Cytochrome P-450 metabolite of arachidonic acid mediates bradykinin-induced negative inotropic effect

R. RASTALDO, N. PAOLOCCI, A. CHIRIBIRI, C. PENNA, D. GATTULLO, and P. PAGLIARO. Cytochrome P-450 metabolite of arachidonic acid mediates bradykinin-induced negative inotropic effect. Am J Physiol Heart Circ Physiol 280: H2823–H2832, 2001.—This study focused on the mechanisms of the negative inotropic response to bradykinin (BK) in isolated rat hearts perfused at constant flow. BK (100 nM) significantly reduced developed left ventricular pressure (LVP) and the maximal derivative of systolic LVP by 20–22%. The cytochrome P-450 (CYP) inhibitors 1-aminobenzotriazole (1 mM and 100 μM) or proadifen (5 μM) abolished the cardiodepression by BK, which was not affected by nitric oxide and cyclooxygenase inhibitors (35 μM Nω-nitro-L-arginine methyl ester and 10 μM indomethacin, respectively). The CYP metabolite 14,15-epoxyeicosatrienoic acid (14,15-EET; 50 ng/ml) produced effects similar to those of BK in terms of the reduction in contractility. After the coronary endothelium was made dysfunctional by Triton X-100 (0.5 μl), the BK-induced negative inotropic effect was completely abolished, whereas the 14,15-EET-induced cardiodepression was not affected. In hearts with normal endothelium, after recovery from 14,15-EET effects, BK reduced developed LVP to a 35% greater extent than BK in the control. In conclusion, CYP inhibition or endothelial dysfunction prevents BK from causing cardiodepression, suggesting that, in the rat heart, endothelial CYP products mediate the negative inotropic effect of BK. One of these mediators appears to be 14,15-EET.

endothelium; epoxyeicosatrienoic acids; myocardial contractility; left ventricular pressure; coronary perfusion pressure

BRADYKININ (BK) is a well-known endothelium-dependent vasodilator that affects coronary resistance and myocardial contractility (3, 12, 17, 33, 34, 38, 39, 47). Its production can increase during myocardial ischemia (39) and angiotensin-converting enzyme inhibition, which protects BK from inactivation (38). While vasodilatation depends on the release of endothelial factors such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) (5, 6, 8, 13–18, 20–22, 35, 36, 38, 49, 50, 54–57), the mechanisms leading to changes in myocardial contractility have not yet been fully investigated.

BK may have a positive inotropic effect, which is attributed to an increase in sympathetic discharge (33, 34) or to Gregg’s phenomenon (34, 39). However, in many preparations in which these two mechanisms are limited, BK has a negative inotropic effect that seems mediated by endothelial factor(s) (3, 12, 17, 44). For example, Ou et al. (44) reported that endothelial factor(s) can induce a reduction in the calcium transient of electrically stimulated cardiomyocytes and that BK further reduces the amplitude of this transient by acting on the endothelium. However, the nature of this cardiodepressive factor is still unknown. It is possible that one of the endothelial vasodilator pathways activated by BK is also responsible for the endothelium-mediated negative inotropic effect.

One of the endothelial factors involved in the cardiodepression could be NO. However, NO has a bi-modal action on contractility that depends on its concentration and the experimental model (3, 4, 17, 27, 28). For example, NO inhibition completely abolishes BK-induced contractile depression in isolated guinea pig cardiomyocytes (3) but not in the ferret heart (17). Moreover, in many species (including humans), the vasodilatation caused by BK is largely dependent on EDHF rather than NO (35, 36, 45); in particular, in the rat heart, NO and prostaglandins have been seen to play a trivial, if any, role in vasodilator responses to BK (20, 21). These findings suggest that the isolated rat heart can be an adequate model to study whether compounds different from NO are involved in the endothelium-mediated cardiodepression induced by BK.

A cytochrome P-450 (CYP) monooxygenase metabolite of arachidonic acid, namely, one of the epoxyeicosatrienoic acids (EETs), has been reported to be responsible for BK-induced vasodilatation in the rat coronary circulation (20, 21) and has been proposed as the most likely candidate for the role of EDHF in the coronary bed of many species (1, 5, 6, 8, 15, 16, 20, 21, 35, 37, 49, 50, 55, 57). In porcine coronary arteries, CYP 2C fulfills the property of EDHF synthase (16). Indeed, BK can activate both CYP and a membrane phospholipase, which

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causes the release of arachidonic acid from phospholipids, thus providing the substrate to CYP for the formation of various EETs (5, 6, 14, 30, 55, 57). These are incorporated into the membrane phospholipids of endothelial and smooth muscle cells, from which they are released by the hydrolizing activity of BK (14, 30, 55, 57). Thus BK could be responsible for both the production and release of various CYP products from the endothelium. Yet while only one EET is likely to be EDHF (5, 6, 15, 16, 21, 49, 50), data on the role of the other CYP products remain scant, many of them concerning solely the vasomotor effects.

Because the relative contribution of EDHF/EET to vasodilatation is greater in microvessels, which lie in juxtaposition to cardiomyocytes (3), than in conduit arteries (8, 35, 36, 49, 50) and because EETs are diffusible compounds that act on a number of membrane channels [sodium (30), Ca\(^{2+}\)-dependent potassium (5, 6, 15, 35, 36), and L-type calcium channels (7)], we hypothesized that one or more EET, released by the endothelium, can influence heart contractility.

Therefore, our study investigated whether the CYP pathway is involved in the negative inotropic response induced by BK and whether EETs mediate this response. In isolated rat hearts perfused at constant flow, we assessed the effects of BK infusion on left ventricular pressure (LVP) and coronary perfusion pressure (CPP) before and after the administration of 1-amino-benzotriazole (ABT) or proadifen, two structurally different inhibitors of CYP activity, with and without inhibition of NO synthase (NOS) and cyclooxygenase (COX). We also investigated whether perfusion with solutions containing EETs induces cardiodepression and/or vasodilatation. Finally, we examined whether the responses of the myocardium and vasculature to BK were affected by the preliminary administration of EETs.

METHODS

Isolated hearts of male Wistar rats (n = 77, 450–550 g body wt) were retrogradely perfused with oxygenated buffer solution at 37°C. Flow was kept constant using a pump titrated to a CPP of 85–90 mmHg. Constant flow was used to limit confounding alterations in contractility due to Gregg’s phenomenon (11, 12). A little hole in the left ventricular wall allowed the drainage of the thebesian flow. A polyvinyl chloride balloon was placed in the left ventricle and connected to an electromanometer to record LVP. The balloon was filled with saline at an end-diastolic LVP of 5–15 mmHg to achieve a maximum isovolumic developed LVP. The hearts were allowed to stabilize for 20–30 min before baseline values were recorded. The following six groups were used:

**Group 1: BK before and during ABT.** In six hearts, a 3-min BK infusion (100 nM) was performed, and a washout period of 20–30 min was monitored. To block CYP activity, ABT, which is reported to be a long-lasting inhibitor of CYP (5, 31, 43), was then infused at the concentration of 1 mM for 10 min and then at the concentration of 100 μM for 5 min. After these 5 min had elapsed, BK (100 nM) was infused for 3 min with ABT (100 μM). The infusion of ABT (100 μM) alone was then continued for 20–30 min to verify the persistence of steady-state conditions. The concentration of ABT was reduced from 1 to 100 μM to limit possible disturbing side effects unrelated with CYP inhibition. Moreover, to determine whether CYP was still blocked after ABT was washed out, we performed six additional experiments in which BK was infused starting after a washout period of 10 min from the end of the administration of ABT. To give these hearts the same amount of ABT received by the other ABT-treated hearts, the substance was infused at a concentration of 1.05 mM for 10 min.

**Group 2: BK before and during proadifen.** To further support the hypothesis that the inhibition of BK effect by ABT was not due to a nonspecific effect of the drug, proadifen, a structurally different inhibitor of CYP (6), was used. In five hearts, a 3-min BK infusion (100 nM) was performed, and a washout period of 20–30 min was monitored. Proadifen (5 μM) was then infused for 10 min. After this period, BK (100 nM) was infused with proadifen for 3 min. The infusion of proadifen (5 μM) alone was then continued for 20–30 min to verify the persistence of steady-state conditions.

**Group 3: BK before and after N\(^{g}\)-nitro-L-arginine methyl ester + indomethacin and after ABT.** In seven hearts, BK infusion (100 nM) was performed before and after 10 min of confusion of N\(^{g}\)-nitro-L-arginine methyl ester (l-NNAME; 35 μM) (29), a NOS inhibitor, plus indomethacin (Indo; 10 μM) (3, 17, 36), a COX inhibitor. When the effects of the second BK infusion were over, ABT was added at the same dose as in group 1, and BK was infused again. In four additional hearts, the same protocol was followed except that Tyrode solution was replaced with Krebs-Henseleit solution.

**Group 4: BK before and after Triton X-100.** In eight hearts, BK infusion (100 nM) was performed before and after the endothelium was made dysfunctional by a 0.1-mL injection of Triton X-100 (5 μL/ml) (12, 17). In four of these hearts, the specificity of the endothelial dysfunction was checked by the infusion of sodium nitropusside (SNP; 100 μM) before and after contrast.
after Triton X-100. Finally, in two of these hearts, at the end of the experiment the cardiodepressive capability of verapamil (2.5 μM) was tested.

Group 5: BK before and after EETs. BK (100 nM) infusion was performed before and after various EETs [8,9-EET (n = 9), 11,12-EET (n = 6), or 14,15-EET (n = 9)] were infused separately at a concentration ranging from 10 to 50 ng/ml (corresponding to 0.03–0.16 μM) for 20 min. To verify whether the effects of EETs were endothelium dependent, in seven additional hearts, 11,12-EET (50 ng/ml, n = 3) and 14,15-EET (50 ng/ml, n = 4) infusions were performed before and after the endothelium was made dysfunctional, as in the hearts of group 4.

Group 6: role of BK tachyphylaxis. To exclude a role of tachyphylaxis, in five hearts, three sequential 3-min BK (100 nM) infusions were performed, separated from each other by 30 min of washout. In five additional hearts, the 3-min BK (100 nM) infusion was performed only after proadifen (5 μM) had been infused for 20 min.

Materials

The drugs used in these experiments were made to required concentration in the buffer solutions. The compounds were obtained from Sigma (St. Louis, MO), ICN (EETs; Costa Mesa, CA), Chiesi (Indo; Parma, Italy), and Roche (heparin; Milan, Italy).

Data analysis

Data are presented as means ± SD. For comparison of paired samples in each group, Student’s t-test for paired data was used. Between intervention effects were first tested by ANOVA, and individual comparisons were then made with Student’s nonpaired t-test, using a Bonferroni correction for multiple comparisons.

RESULTS

After stabilization, in the 73 hearts perfused with Tyrode solution, developed LVP was 71 ± 8 mmHg, dP/dt max was 1,075 ± 167 mmHg/s, and CPP was 87 ± 9 mmHg. These values were not statistically different from those of the control conditions of each group. In 61 hearts in which the 3-min BK infusion was performed in control conditions, a significant (P < 0.001) reduction in developed LVP (−21 ± 7%), dP/dt max (−22 ± 10%), and CPP (−23 ± 11%) occurred, which recovered within 20–30 min. The BK effects in control conditions and after the administration of the various agents are reported in Table 1.

Group 1: BK Before and During ABT

Figure 1A displays an example of developed LVP and CPP responses to BK before and during ABT. The results obtained in these hearts are summarized in Table 1 and Fig. 1B.

In these hearts, BK infusion caused a significant reduction of developed LVP (from 72 ± 13 to 55 ± 20 mmHg, P < 0.01), dP/dt max (from 998 ± 174 to 769 ± 282 mmHg/s, P < 0.05), and CPP (from 89 ± 7 to 75 ± 9 mmHg, P < 0.05). During ABT infusion and before the second BK infusion, ventricular and perfusion pressure values, after an initial transient (2–4 min) increase, were not significantly different from the con-
trol. The second infusion of BK, given in coinfusion with ABT, did not affect developed LVP, dP/dt max, and CPP (Table 1). In six additional hearts in which the administration of ABT was followed by the washout period, the first BK infusion reduced developed LVP, dP/dt max, and CPP by 20–25%. Ten minutes after the end of ABT infusion, the second administration of BK did not alter developed LVP, dP/dt max, and CPP (Fig. 2).

**Group 2: BK Before and During Proadifen**

Figure 3A displays an example of developed LVP and CPP responses to BK during proadifen infusion. The results of this group are summarized in Table 1 and Fig. 3B.

In this group, BK infusion caused a significant reduction of developed LVP (from 70 ± 11 to 55 ± 15 mmHg, P < 0.01), dP/dt max (from 988 ± 154 to 759 ± 272 mmHg/s, P < 0.05), and CPP (from 90 ± 7 to 75 ± 10 mmHg, P < 0.05). During proadifen and before the second BK infusion, developed LVP and dP/dt max were not significantly different from the control. Also in this group, the second infusion of BK, given in coinfusion with proadifen, did not affect developed LVP, dP/dt max, and CPP (Table 1).

**Group 3: BK Before and After L-NAME + Indo and After ABT**

Figure 4A displays an example of developed LVP and CPP responses to BK in this group. The results are summarized in Table 1 and Fig. 4B.

In the seven hearts in which the Tyrode solution perfusate was used, the first BK infusion caused the usual significant reduction of developed LVP (from 69 ± 6 to 56 ± 8 mmHg, P < 0.01) and dP/dt max (from 1,249 ± 197 to 1,032 ± 209 mmHg/s, P < 0.01). After NOS + COX inhibition, the second BK infusion also significantly reduced developed LVP and dP/dt max (Table 1). It is noteworthy that NOS + COX inhibition “per se” did not alter basal contractility (dP/dt max 1,249 ± 197 vs. 1,276 ± 198 mmHg/s).

CPP, which was significantly (P < 0.005) reduced (from 97 ± 7 to 77 ± 4 mmHg) by the first BK infusion, was significantly increased (+39%, P < 0.02) by NOS + COX inhibition and was not affected by the second BK infusion (Table 1).

After the effects of the second BK infusion were over, ABT did not significantly affect developed LVP, dP/dt max, and CPP. In the presence of ABT, the third BK infusion was ineffective on both contractility and CPP (Table 1).

The failure of BK to induce evident vasodilatation suggested a possible alteration of the endothelial function by interaction of NOS + COX inhibition with HEPES (25). To test this hypothesis, experiments were performed using the Krebs-Henseleit solution. In the four Krebs-Henseleit solution-perfused hearts, after stabilization, developed LVP was 67 ± 2 mmHg, dP/dt max was 1,058 ± 209 mmHg/s, and CPP was 94 ± 6 mmHg. The 3-min BK infusion, performed in control conditions, induced the usual significant (P < 0.05) reduction in developed LVP (−22 ± 5%), dP/dt max
After NOS inhibition, in these hearts, BK also reduced the contractility (developed LVP from 65 ± 6 to 54 ± 4 mmHg, \(P < 0.05\), and \(dP/dt_{\text{max}}\) from 950 ± 99 to 792 ± 86 mmHg/s, \(P < 0.05\)) but did not alter CPP significantly (from 156 ± 28 to 151 ± 24 mmHg, \(P = 0.15\)). Thus these results supported the idea that the absence of vasodilator response could not be ascribed to altered endothelial function by HEPES.

**Group 4: BK Before and After Endothelium Was Made Dysfunctional by Triton X-100**

Whereas in baseline conditions BK infusion had the usual reducing effect on developed LVP, \(dP/dt_{\text{max}}\), and CPP (e.g., developed LVP decreased from 69 ± 7 to 56 ± 9 mmHg, \(-17\%, P < 0.005\)), it did not produce any effect on myocardial contractility and CPP after the endothelium was made dysfunctional by Triton X-100 (Table 1). The results of this group are similar to those reported by other authors (12, 17). It is noteworthy that Triton X-100 did not affect basal contractility, whereas it increased CPP similar to L-NAME + Indo. Despite the similarity of the effects induced by the two treatments, the subsequent infusion of BK was unable to reduce both contractility and coronary resistance only in the presence of endothelial dysfunction by Triton X-100.

In four of these hearts, the endothelium-independent vasodilator effect of SNP was not affected by Triton X-100 pretreatment. SNP reduced CPP by 25% but did not significantly affect heart contractility. Furthermore, at the end of the experiments, verapamil (an endothelium-independent cardiodepressor) reduced
developed LVP by 40%, thus showing that Triton X-100 was effective in selectively disrupting the coronary vascular endothelium without affecting the myocardium.

**Group 5: BK Before and After EETs**

A 20-min infusion of 8,9-EET \( n = 3 \), 11,12-EET \( n = 3 \) (10–20 ng/ml, for all EETs) did not cause any change in CPP and LVP. The ineffectiveness of EET infusion is likely to depend on their incorporation in vascular membrane phospholipids \( 14, 30, 55, 57 \). It has been reported that stored EETs can be released after phospholipid hydrolysis induced by BK \( 14, 30, 55, 57 \). Therefore, to bypass vascular incorporation, higher doses of various EETs were used; 8,9-EET \( n = 6 \), 11,12-EET \( n = 3 \), and 14,15-EET \( n = 6 \) were infused at the concentration of 50 ng/ml (corresponding to 0.16 \( \mu \)M) for 20 min, and the effects of BK infusion before and after various EETs were compared.

Of the various EET regioisomers tested, only 14,15-EET (50 ng/ml, \( n = 6 \)) caused a reduction in contractility.

Figure 5A displays an example of developed LVP and CPP responses to BK in the hearts in which 14,15-EET was infused. The results are summarized in Table 1 and Fig. 5B.

The first BK infusion reduced developed LVP (from 75 ± 14 to 59.5 ± 11 mmHg, \( P < 0.001 \)) and \( \frac{dP}{dt_{\text{max}}} \) (from 1,026 ± 228 to 795 ± 223 mmHg/s, \( P < 0.001 \)). After washout of BK effects, 14,15-EET infusion reduced developed LVP (from 74 ± 12 to 62 ± 12 mmHg, \( P < 0.005 \)) and \( \frac{dP}{dt_{\text{max}}} \) (from 981 ± 208 to 818 ± 213, \( P < 0.005 \)). These reductions were significantly lower \((-21\%, P = 0.033, \text{and} -26\%, P = 0.036, \text{respectively})\) than those obtained with BK infusion. After 5 min of washout to allow the recovery of contractile function, a second BK infusion showed potentiated effects in reducing myocardial contractility (Fig. 5B). In particular, the second BK infusion reduced developed LVP and \( \frac{dP}{dt_{\text{max}}} \) (Table 1) to a greater extent than the first one \((+35\%, P = 0.015, \text{and} +15\%, P = 0.036, \text{respectively})\).

The first BK infusion significantly reduced \((P < 0.05)\) CPP. No effect on CPP was observed during 14,15-EET, whereas the second BK infusion again caused a significant \((P < 0.05)\) reduction. No significant difference was seen between the effects of the two BK infusions on CPP.

In seven additional hearts, 11,12-EET (50 ng/ml, \( n = 3 \)) and 14,15-EET (50 ng/ml, \( n = 4 \)) were infused before and after the endothelium was made dysfunctional. It was observed that 11,12-EET was ineffective in altering heart contractility both before and after Triton X-100. On the contrary, the infusion of 14,15-EET induced a negative inotropic effect, which was similar before and after the endothelium was made dysfunctional. In particular, developed LVP and \( \frac{dP}{dt_{\text{max}}} \) were significantly reduced by 15% before and after Triton X-100.

**Group 6: Role of BK Tachyphylaxis**

BK induced a similar significant \((P < 0.05)\) reduction by \(-20\%\) in developed LVP during each of the three sequential infusions. In the subset of hearts in which BK was infused only in the presence of proadifen,
developed LVP was not affected, being 68 ± 2 mmHg before and 66 ± 3 mmHg during BK. From these results and those of group 5, in which the second dose of BK was more effective than the first one, any role of tachyphylaxis in the abrogation of BK-induced cardiodepression can be excluded.

DISCUSSION

The major novel finding of this study is that the cardiodepressive effect caused by intracoronary administration of BK is the result of the endothelial release of CYP-dependent metabolites, one of which is likely to be 14,15-EET. The present results also indicate that the extent of BK-induced negative inotropic effect is enhanced by prior administration of 14,15-EET (Table 1 and Fig. 5). Because 14,15-EET did not induce evident vasodilatation, this particular EET is not likely to be the putative vasodilating EDHF. This last hypothesis is in agreement with many reports (e.g., Refs. 6, 15, 16, 20, and 21) that identified 5,6- or 11,12-EET as the most likely candidate for EDHF.

BK exerts an evident negative inotropic action only in isolated cardiomyocytes (44) or in hearts perfused at constant flow (3, 12, 17), i.e., when perfusion-dependent changes in contractility (Gregg's phenomenon) and sympathetic stimulation are limited. However, in isolated hearts, perfusion-induced changes in contractility do not seem completely prevented when a reduction in CPP occurs despite a constant flow (11). Because in our experiments both 14,15-EET alone and BK after NOS + COX inhibition induced a reduction in contractility despite unchanged CPP and flow, perfusion-related cardiodepression can be excluded as an effect of BK and/or 14,15-EET.

BK is unable to reduce contractility only after CYP inhibition or in the presence of endothelial dysfunction obtained with Triton X-100. These findings show for the first time that the endothelial CYP pathway is involved in BK-induced cardiodepression. Although CYP is an ubiquitous enzyme, EET involved in the BK-induced response must be of endothelial origin. This hypothesis is reinforced by the fact that the CYP enzyme system is more concentrated in vascular endothelial cells than in cardiomyocytes (47). Moreover, the endothelium of microvessels (<100 μm), which lies close to cardiomyocytes (3), releases more EETs than NO (8, 35, 36, 49, 50). Furthermore, in the presence of endothelial dysfunction, 14,15-EET still causes a reduction in contractility. This result reinforces the hypothesis that this particular EET is released by the endothelium in BK-induced cardiodepression. Finally, the observation that L-NAME + Indo did not prevent BK cardiodepression is also consistent with the study of Fort and Lewis (17), who reported that higher doses of BK (10^-6 M) have an NO-independent cardiodepressive effect and excluded any role of endothelial autacoids different from CYP-dependent metabolites in the reduction of contractility by BK. This conclusion is based on the reported effectiveness of 35 μM L-NAME in inhibiting NOS and reducing CF in isolated rat hearts (29). Although higher doses may be required in some different preparations (9, 36), in our study, the fact that the addition of the CYP inhibitor ABT after NOS + COX inhibition abolished the cardiodepressive response to BK further supports the hypothesis that the response was not due to a residual NO release after L-NAME (9). Furthermore, it has been reported that the BK-induced cardiodepression is unaffected by COX
inhibition with Indo (3, 17). However, the simultaneous 
inhibition of NOS and COX is required to ascertain the role 
of CYP products on the regulation of vasomotor tone 
(1, 5, 16, 38).

The endothelium-dependent coronary vasodilator ef-
fect of BK is reported to be largely dependent on EDHF 
rather than NO (20, 21, 35, 36, 45). In particular, in the 
rat heart, NO has been seen to play a trivial, if any, 
role in the vasodilatation by BK (20, 21). These observa-
tions are consistent with our finding that BK has no 
vasodilator effect after CYP inhibition. On the basis of 
these observations one might argue that, in the rat, 
CYP products (including EDHF) could be the sole en-
dothelial factors involved in BK-mediated vasodilata-
tion and cardiodepression. However, in apparent con-
tраст to the above findings, vasodilatation by BK but 
not cardiodepression is also abolished by only NOS + 
COX inhibition regardless of the buffer used. A similar 
result has been obtained in a previous study (45) on 
anesthetized dogs to which low (intracoronary) doses of 
BK were given. This apparent discrepancy may reflect 
a dose- or a species-dependent effect. We can speculate 
that, depending on the experimental model, a concom-
itant release of NO, prostacyclin, and EET/EDHF may 
be required to induce an evident vasodilatation by BK. 
In fact, BK is responsible for the release of NO (3, 22) 
and of arachidonic acid from phospholipids, thus pro-
viding the substrate to activated CYP (5, 6, 21, 30, 47) 
for the formation of various EETs and to COX for the 
formation of prostacyclin (47). Inhibition of either or 
both pathways in the experimental conditions listed 
above fully prevents BK-induced vasodilatation. On 
the contrary, only the release of CYP products (includ-
ing 14,15-EET) seems to be responsible for BK-induced 
cardiodepression in the rat. The CYP products, differ-
ent from EDHF, can also counteract the potential pos-
tive inotropic effect of a low concentration of NO (28) 
without contributing to the vasodilatation.

Regarding the precise mechanisms by which BK/
14,15-EET induces myocardial depression, several al-
ternative hypothesis should be entertained. According 
to Chen et al. (7), a role for EETs could derive from 
their ability to reduce the open probability of myocar-
dial L-type Ca2+
channels. It has also been reported 
that EDHF/EET acts on the smooth muscles by opening 
Ca2+-dependent potassium channels (5, 6, 15, 35, 
36). By opening the myocardial Ca2+-dependent potas-
sium channels (23), EETs could cause an early rep-
olarization, with shortening of the plateau of the action 
potential and reduction of cellular calcium influx and 
concentration. This hypothesis is supported by recent 
data of Paolocci et al. (46), who have shown a role for 
Ca2+-dependent potassium channels in BK-induced 
cardiodepression. Although this may be true, the role 
of EETs on myocardial calcium influx is still contro-
versial. In particular, 5,6- and 11,12-EET increase cell 
shortening and intracellular calcium concentrations, 
whereas low doses of 8,9- or 14,15-EET (1–16 ng/ml) 
are ineffective on these parameters (37). Recently, it 
has been demonstrated an effect of EETs, in particular 
8,9-EET, reduces the open probability of sodium chan-
nels in isolated cardiomyocytes (30). All these findings 
(5–7, 15, 30, 35, 36, 47) suggest that EETs have ion 
channels as major targets and may be involved not only 
in the regulation of the contractile properties of myo-
cardium but also in the stabilization of cardiac electrophysiology. However, the effects of the individual EETs 
on cardiac ion channels and functional consequences 
are not yet completely clear. Furthermore, the observa-
tion that CYP inhibition by imidazole antimycotics 
(59) suppresses L-type Ca2+
 current in cardiomyocytes 
indicates that further studies are required to clarify 
which channels and mechanisms are involved in the 
cardiodepression by BK and 14,15-EET.

The inhibitors we used are not likely to affect myo-
cardial L-type Ca2+
 current; in fact, ABT and proadifen 
per se do not modify contractility. Unfortunately, the 
majority of CYP inhibitors have nonspecific inhibitory 
effects and do not solely block CYP activity but also 
inhibit ATP-sensitive potassium channels (5, 19, 54, 
56). Hence, we used two structurally unrelated inhibi-
tors, one of which (ABT) has a broad substrate speci-
city (43) and does not block potassium channels (5, 54, 
56). Because only specific CYP inhibition should be 
long lasting, the efficacy of ABT even after a washout 
period of 10 min supports the hypothesis of an involve-
ment of CYP products in BK-induced cardiodepression. 
Nevertheless, the fact that 14,15-EET reproduces and 
potentiates negative inotropic effects of BK further 
supports this hypothesis and limits the importance of 
possible side effects of the inhibitors.

The influence of the basal release of endothelial 
factors on contractility remains uncertain (for reviews, 
see Refs. 4, 27, and 28). Brutsaert et al. (4) reported 
that simultaneous inhibition of NO, PGI2, and endo-
thenin-1 in isolated papillary muscle results in no 
change of baseline contractile performance. The 
present study also demonstrates that the basal release 
of CYP products does not affect baseline contractility 
because proadifen and ABT (alone or in association 
with L-NAME + Indo) do not significantly affect heart 
performance.

The potentiation of BK-induced cardiodepression by 
14,15-EET pretreatment seems to depend on the fact 
that part of the 14,15-EET infused is stored in the 
membrane phospholipids of vascular endothelial and 
smooth muscle cells and is then released when BK is 
given (14, 55, 57). Thus, when infused at low doses, 
EETs cannot reach the smooth muscle fibers and myo-
cardium. Moffat et al. (37) reported that, in the guinea 
pig, low doses of EETs do not change cardiac function 
and coronary resistance, whereas after pretreatment 
with EETs, an exacerbation of the ischemia-reper-
fusion cardiodepression occurs. Because during ische-
mia-reperfusion BK can be released by the activity of a 
kininogenase on kininogen (30, 60), a BK-induced re-
lease of stored 14,55-EET can explain this exacerba-
tion of cardiodepression. In addition, during ischemia, 
the cellular concentration of EETs and its release may 
be enhanced as a consequence of an increased release 
of arachidonic acid with subsequent CYP epoxxygen-
aton, activation of phospholipases, and oxidation-hy-
drolysis of membrane phospholipids (30, 41). Indeed, the amount of EETs released by the vascular wall is unknown, but in the human platelet it has been estimated to reach the micromolar range (61).

From our results, it seems that, at the dose used (nM range), EET isomers different from 14,15-EET are not involved in the negative inotropic effect of BK. The ineffectiveness of 8,9- and 11,12-EETs could be derived from the fact that they are more avidly taken up by membrane phospholipids (15, 55, 57). Thus greater doses may be required to be effective or, alternatively, they are not involved in the cardiodepression induced by BK in the rat, as indicated by the fact that 20–30 nM of 11,12-EET induced an increase in L-type calcium current in isolated guinea pig (37) and rat (59) cardiomyocytes, whereas lower doses (2 nM) were ineffective (59).

**Clinical Implications**

CYP activity have been identified in hearts from several mammalian species, including rats and humans (24, 58), and may play an important role in the development of some diseases. In isolated guinea pig hearts, the exacerbation of the effects of ischemia-reperfusion by EETs supports this hypothesis (43). Furthermore, in many diseases (10, 18, 26, 53), a reduced NO-dependent vasodilatation has been described. Such a reduction is reported to lead to a disinhibition of CYP (1, 42), thus enhancing the release of CYP products. This mechanism can not only be responsible for the enhanced hyperpolarizing response, as seen in the hypercholesterolemic rabbit (40), but can also enhance the potential negative inotropic effect of the substances capable to activate CYP, as BK is.

In conclusion, this study provides a novel explanation for the negative inotropic effect of BK, which persists after NO inhibition, suggesting that, in the rat, the CYP pathway is the major physiological mechanism of BK-induced cardiodepressive response and that the relevant cytochrome is of vascular endothelial origin. The main product of CYP involved in the response may be 14,15-EET, which, on the other hand, is not likely to be responsible for vasodilatation. Finally, we cannot exclude that other CYP products, different from 14,15-EET, could be involved in the cardiodepressive response induced by BK.

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