Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide

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Abstract

The aim of the present study was to test the hypothesis that bradykinin-stimulated release of nitric oxide (NO) and prostacyclin from endothelium blocks myocyte hypertrophy in vitro. Angiotensin II increased [3H]phenylalanine incorporation by 21 ± 2% in myocytes cocultured with endothelial cells; this was abolished by bradykinin in the presence of endothelial cells. Bradykinin increased cytosolic concentrations of cGMP by 29 ± 4% in myocytes cocultured with endothelial cells. This was abolished by inhibition of NO synthase and by a cyclooxygenase inhibitor. Angiotensin II also increased [3H]phenylalanine incorporation by 28 ± 3% in myocytes cultured in the absence of endothelial cells. This effect of angiotensin II in monocyte culture was abolished by donors of NO but not by bradykinin. Neither the stable analog of prostacyclin (iloprost) nor the prostacyclin second messenger analog 8-bromo-cAMP (8-BrcAMP) blocked the effect of angiotensin II. Furthermore, 8-BrcAMP and iloprost individually increased [3H]phenylalanine incorporation. The anti-hypertrophic effects of bradykinin are critically dependent on endothelium-derived NO.

Keywords: angiotensin converting enzyme inhibitors; bradykinin; prostacyclin; endothelium

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (ACEI) have an important place in the management of patients after myocardial infarction and patients with cardiomyopathies, for these drugs effectively prevent left ventricular hypertrophy and reduce ventricular remodeling (15, 18, 22). ACEI diminish catabolism of bradykinin (BK), resulting in tissue accumulation of BK (1, 7, 11, 14), and they inhibit formation of ANG II. Both mechanisms may contribute to the resultant beneficial effects of ACEI treatment. B2-kinin receptors have been identified on ventricular cardiomyocytes (VCM; see Ref. 21), and an antagonist for these receptors, HOE-140, blocks the antihypertrophic and other beneficial effects of ACEI (10, 13, 15, 19). These findings suggest the potential for a prominent role for BK in the antihypertrophic effects of ACEI independent of inhibition of ANG II production.

We have previously demonstrated that BK blocks hypertrophy induced by ANG II in an in vitro model system, adult rat VCM. ANG II increases [3H]phenylalanine incorporation (an in vitro marker of hypertrophy) in myocytes in monocyte culture and in myocytes cocultured with endothelial cells (EC). BK abolished this ANG II-induced hypertrophy only in the presence but not in the absence of EC. This suggests that BK-stimulated release of paracrine factor(s) from endothelial cells is required for BK, and hence the ACEI-induced increased local concentration of BK, to block hypertrophy (24).

Activation of endothelial cell B2-kinin receptors by BK initiates production of prostacyclin (PGI2) and release of nitric oxide (NO) via activation of constitutive NO synthase (14, 19, 25). ACEI also have been demonstrated to increase the release of both paracrine factors (11, 14, 19, 29). It is likely that BK-induced release of one of these factors from the EC adjacent to the VCM is responsible for the block of ANG II-mediated hypertrophy. For example, in vitro studies have inferred that BK may not be able to release NO directly from myocytes but can elicit responses via release of NO from the adjacent cardiac EC (1, 3).

The potential for either NO and/or PGI2 to contribute to the beneficial effects of ACEI in the heart has been demonstrated in the attenuation of myocardial stunning and arrhythmias in ischemia-reperfusion (7, 13, 19, 20) and left ventricular relaxation in isolated hearts (1), but their contribution to the antihypertrophic effects of ACEI has not been investigated previously. Therefore, because BK stimulates elaboration of NO and PGI2 from EC, the objective of the present study was to test the hypothesis that NO and/or PGI2 block hypertrophy of VCM induced by ANG II in vitro.

METHODS

Cell culture. Myocytes from adult male Sprague-Dawley (200–250 g) rat hearts were enzymatically dissociated and plated onto laminin-coated (Collaborative Biomedical Products, Bedford, MA) six-well tissue culture plates (Falcon; Becton-Dickinson) in serum-free medium 199, with >93% myocyte content as previously described (24). Cells were plated at a density of 1 × 105 cells/35-mm well. VCM were incubated at 37°C until required, 2–24 h.

EC derived from rat heart were cultured in Dulbecco's modified Eagle's medium (GIBCO-BRL) containing 7% serum as previously described (24). Passage levels of 30–50 were utilized for this study. EC were plated onto 0.45 µm × 30-mm mixed cellulose ester culture plate inserts (Millipore, Bedford, MA) and incubated at 37°C until confluent.

[3H]phenylalanine incorporation. For studies of adult VCM in monocyte culture, myocytes were incubated with [3H]phenylalanine (1.5 µCi/ml; specific activity 132 Ci/mmol; Amersham, Arlington Heights, IL) with or without study drugs in serum-free medium 199 for 2 h at 37°C. The incubation was initiated 2–3 h after isolation and planting of the cells. As previously described, [3H]phenylalanine incorporation was determined in samples that had been trichloroacetic acid-precipitated before resuspension in sodium hydroxide. Results were normalized to nanograms DNA per well to correct for cell.
and stored at −80°C. The cells were lysed. Lysates were dried under vacuum and then resuspended in 0.05 mol/l sodium acetate, pH 6.2, and assayed for cGMP content by radioimmunoassay as previously described (12). Individual experiments were conducted with six replicates and expressed as a percentage of control for that experiment (24).

Studies of adult VCM cocultured with EC utilized the same methodology, except that, immediately before study, culture plate inserts coated with EC were washed with serum-free medium 199 and placed in six-well plates plated with adult VCM. Serum-free medium 199 containing [3H]phenylalanine with or without study drug(s) was added to the medium bathing VCM and the EC, as previously described (24). Determination of cGMP. Studies of cytosolic cGMP content in adult VCM cocultured with EC utilized culture plate inserts coated with EC. The inserts were rinsed with serum-free medium 199 and placed into six-well plates containing adult rat VCM just before study. Medium 199 containing study drug(s) was added to both the wells (1.5 ml) containing VCM and to the inserts (a further 1.5 ml), as for the [3H]phenylalanine incorporation studies, before incubation at 37°C. Inhibitors were added 30 min before BK, and the cGMP response was assayed at 15 min for the cyclooxygenase study and at 2 h for the NO synthase inhibition study. An aliquot of 3-isobutyl-1-methylxanthine (IBMX) was added to the cells for the final 30 min of the incubation period (final concentration 1 mmol/l) to prevent degradation of cGMP. Adult VCM were then thoroughly washed with ice-cold phosphate-buffered saline (pH 7.4) before precipitation with 1.0 ml 100% ethanol and 100% methanol were from Aaper Alcohol and Chemical (Shelbyville, KY). Iloprost and meclofenamate (MF) ethanol and 100% methanol were from Aaper Alcohol and Chemical (Shelbyville, KY). Iloprost and meclofenamate (MF) were kindly provided by Berlex Laboratories (Wayne, NJ) and theNO scavenger Hb (3.3 mg/ml) also restored the ANG II-mediated increase in [3H]phenylalanine incorporation in the presence of BK to 22 ± 3% (n = 3). BK alone has no effect on [3H]phenylalanine incorporation in these coculture studies. Also, L-NMMA alone and Hb alone had no effect (data not shown).

ANG II (1 µmol/l) also increased [3H]phenylalanine incorporation by 28 ± 3% in VCM studied in monolulture (Fig. 3). As we have previously described, in the absence of EC not only does BK fail to block the ANG II-elicited increase in [3H]phenylalanine incorporation, but BK itself increases protein synthesis by 24 ± 3%. However, when each of two distinct donors of NO, SNP (30 µmol/l, n = 7) and SIN-1 (30 µmol/l, n = 2), were added to the monolulture of myocytes the hypertrophic response to ANG II was abolished. To determine if there was a nonspecific effect of SNP or of SIN-1 on protein synthesis, SNP (n = 8) and SIN-1 (n = 3) individually were added to monocultures; each had no effect on [3H]phenylalanine incorporation (Fig. 3).

BK-mediated inhibition of hypertrophy: Role of PGI2. We next determined the effect of cyclooxygenase inhibition on ANG II-mediated increases in [3H]phenylalanine incorporation to test the hypothesis that BK-mediated inhibition of hypertrophy: Role of NO. To test the hypothesis that BK mediates an antihypertrophic effect on cardiac myocytes via release of NO from EC, we determined the influence of NO inhibition on ANG II-mediated increases in [3H]phenylalanine incorporation in myocytes. As shown in Fig. 1, ANG II (1 µmol/l) increased [3H]phenylalanine incorporation by 21 ± 2% (n = 16) in VCM cocultured with EC. Addition of BK abolished the increase in [3H]phenylalanine incorporation (n = 16). The hypertrophic response to ANG II was restored by the addition of the NO synthase inhibitor L-NMMA (100 µmol/l, n = 8, Fig. 1). This concentration of L-NMMA blocked the BK-stimulated rise in myocyte cytosolic cGMP, a marker of NO-stimulated guanylyl cyclase (Fig. 2). In addition, the NO scavenger Hb (3.3 mg/ml) also restored the ANG II-mediated increase in [3H]phenylalanine incorporation in the presence of BK to 22 ± 3% (n = 3). BK alone has no effect on [3H]phenylalanine incorporation in these coculture studies. Also, L-NMMA alone and Hb alone had no effect (data not shown).

Fig. 1. Inhibition of nitric oxide (NO) synthase blocks prevention of hypertrophy in coculture. ANG II (1 µmol/l) increases [3H]phenylalanine incorporation in adult rat ventricular cardiomyocytes (VCM) cocultured with endothelial cells (EC; n = 16). This is blocked by bradykinin (BK; 10 µmol/l; n = 16) and restored by the addition of NO synthase inhibitor N0-monomethyl-L-arginine (L-NMMA; 100 µmol/l; n = 8). *Significant change compared with control at the P < 0.05 level.

RESULTS

BK-mediated inhibition of hypertrophy: Role of NO. To test the hypothesis that BK mediates an antihypertrophic effect on cardiac myocytes via release of NO from EC, we determined the influence of NO inhibition on ANG II-mediated increases in [3H]phenylalanine incorporation in myocytes. As shown in Fig. 1, ANG II (1 µmol/l) increased [3H]phenylalanine incorporation by 21 ± 2% (n = 16) in VCM cocultured with EC. Addition of BK abolished the increase in [3H]phenylalanine incorporation (n = 16). The hypertrophic response to ANG II was restored by the addition of the NO synthase inhibitor L-NMMA (100 µmol/l, n = 8, Fig. 1). This concentration of L-NMMA blocked the BK-stimulated rise in myocyte cytosolic cGMP, a marker of NO-stimulated guanylyl cyclase (Fig. 2). In addition, the NO scavenger Hb (3.3 mg/ml) also restored the ANG II-mediated increase in [3H]phenylalanine incorporation in the presence of BK to 22 ± 3% (n = 3). BK alone has no effect on [3H]phenylalanine incorporation in these coculture studies. Also, L-NMMA alone and Hb alone had no effect (data not shown).
mediates at least part of its antihypertrophic effects via an endothelium-derived cyclooxygenase product (possibly PGI₂). Figure 4 demonstrates that the usual hypertrophic response to ANG II (Figs. 1 and 3) is abolished by BK (Fig. 4, second bar). However, in the presence of BK, the hypertrophic response to ANG II is restored by either meclofenamate (Fig. 4, third bar) or by Indo (fourth bar). The quantitative response (~125% of control) is very similar to that of ANG II alone (Figs. 1 and 3, ~123%). Neither meclofenamate alone nor Indo alone produced a hypertrophic response (data not shown). Thus it appears that inhibition of formation of a cyclooxygenase product at least partially blocks BK-mediated inhibition of hypertrophy. The cyclooxygenase inhibitor meclofenamate (10 µmol/l) also decreased myocyte cGMP production in coculture (Fig. 2).

However, in VCM studied in monoculture, neither the stable analog of 8-BrcAMP (1 mmol/l, n = 8, Fig. 5A), the second messenger for PGI₂, nor the PGI₂ analog iloprost (1 µmol/l, n = 8, Fig. 5B) inhibited ANG II-stimulated [³H]phenylalanine incorporation. In fact, both 8-BrcAMP (n = 11) and iloprost (n = 8) individually increased [³H]phenylalanine incorporation by 25 ± 2 and 22 ± 8%, respectively.

DISCUSSION

There are several new, significant findings of this study. The present investigation demonstrates that the antihypertrophic effect of BK in VCM observed in the presence of endothelial cells was abolished by an inhibitor of NO synthesis, by an NO scavenger (l-NMMA and Hb, Fig. 1), or by cyclooxygenase inhibition (MF or Indo, Fig. 4). In studies of VCM in monoculture, donors of NO (SNP or SIN-1, Fig. 2), but not surrogates of PGI₂ (iloprost and 8-BrcAMP, Fig. 5), abolished increases in [³H]phenylalanine incorporation. Also, our data suggest that cGMP plays a role in the antihypertrophic effects of BK.

To date, there has been little evidence for a direct antihypertrophic effect of NO in cardiac muscle. We have shown previously that the phenylephrine-mediated increase in protein content in neonatal VCM is attenuated by another source of NO, glycerol trinitrate (12). The significant contribution of NO to the antihypertrophic effects of BK demonstrated in the present study is further supported by indirect evidence from other investigators. In other cell types, NO blockers increase in protein and DNA synthesis, phosphatidylcholine and phosphatidylinositol hydrolysis, phospholipase D activation, and vascular smooth muscle cell migration induced by ANG II and other hypertrophic stimuli (2, 4, 8, 9, 17). Furthermore, NO synthase inhibitors induce modest hypertrophy of the left ventricle in vivo with long-term administration and prevent the reduction in infarct size induced by ramiprilat in ischemia-reperfusion.
Our data suggest that NO plays a role in the antihypertrophic effect of BK. Unlike the definitive antihypertrophic effect of NO, the potential for a role of PGI2 in the antihypertrophic effects of BK in the present study is less clear cut. On the basis of the observation that in VCM-EC cocultures cyclooxygenase inhibition abolishes the antihypertrophic effect of BK (Fig. 4), PGI2 (or another product of cyclooxygenase) appears to have an antihypertrophic effect when endothelial cells are present. However, the observation that iloprost and 8-BrcAMP, when added to VCM monocultures under the conditions of these experiments, actually increase [3H]phenylalanine incorporation makes it clear that the effects of cyclooxygenase inhibition are not straightforward; there may be an important time and concentration dependency of effect, or another cyclooxygenase product may be playing a role. Our data raise the possibility that, in coculture of EC with myocytes, a cyclooxygenase product may be necessary for BK-induced activation of NO synthase in the EC.

We observed a direct hypertrophic effect of cAMP. There is a precedent for this. Interventions that elevate intracellular cAMP concentrations increase protein synthesis in Langendorff-perfused rat hearts (28) and increase DNA synthesis and activate the mitogen-activated protein kinase and p70 S6 kinase cascades in Swiss 3T3 cells (27), similar to known hypertrophic agents. Our data also support a direct hypertrophic effect of PGI2, at least under some conditions; the concentration of PGI2 or another cyclooxygenase product appears to be sufficient to attenuate the inhibitory effect of BK on ANG II-induced hypertrophy. When cyclooxygenase is inhibited, there is a further hypertrophic response to ANG II.

The importance of the endothelium as a secretory organ in the cardiovascular system is well established (5, 6). In addition to vasomotor tone, myocardial growth and hypertrophy also are influenced by the paracrine function of the endothelium. Thus, in patients with normally functioning endothelium, angiotensin-converting enzyme inhibition may be beneficial by dual mechanisms: blockade of ANG II production and enhanced BK concentration with BK-stimulated release of NO from EC adjacent to VCM. ACEI clearly are of clinical benefit in diseases such as hypertension and heart failure. Nonetheless, the beneficial effects of angiotensin-converting enzyme inhibition may be diminished in patients with cardiovascular disorders in which endothelial function is compromised (e.g., hypertension, hyperlipidemia, coronary artery disease, and diabetes) and in patients receiving concomitant therapy with a cyclooxygenase inhibitor. In a canine model of heart failure, endothelium-dependent dilation of coronary arteries is depressed (26), and recent data demonstrate that human coronary microvessels from failing heart fail to generate less NO than those from normal heart (16).

In conclusion, the present investigation demonstrates that the antihypertrophic effects of BK in vitro are critically dependent on the release of NO from EC. PGI2 or another cyclooxygenase product may possibly play an antihypertrophic effect as well. Our current findings strongly support an important role for BK-mediated release of NO in ACEI-induced inhibition of ventricular hypertrophy.

Fig. 5. cAMP and iloprost do not block ANG II-mediated hypertrophy. ANG II (1 µmol/l)-induced [3H]phenylalanine incorporation for myocytes in monoculture is shown for control cells and for cells treated with 8-bromo-cAMP (8-BrcAMP; A) or for cells treated with iloprost (B). Neither 8-BrcAMP nor iloprost abolished the hypertrophic effect of ANG II and individually increased [3H]phenylalanine incorporation. * Significant change compared with control at the P < 0.05 level.

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