muscle. Because homocysteine has been reported to elicit numerous effects on the vascular endothelium (9, 32, 33), subsequent experiments were designed to determine whether the acute inotropic actions of homocysteine could be observed in the absence of coronary perfusion. Contractility was monitored in superfused papillary muscle before and during exposure to the

amino acid. As shown in Fig. 2, homocysteine did not affect contractile function in these preparations. No significant inotropic effect was observed during a 30-min exposure to 100 μM homocysteine or with cumulative exposure to concentrations ranging from 10 to 300 μM (allowing a 10-min exposure to each concentration). Similarly, homocysteine had no effect on resting tension in isolated papillary muscle (data not shown).

Effects of Langendorff effluent on papillary muscle. Continued experiments examined contractile function in papillary muscle exposed to effluent collected from Langendorff-perfused hearts. Figure 3 shows that effluent collected from hearts perfused with control buffer tended to increase developed tension in papillary muscle; however, this effect was not statistically significant. When homocysteine was added to the buffer perfusing the Langendorff preparations, the effluent elicited a decrease in contractility that increased in a dose-dependent manner with the concentration of homocysteine. Statistically significant differences were first observed using effluent obtained from hearts exposed to 100 μM homocysteine. Similar results were obtained in papillary preparations pretreated with homocysteine before exposure to effluent. The effluent had no effect on resting tension (data not shown).

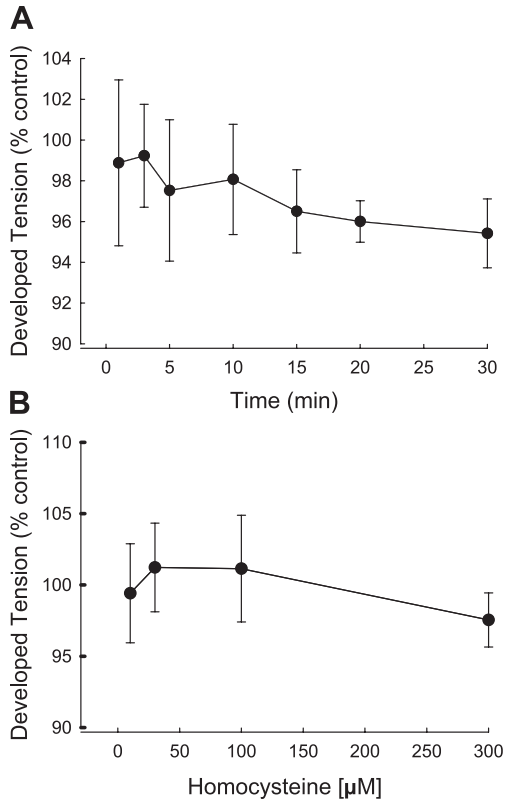

Fig. 2. Effects of homocysteine on developed tension in papillary muscle isolated from rat heart. Preparations were bathed in an oxygenated Krebs-Henseleit solution (37°C) and paced electrically at 180 beats/min by field electrodes. *A*: time-dependent effects of 100 μM homocysteine. Data are presented as a percentage of prehomocysteine control values (0.56 ± 0.07 g; $n = 5$). No significant difference was observed at any time. *B*: concentration-dependent effects of homocysteine as examined using cumulative addition. Data are presented as a percentage of prehomocysteine control values (0.58 ± 0.05 g; $n = 5$). Vertical bars represent means ± SE. No significant effect was observed at any concentration.

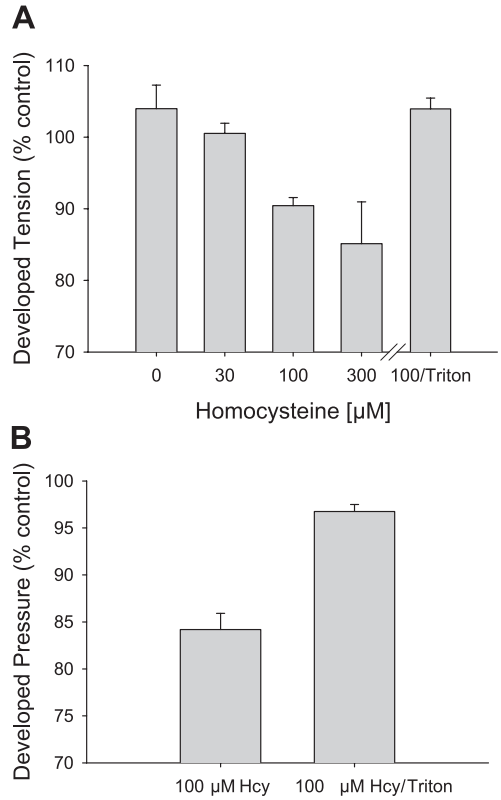

Fig. 3. *A*: negative inotropic effects of effluent collected from Langendorff-perfused rat heart on rat papillary muscle. Experiments were performed using heart and papillary preparations similar to those described in Figs. 1 and 2. Effluent collected from the coronary-perfused heart preparation was superfused onto the papillary muscle before and after cumulative addition of homocysteine to the buffer perfusing the heart. Data for developed tension in the papillary muscle are presented as a percentage of the control values obtained before exposure to effluent (0.68 ± 0.07 g; $n = 5$). The bar on the far right shows developed tension in a different group of papillary preparations that were exposed to effluent obtained from Langendorff hearts that were pretreated with Triton X-100 before exposure to 100 μM homocysteine (control value: 0.70 ± 0.06 g; $n = 5$). Significant reductions ($P < 0.05$) in developed tension were observed with effluent collected from hearts exposed to 100 and 300 μM homocysteine; this effect was eliminated by Triton X-100 pretreatment of the hearts. *B*: effects of 100 μM homocysteine on developed pressure in hearts before and after treatment with Triton X-100. The Triton treatment consisted of a 1-s exposure to a 1:200 dilution introduced as 1% of the flow rate of the Krebs-Henseleit buffer. Data are presented as a percentage of prehomocysteine control values (104.5 ± 4.8 and 109.9 ± 4.2 mmHg before and after Triton treatment, respectively; $n = 5$). The significant decrease ($P < 0.05$) in developed pressure elicited by 100 μM homocysteine was eliminated by Triton X-100 pretreatment. Vertical bars represent means ± SE.

Effects of Triton X-100 treatment on inotropic actions of homocysteine. To further verify that the inotropic actions of homocysteine were mediated by the vascular endothelium, experiments utilized Langendorff-perfused hearts that were pretreated with Triton X-100 (1:200 dilution introduced as 1% of the buffer flow for 1 s) to inactivate endothelial function (14, 20). The inotropic effects of 100 μM homocysteine were examined before and 25 min after the Triton X-100 treatment. The detergent treatment itself had little effect on contractile function; developed pressure before and after Triton treatment was 104.5 ± 4.8 and 109.9 ± 4.2 mmHg, respectively ($n = 5$) with no change in diastolic pressure. However, as shown in Fig. 3*B*, the nearly 15% reduction in developed pressure elicited by homocysteine before treatment with Triton X-100

was almost eliminated by endothelial inactivation; as in naïve hearts, homocysteine had no effect on diastolic pressure in Triton-treated hearts (data not shown). Time-control studies showed that the response to homocysteine was not significantly different during a second exposure if the hearts were not treated with the detergent after the first exposure (data not shown). In contrast to its effect on the response to homocysteine, Triton treatment did not significantly affect the response to a relatively low concentration of the calcium channel blocker nifedipine (0.03 μM), which reduced developed pressure by 22.4 ± 4.9% and 28.8 ± 5.5% before and after endothelial activation ($n = 4$). Coronary pressure was increased by the detergent treatment (from 67.9 ± 2.6 to 81.7 ± 4.4 mmHg; $n = 5$), and the endothelial inactivation was supported by the fact that Triton X-100 treatment decreased the nitrate/nitrite concentration (a measure of NO release) of the Langendorff effluent from 0.99 ± 0.06 to 0.20 ± 0.07 μM ($n = 5$). In contrast to its effects in untreated hearts, homocysteine had no effect on coronary pressure after Triton X-100 treatment (81.7 ± 4.4 and 80.6 ± 6.6 mmHg before and after homocysteine; $n = 5$).

The effects of Langendorff effluent on papillary muscle were also examined after Triton X-100 pretreatment of hearts. As shown in Fig. 3*A*, the negative inotropic effect of effluent from hearts treated with 100 μM homocysteine was eliminated when hearts were treated with Triton X-100 before homocysteine administration.

Effects of N^{ω}-nitro-L-arginine and indomethacin on inotropic actions of homocysteine. Continued experiments were designed in an attempt to determine the mechanism underlying the observed negative inotropic action of homocysteine. Because the action was antagonized by inactivation of the endothelium, it seemed possible that the amino acid was acting to release NO, an agent with negative inotropic effects, from the vascular endothelium. Thus experiments compared the concentration-dependent inotropic effects of homocysteine in Langendorff-perfused hearts in the presence and absence of 0.1 mM N^{ω}-nitro-L-arginine (L-NNA), a NO synthase (NOS) inhibitor. The inhibitor was added to the buffer solution 30 min before cumulative addition of homocysteine. L-NNA alone increased coronary pressure from 76.7 ± 5.7 to 96.6 ± 8.1 mmHg and decreased developed pressure from 103.6 ± 10.2 to 92.9 ± 9.2 mmHg ($n = 5$) before treatment with homocysteine. As shown in Fig. 4, hearts treated with L-NNA showed an enhanced negative inotropic response to homocysteine that was significantly greater than that observed in control hearts at concentrations of 30 μM and above. Coronary pressure was not affected by homocysteine in the presence of L-NNA (e.g., coronary pressures were 96.6 ± 8.1 and 94.9 ± 6.7 mmHg before homocysteine exposure and after treatment with 300 μM homocysteine; $n = 5$).

Because arachidonic acid derivatives have also been reported to affect cardiac contractile function (5, 13), continued experiments were designed to determine whether the proposed endothelium-derived paracrine agent is a product of the cyclooxygenase pathway. Indomethacin (50 μM), a cyclooxygenase inhibitor, was added to the perfusion buffer 20–30 min before the cumulative addition of homocysteine. This concentration of indomethacin was shown in preliminary studies to essentially eliminate the acute negative inotropic action elicited by arachidonic acid in Langendorff-perfused hearts (10 μM arachidonic acid elicited a 14.3 ± 3.1% decrease in developed pressure under control conditions and only a 1.5 ± 1.1% decrease after pretreatment with 50 μM indomethacin). Indomethacin alone did not affect contractile function or coronary pressure in the Langendorff-perfused hearts, and had no effect on the negative inotropic response to homocysteine (Fig. 4). As in the absence of indomethacin, coronary pressure was decreased by homocysteine in the presence of the cyclooxygenase inhibitor (e.g., from 81.2 ± 6.1 to 73.4 ± 4.4 and 71.0 ± 5.4 mmHg before homocysteine exposure and after treatment with 30 and 300 μM homocysteine, respectively; $n = 5$).

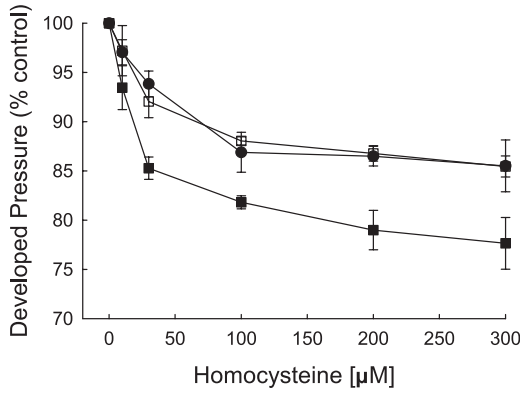

Fig. 4. Effects of N^{ω}-nitro-L-arginine (L-NNA) and indomethacin on the negative inotropic actions of homocysteine in Langendorff-perfused rat hearts. The hearts were prepared as described in Fig. 1. L-NNA (0.1 mM; ■; $n = 5$), indomethacin (50 μM; □; $n = 5$) or vehicle (●; $n = 5$) was added to the buffer 20–30 min before the cumulative addition of homocysteine. Data are presented as a percentage of values obtained immediately before the addition of the first concentration of homocysteine (see text for effects of L-NNA and indomethacin alone). Vertical bars represent means ± SE. L-NNA enhanced ($P < 0.05$) the effect of homocysteine at concentrations of 30 μM and above. No differences were observed when control and indomethacin-treated hearts were compared.

DISCUSSION

Results of this study demonstrate that homocysteine elicits an acute negative inotropic effect on ventricular myocardium and that this effect is observed at concentrations similar to the homocysteine levels observed in individuals with Hhe. The action appears to be specific for homocysteine because it was not observed with identical concentrations of cysteine. The data also suggest that the acute negative inotropic action of homocysteine is not mediated by the release of NO or cyclooxygenase products from the coronary vascular endothelium. The effect of homocysteine was not observed in superfused papillary muscle, but it was observed in papillary muscle exposed to coronary effluent collected from Langendorff-perfused hearts. In addition, the decrease in contractility was eliminated when hearts were preperfused with Triton X-100, a method of inactivating endothelial function. The magnitude of the negative inotropic action is somewhat limited, reaching approximately a 15% depression of contractility at 100–300 μM homocysteine; however, even minimal acute decreases in contractile function can be clinically relevant over a long period of time and contribute to the recently reported link between Hhe and cardiac dysfunction (11, 12, 30).

As mentioned above, Tyagi et al. (27), by using ex vivo cardiac rings of the rat ventricle, showed that homocysteine

enhances diastolic tension and that this action is enhanced by removal of the endocardial endothelium. Current results showed no significant effect of acute homocysteine exposure on left ventricular diastolic pressure in the electrically stimulated whole heart preparation or on resting tension in electrically paced papillary muscle (data not shown). The cause of this disparity is unknown but is likely caused by the differing preparations and conditions used in the two studies. The absence of direct effects of homocysteine on contractile function in papillary muscle and Triton-pretreated whole heart suggests that previously reported actions of the amino acid on Na^+-K^+-ATPase activity (24), intracellular calcium concentration (28), and I_{to} (22) were not elicited by concentrations up to 300 μM in intact tissue maintained under the chosen experimental conditions. Nonetheless, it is possible that positive inotropic (inhibition of Na^+-K^+-ATPase and I_{to}) and negative inotropic (decreased intracellular calcium) influences were offsetting, thereby resulting in no detectable change in contractile function.

The concentration dependency of the negative inotropic action of homocysteine observed in this study falls within the range observed in individuals diagnosed with Hhe. Plasma protein binding accounts for >70% of circulating homocysteine levels in humans with ~25% being in free oxidized forms and ~3% being in free reduced forms (6). Thus plasma concentrations of total homocysteine in individuals with moderate (30–100 μM) and severe (>100 μM) Hhe would result in free concentrations similar to those that elicited the acute effects in current experiments (10–300 μM with the first statistically significant effect being observed at 30 μM). Continued experiments are required to determine the relative concentrations of the oxidized and reduced forms of homocysteine in physiological buffers, as well as the role of these different forms in observed effects.

The mechanism by which homocysteine acts to elicit the release of the proposed endothelial mediator has not been elucidated, nor has the identity of the proposed paracrine agent or its mechanism of action. L-NNA, a NOS inhibitor, did not antagonize the inotropic action of homocysteine, indicating that the endothelial factor is not NO. This is not too surprising in light of data showing that homocysteine acts acutely to inactivate NO via oxidative mechanisms (29) and impair NO synthesis via production of asymmetric dimethylarginine (25). Hhe has been associated with increased levels of NOS, both endothelial NOS and inducible NOS (31), but this requires more prolonged exposure. In addition, indomethacin did not alter the response of Langendorff-perfused heart to homocysteine. This suggests that the endothelial factor is not a product of cyclooxygenase such as PGE_1, which has been shown to decrease contractile function in the rat heart (13). It is possible that other metabolites of arachidonic acid, such as the negative inotropic leukotrienes (5) or products of cytochrome *P*-450, are involved. Current experiments were restricted to examining the possible role of cyclooxygenase products because preliminary studies showed that indomethacin nearly eliminated the previously reported acute negative inotropic response to arachidonic acid (26) under the experimental conditions used in this study.

Brief Triton X-100 exposure of the coronary vasculature was utilized to inactivate the endothelium. Previous studies (14, 20) demonstrated the effectiveness of this treatment by showing that it inhibits the response to endothelium-dependent vasodilators without producing morphological changes in the heart. Similarly, current data indicated a significant reduction in endothelial function after Triton X-100 treatment. Coronary pressure rose significantly following treatment with the increase being similar to that elicited by L-NNA. In addition, experiments showed that the Triton X-100 exposure reduced NO release into the effluent by ~80%, while having little effect on left ventricular developed pressure (eliciting approximately a 5% increase) suggesting that it did not damage the endothelium to the point of altering barrier function or damaging cardiomyocytes.

Current data would seem to suggest that the acute effect of homocysteine on the Langendorff-perfused heart is more potent than the effect of homocysteine-containing effluent on papillary muscle. The first statistically significant effect of homocysteine on heart was observed at 30 μM, whereas the first significant change in papillary muscle was observed with effluent containing 100 μM homocysteine. Efficacy did not seem to be different as both preparations showed approximately a 15% decrease in contractile function at 300 μM. Although the cause of this slight rightward shift is not understood, it may reflect a simple dilution of the paracrine mediator in the buffer compared with its local concentration in the heart. Alternatively, it is possible that the paracrine mediator is unstable in aqueous solution.

Previous studies (20) have demonstrated that even under control conditions the coronary vascular endothelium releases both positive and negative inotropic agents with the relative release depending in part on coronary flow and oxygen tension. A review by Brutsaert (4) suggests that NO, angiotensin II, endothelin, prostanoids, and even polypeptides may be involved. In current experiments, the effluent collected from Langendorff-perfused hearts before exposure to homocysteine tended to increase systolic function in the papillary muscle even though this trend was not statistically significant. Future studies are required to identify the mediators of the endothelial action as well as the mediator released by homocysteine.

In addition to its acute negative inotropic effects homocysteine altered coronary pressure in Langendorff-perfused hearts. It decreased coronary pressure ~13% in control conditions, but had no significant effect after Triton X-100 or L-NNA pretreatment. This would seem to suggest that the coronary dilation elicited by homocysteine was mediated via an increase in NO release or via the acute formation of *S*-nitroso-homocysteine, a potent vasodilator (15, 23). Somewhat in contrast, other reports suggest that homocysteine acts acutely to inactivate NO (29) and impair its synthesis (25). Future studies are required to clarify this issue. Nonetheless, the more important question for the current study is whether the changes in coronary pressure played a role in observed inotropic actions. Gregg (10) reported in 1963 that changes in coronary perfusion can affect cardiac contractility, and subsequent work suggested that this effect may be caused by changes in oxygen supply (8) or the "garden hose" effect (1) with more recent studies indicating that it is related to capillary perfusion (7). Although current experiments were performed under constant coronary flow, it is possible that changes in arteriolar diameter may have influenced left ventricular systolic function by changing flow distribution. This, however, seems unlikely as homocysteine either decreased or had no effect on coronary pressure while decreasing systolic function. Similarly, it might be proposed

that the relatively large increase in coronary pressure elicited by L-NNA may have contributed via this mechanism to the concurrent fall in systolic function; however, a similar fall in cardiac contractility did not occur when Triton X-100 increased coronary pressure.

In summary, this study shows that pathophysiological concentrations of homocysteine elicit an acute negative inotropic effect on ventricular myocardium that is mediated by a coronary endothelium-derived agent other than NO or cyclooxygenase products. Future studies are required to elucidate the mechanism by which homocysteine acts to elicit the release of the proposed endothelial mediator, the identity of the proposed paracrine agent, and the mechanism of its negative inotropic action. Possible mechanisms of its inotropic effect include actions on voltage-dependent calcium influx, sarcoplasmic reticular calcium release and/or reuptake, calcium efflux (most likely via the Na/Ca exchanger), and/or myofilament calcium sensitivity.

ACKNOWLEDGMENTS

The authors thank Kerrey Roberto, Dawn McNeice, and Amanda Clausen for excellent technical assistance.

GRANTS

This work was supported in part by a Grant-in-Aid from the American Heart Association, Heartland Affiliate (to R. H. Kennedy), and a Beginning Grant-in-Aid from the American Heart Association, Heartland Affiliate (to J. Joseph).

REFERENCES

1. **Arnold G, Kosche F, Miessner E, Neitzert A, and Lochner W.** The importance of perfusion pressure in the coronary arteries for the contractility and the oxygen consumption of the heart. *Pflügers Arch* 299: 339–356, 1968.
2. **Blacher J, Demuth K, Guerin AP, Vadez C, Moatti N, Safar ME, and London GM.** Association between plasma homocysteine concentrations and cardiac hypertrophy in end-stage renal disease. *J Nephrol* 12: 248–255, 1999.
3. **Boushey CJ, Beresford SA, Omenn GS, and Motulsky AG.** A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intake. *JAMA* 274: 1049–1057, 1995.
4. **Brutsaert DL.** Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance and rhythmicity. *Physiol Rev* 83: 59–115, 2002.
5. **Burke JA, Levi R, Guo ZG, and Corey EJ.** Leukotrienes C_4, D_4 and E_4: effects on human and guinea-pig cardiac preparations in vitro. *J Pharmacol Exp Ther* 221: 235–241, 1982.
6. **Chwatki G and Bald E.** Determination of different species of homocysteine in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr A* 949: 141–151, 2002.
7. **Dijkman MA, Heslinga JW, Sipkema P, and Westerhof N.** Perfusion-induced changes in cardiac contractility depend on capillary perfusion. *Am J Physiol Heart Circ Physiol* 274: H405–H410, 1998.
8. **Downey JM.** Myocardial contractile function as a function of coronary blood flow. *Am J Physiol* 230: 1–6, 1976.
9. **Eberhardt RT, Forgione MA, Cap A, Leopold JA, Rudd MA, Trolliet M, Heydrick S, Stark R, Klings ES, Moldovan NI, Yaghoubi M, Goldschmidt-Clermont PJ, Farber HW, Cohen R, and Loscalzo J.** Endothelial dysfunction in a murine model of mild hyperhomocyst(e)inemia. *J Clin Invest* 106: 483–491, 2000.
10. **Gregg DE.** Effect of coronary perfusion pressure or coronary flow on oxygen usage of the myocardium. *Circ Res* 13: 497–500, 1963.
11. **Joseph J, Joseph L, Shekhawat NS, Devi S, Wang J, Melchert RB, Hauer-Jensen M, and Kennedy RH.** Hyperhomocysteinemia leads to pathologic ventricular hypertrophy in normotensive rats. *Am J Physiol Heart Circ Physiol* 285: H679–H686, 2003.
12. **Joseph J, Washington A, Joseph L, Koehler L, Fink LM, Hauer-Jensen M, and Kennedy RH.** Hyperhomocysteinemia leads to adverse cardiac remodeling in hypertensive rats. *Am J Physiol Heart Circ Physiol* 283: H2567–H2574, 2002.
13. **Karmazyn M, Leung CKH, and Dhalla NS.** Prostaglandin actions and interactions on isolated perfused rat hearts. *Can J Physiol Pharmacol* 57: 1275–1282, 1979.
14. **Li K, Rouleau JL, Andries LJ, and Brutsaert DL.** Effect of dysfunctional vascular endothelium on myocardial performance in isolated papillary muscles. *Circ Res* 72: 768–777, 1993.
15. **Loscalzo J.** The oxidant stress of hyperhomocysteinemia. *J Clin Invest* 98: 5–7, 1996.
16. **McCully KS.** Vascular pathology of hyperhomocysteinemia: implications for the development of arteriosclerosis. *Am J Pathol* 56: 111–128, 1969.
17. **Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, and Vollset SE.** Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 337: 230–236, 1997.
18. **Nygard O, Vollset SE, Refsum H, Brattstrom L, and Ueland PM.** Total homocysteine and cardiovascular disease. *J Intern Med* 246: 425–454, 1999.
19. **Pratt PF, Nithipatikom K, and Campbell WB.** Simultaneous determination of nitrate and nitrite in biological samples by multiflow injection analysis. *Anal Biochem* 231: 383–386, 1995.
20. **Ramaciotti C, McClellan G, Sharkey A, Rose D, Weisberg A, and Winegrad S.** Cardiac endothelial cells modulate contractility of rat heart in response to oxygen tension and coronary flow. *Circ Res* 72: 1044–1064, 1993.
21. **Schnyder G, Orf M, Pin R, Flapper Y, Lange H, Eberle FR, Meier B, Tru SG, and Hess OM.** Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med* 345: 1593–1600, 2001.
22. **Shontz RD, Xu Z, Patel KP, and Rozanski GJ.** Inhibition of K^+ currents by homocysteine in rat ventricular myocytes. *J Cardiovasc Electrophysiol* 12: 175–182, 2001.
23. **Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, and Loscalzo J.** Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 91: 308–318, 1993.
24. **Streck EL, Zugno AI, Tagliari B, Sarkis JJF, Wajner M, Wannmacher CMD, and Wyse ATS.** On the mechanism of the inhibition of Na^+,K^+-ATPase activity caused by homocysteine. *Int J Dev Neurosci* 20: 77–81, 2002.
25. **Stühlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, and Cooke JP.** Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 104: 2569–2575, 2001.
26. **Szekeres L, Borbola J Jr, and Papp JG.** Cardiac actions of arachidonic acid. *Acta Biol Med Ger* 35: 1119–1126, 1976.
27. **Tyagi SC, Smiley LM, and Mujumdar VS.** Homocyst(e)ine impairs endocardial endothelial function. *Can J Physiol Pharmacol* 77: 950–957, 1999.
28. **Ungvari Z and Koller A.** Homocysteine reduces smooth muscle $[Ca^{2+}]_i$ and constrictor responses of isolated arterioles. *J Cardiovasc Pharmacol* 37: 705–712, 2001.
29. **Upchurch GR, Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF, and Loscalzo J.** Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 272: 17012–17017, 1997.
30. **Vasan RS, Beiser A, D'Agostino RB, Levy D, Selhub J, Rosenberg IH, and Wilson PWF.** Plasma homocysteine and risk for congestive heart failure in adults without prior myocardial infarction. *JAMA* 289: 1251–1257, 2003.
31. **Wang S, Wright G, Harrah J, Touchon R, McCumbee W, Geng W, Fultz ME, Abdul-Jalil MN, and Wright GL.** Short-term exposure to homocysteine depresses rat aortic contractility by an endothelium-dependent mechanism. *Can J Physiol Pharmacol* 78: 500–506, 1999.
32. **Weiss N, Zhang YY, Heydrick S, Bierl C, and Loscalzo J.** Overexpression of cellular glutathione peroxidase rescues homocysteine-induced endothelial dysfunction. *Proc Natl Acad Sci USA* 98: 12503–12508, 2001.
33. **Zheng H, Dimayuga C, Hudaihed A, and Katz SD.** Effect of dexrazoxane on homocysteine-induced endothelial dysfunction in normal subjects. *Arterioscler Thromb Vasc Biol* 22: E15-E18, 2002.