ATP is involved in myocardial and vascular effects of exogenous bradykinin in ejecting guinea pig heart

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MATERIALS AND METHODS
Ejecting Heart Preparation

All experiments conform to the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892]. Methods detailing the use of the isolated ejecting guinea pig heart have been described previously (15, 16). Briefly, hearts were excised from anticoagulated, anesthetized guinea pigs of either gender (350–450 g; 300 U iv heparin and 60 mg/kg iv pentobarbitone sodium) and immersed in ice-cold Krebs-Henseleit buffer solution. The composition of the buffer was (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO4·7H2O, 24 NaHCO3, 1.1 KH2PO4, 10 glucose, and 2.5 CaCl2·2H2O, with added acetobutol (0.1 µM) and indomethacin (1 µM) to inhibit β-adrenergic and prostaglandin effects, respectively, and constantly gassed with 95% O2-5% CO2. Hearts were initially perfused retrogradely via the aorta (Langendorff mode) at a constant pressure of 70 cmH2O with Krebs-Henseleit solution at 37°C. After cannulation of the left atrium, hearts were switched to the recirculating ejecting mode by using a left atrial filling pressure of 10 cmH2O and an aortic afterload of 70 cmH2O. Heart rate was maintained constant by pacing the right atrium at ~10% above the intrinsic rate. Timed collection of pulmonary artery effluent allowed measurement of coronary flow. Aortic flow was mea-

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Sure with a flotation flowmeter (KDG Flowmeters), and stroke volume was calculated by dividing the sum of aortic and coronary flows by heart rate. High-fidelity LV pressure was recorded with a 2 F micromanometer-tipped catheter-transducer (Millar) inserted directly into the LV cavity via the apex, with care taken to avoid leakage of fluid around the catheter. LV pressure was sampled at 4 kHz with a MacLab 4 data acquisition module (Analog Digital Instruments, Australia) coupled to a Macintosh personal computer. The peak rate of rise of LV pressure (LV dP/dt max) was obtained from the first derivative of the LV pressure signal. LV end-diastolic pressure was measured as the pressure at the time of the initial upward deflection on the dP/dt trace. We previously reported the characterization of biphasic LV pressure fall in this preparation by the calculation of exponential time constants: T E for the early phase of pressure decline and T L for the later phase, which corresponds approximately to isovolumic relaxation (15, 16).

Measurement of ATP

ATP was measured with the luciferin-luciferase assay essentially as described by Kirkpatrick and Burnstock (21). Gassed unperfused Krebs solution was collected to determine the background level of ATP, which was subtracted from the values obtained in the experimental samples. Samples (200 µl) were collected from coronary effluent, which was facilitated by occluding the pulmonary artery. The samples were snap-frozen using solid CO2 and stored for up to 14 days at −70°C before assay. For assay the samples were passed through a Packard luminometer, during which 1 ml of luciferin-luciferase mixture was added to each sample. By use of linear regression, a standard curve was prepared with samples containing known quantities of ATP, from which ATP quantities in the samples were calculated. The limit of detection for ATP was −1 nM.

Protocol

Only those hearts in which baseline LV pressure and aortic and coronary flows were stable for an equilibration period of 12 min were included for study. Study drugs (0.15 ml volume) were introduced into the gassing chamber, and hemodynamic parameters were monitored subsequently. The following groups were studied: 1) control hearts, treated with 0.15 ml of distilled water, 2–5) hearts treated with exogenous bradykinin (1 or 100 nM) alone or in the presence of the nonselective P2 purinoceptor antagonist suramin sodium (1 µM); 6) hearts treated with bradykinin (100 nM) in the presence of the selective P2y purinoceptor antagonist pyridoxal phosphate 6-azophenyl-2,4'-disulfonic acid (PPADS, 1 µM); and 7) hearts treated with bradykinin (100 nM), in which samples of coronary effluent were taken for determination of ATP concentrations. The baseline characteristics of hearts before addition of the study drug are given in Table 1. Suramin and PPADS were added 3–4 min before addition of bradykinin. Neither suramin nor PPADS had any significant effect on basal cardiac function (Table 1).

Drugs and Chemicals

Bradykinin, acebutolol, suramin, and indomethacin were obtained from Sigma Chemical. PPADS was obtained from Cookson Chemicals. All drugs were dissolved in distilled water, with the exception of indomethacin, which was dissolved in 100% ethanol. The final concentration of ethanol was 0.01% and was without effect on the hearts. All other chemicals were of the purest reagent grade available.

Statistics

For LV pressure data, measurements from at least four consecutive beats were averaged, and the percent change from baseline was calculated. Within-group comparisons were performed on the absolute values with use of Student’s paired t-test followed by Dunnett’s correction for multiple tests. Between-group comparisons were performed by a repeated-measures ANOVA followed by a post-Student-Newman-Keuls test to isolate differences.

RESULTS

Control Hearts

All parameters remained stable in the control group of hearts, with no significant changes during the time course of the experiments (Fig. 1).

Effect of Bradykinin

Bradykinin (1 and 100 nM) induced characteristic changes in LV relaxation, as previously reported (15). Representative LV pressure traces showing the typical effect of bradykinin (100 nM) are shown in Fig. 2. LV relaxation. Early LV pressure decline was significantly accelerated, i.e., a reduction in the time constant of early relaxation (T E) after exposure to 100 nM bradykinin: −17.1 ± 4.0% after 4 min (P < 0.05 vs. control group; Fig. 1). Bradykinin at 1 nM had a similar, but smaller, effect on T E (Fig. 1). Interestingly, the time constant of late relaxation (T L) was unaffected by high-dose bradykinin (100 nM), whereas low-dose

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<th>Table 1. Baseline characteristics of isolated ejecting guinea pig heart preparations</th>
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Values are means ± SE; n, number of guinea pigs. LVP, peak left ventricular pressure; dP/dt max, peak rate of rise of LVP; LVEDP, left ventricular end-diastolic pressure; SV, stroke volume; CF, coronary flow; T E, time constant of early relaxation; T L, time constant of late relaxation; PPADS, pyridoxal phosphate 6-azophenyl-2,4'-disulfonic acid.
bradykinin (1 nM) induced a significant fall in $T_L$ at 8, 12, and 16 min (Fig. 1).

Systolic parameters. Bradykinin at 100 nM caused a small significant rise in stroke volume (12.49 ± 7.6%, $P < 0.05$ vs. control group) and peak LV pressure (Fig. 1) at 2 min only. A significant rise in $dP/dt_{\text{max}}$ was also observed at 2 and 4 min: 9.58 ± 4.5% at 4 min ($P < 0.05$ vs. control group). Bradykinin at 1 nM had no effect on peak LV pressure (Fig. 1), stroke volume or $dP/dt_{\text{max}}$: 4.67 ± 2.1 and 0.74 ± 0.96%, respectively, both at 2 min (both not significant). No changes in LV end-diastolic pressure or time to peak pressure were observed in either group.

Coronary flow. Bradykinin at 100 nM induced a rapid, transient increase in coronary flow, with the peak effect observed within 2 min (Fig. 1). Similarly, bradykinin at 1 nM induced a smaller, more short-lived transient increase in coronary flow, with a peak increase at 2 min (Fig. 1). It has also been previously demonstrated that this bradykinin-induced rise in coronary flow is concentration dependent and unrelated to the accompanying fall in $T_E$ (15).

Effect of Suramin

In the presence of the nonselective $P_2$ purinoceptor antagonist suramin, the effect of 100 nM bradykinin on $T_E$ was significantly inhibited, although a small initial reduction was still observed (Fig. 3). The maximal response was reduced by approximately 50%. Similarly, the bradykinin-induced rise in coronary flow was significantly reduced at all time points by approximately 50% (Fig. 3). LV pressure and $T_L$ were unaffected (Fig. 3). Suramin also completely inhibited the fall in $T_E$ and rise in coronary flow induced by 1 nM bradykinin (Fig. 4). The fall in $T_L$ observed with 1 nM bradykinin was also inhibited by suramin, whereas LV pressure was unchanged (Fig. 4).

Effect of PPADS

The selective $P_{2x}$ purinoceptor antagonist PPADS had no effect on the 100 nM bradykinin-induced fall in $T_E$ or rise in coronary flow (Fig. 5). LV pressure and $T_L$ were also unchanged (Fig. 5).
Fig. 3. Percent changes from baseline (0 min) after addition of 100 nM bradykinin alone (○) and in presence of 1 µM suramin (●) on peak LVP, CF, TE, and TL. Values are means ± SE. *P < 0.05 compared with control group (□); †P < 0.05 compared with bradykinin alone; all at equivalent time points.

Fig. 4. Percent changes from baseline (0 min) after addition of 1 nM bradykinin alone (○) and in presence of 1 µM suramin (●) on peak LVP, CF, TE, and TL. Values are means ± SE. *P < 0.05 compared with control group (□); †P < 0.05 compared with bradykinin alone; all at equivalent time points.
Administration of 100 nM bradykinin resulted in a rise in ATP levels in coronary effluent above baseline values. This increase was approximately twofold and was sustained for the duration of the experiment: peak concentration was 5.43 ± 1.34 nM at 2 min (P < 0.05 vs. baseline; Fig. 6). With the large rise in coronary flow observed with 100 nM bradykinin taken into account, expressing the data as ATP levels per minute indicated a much greater increase in total ATP release after addition of bradykinin (Fig. 6).

DISCUSSION

It has been previously shown using the ejecting guinea pig heart preparation that substance P and exogenous and endogenous bradykinin accelerate the early phase of LV pressure decline and that this response is at least partly mediated by NO (3, 15). Similar observations in isolated ferret and cat papillary muscles with the use of both of these agents (27–29) and the knowledge that bradykinin releases NO from cultured coronary vascular endothelial cells (23) and endocardial endothelium (29) allow us to speculate that the NO responsible for this effect is endothelium derived. It is also well known that bradykinin releases factors other than NO from endothelial cells (20, 31). These agents appear to be primarily vasoactive, and we previously published data that are consistent with this finding. For instance, in the isolated Langendorff-perfused ferret heart, it was demonstrated that brady-

Coronary ATP Concentrations

Fig. 5. Percent changes from baseline (0 min) after addition of 100 nM bradykinin alone (—) and in presence of 1 µM pyridoxal phosphate 6-azophenyl-2',4'-disulfonic acid (PPADS, •) on peak LVP, CF, T_E, and T_L. Values are means ± SE. *P < 0.05 compared with control group (□) at equivalent time points.

Fig. 6. Total ATP [expressed in nM (A) and nmol/min (B)] in coronary effluent at baseline (0 min) and after addition of 100 nM bradykinin (BK). *P < 0.05 vs. baseline.
that cultured endothelial cells release ATP in response to bradykinin in the absence of flow or shear stress.

Vasodilatory Effects

The significant inhibition of the 100 nM bradykinin-induced rise in coronary flow by suramin suggests the involvement of ATP/ADP in this response as well. ATP and ADP are potent vasodilators, stimulating the release of ADP from the endothelium via P2Y purinoceptors (see above). However, unlike the myocardial effects of bradykinin, which appear to be mediated entirely by NO, the vascular effects of bradykinin appear to also involve other agents. We therefore also investigated the mechanism of the NO-independent increase in coronary flow induced by 1 nM bradykinin previously observed by us in this preparation (15) and found that this effect was abolished by suramin. This suggests that ATP may also account for some, or possibly all, of the NO-independent effects of bradykinin in the isolated guinea pig heart. It has also been observed that ATP-induced vasodilation in the guinea pig involves mechanisms other than the release of NO (6, 31). One study demonstrated that prostaglandins account for one-third of the vasodilator effect of ATP. Our experiments were, however, performed in the presence of indomethacin, discounting this possibility. In the heart the actions of adenyl purines are complicated by their rapid sequential degradation from ATP to ADP to AMP to adenosine and future work is required to determine the relative contributions of these metabolites to the overall vasodilation in the guinea pig coronary artery (24).

Myocardial Effects

It is now widely accepted that adenyl purines such as ATP can be released from sympathetic nerves as a cotransmitter with norepinephrine (7) and from the endothelium as a mediator in the control of vascular tone (4, 8). Exogenous ATP has also been demonstrated to increase contractile amplitude in adult ventricular myocytes (11) and to exert a positive inotropic effect in rat isolated papillary muscles (26). In the present study the significant inhibition of the LV relaxant effects of bradykinin by suramin suggests the involvement of ATP/ADP in this response. The additional observation that bradykinin induces a significant increase in the concentration of ATP from coronary effluent further supports this hypothesis. Furthermore, with consideration of the accompanying rise in coronary flow observed after bradykinin administration, total ATP release is far greater than the twofold concentration increase observed with 100 nM bradykinin. The observed increase in ATP levels in the present study agrees with the findings by Yang and colleagues (38) showing that bradykinin could induce a rapid release of adenyl purines (ATP/ADP) from cultured guinea pig vascular endothelial cells.

There are two general subtypes of P2 purinoceptors that are stimulated by adenyl purines: the P2x and the P2y purinoceptor (24). In the vasculature, P2y purinoceptors are mostly located on the endothelium, whereas P2x purinoceptors are located on smooth muscle and the endothelium (24). The lack of any observed inhibitory effect with the selective P2x antagonist PPADS (39) implies that the effect of ATP/ADP is mediated by the endothelial P2y purinoceptor. However, this hypothesis may also be open to question, inasmuch as Brown and colleagues (5) recently demonstrated that PPADS can also inhibit P2y purinoceptors. A third endothelial P2 purinoceptor, the P2u purinoceptor, has also been described (35) and is inhibited by suramin (13), but not by PPADS (5, 37). Hence, the P2u purinoceptor may be responsible for the effects of bradykinin.

We previously demonstrated that the myocardial relaxant effect of bradykinin is mediated by NO (15). The present results confirm observations by other researchers that ATP and ADP stimulate the release of NO via the activation of endothelial P2y purinoceptors (31, 33). Thus bradykinin may release NO directly via B2-kinin receptors and indirectly via the release of adenyl purines (ATP/ADP), which themselves release NO through the activation of endothelial P2 purinoceptors. Another possibility is that ATP is indirectly released by bradykinin in response to an increase in shear stress. This, however, does not seem essential, inasmuch as Yang and colleagues (38) demonstrated
by NO, whereas the vascular effects can also be influenced by these other endogenous factors.

The picture is further complicated by the degradation of ATP to ADP to AMP to adenosine. Adenosine also has vasodilatory effects, releasing NO (1, 30), and has been implicated in the cardioprotective effects of "ischemic preconditioning" (25). It has also been shown that endogenous bradykinin can mediate the cardioprotective effects of ischemic preconditioning (14, 34). Because it has been shown that increased quantities of bradykinin are released during ischemia (35), it is tempting to speculate that bradykinin may act as a trigger to stimulate the release of adenyl purines, which are degraded to adenosine, which then exert their protective effect on the underlying myocardium.

Endogenous bradykinin has also been implicated in the mechanism of action of angiotensin-converting enzyme (ACE) inhibitors (22). We previously showed that the ACE inhibitor captopril exerts a selective LV relaxant effect, which is mediated via endogenous bradykinin and NO (3). Preliminary data using suramin demonstrated that this effect also appears to involve endogenous ATP/ADP (2). Further evidence for the involvement of adenyl purines with bradykinin and ACE inhibition was provided by the observation by Vidal and colleagues (33a) that the ACE inhibitor trandolapril could enhance the relaxant effects of ADP in rings of canine femoral arteries. The authors had no explanation for the effects observed, but from the data presented here, the simplest explanation is that bradykinin stimulates the release of adenyl purines, leading to increased activation of endothelial P2 purinoceptors and the release of NO. Thus, Vidal and colleagues were probably observing an additive effect with trandolapril, with increased levels of endogenous bradykinin causing increased release of adenyl purines (i.e., ATP/ADP), which contributed to the enhanced vasodilatory effect observed. From this evidence, it seems reasonable to postulate that ACE inhibitors may exert their effect not simply via a bradykinin-NO pathway but also through a bradykinin-purinoceptor mechanism of action.

The data presented here demonstrate that, as well as releasing NO, exogenous bradykinin releases adenyl purines and that these mediators contribute to the myocardial and vascular effects of bradykinin in the isolated ejecting guinea pig heart.

This work was supported by the British Heart Foundation (BHF) and the Medical Research Council (MRC). P. B. Anning was the recipient of a BHF Ph.D. Studentship, B. D. Prendergast the recipient of a BHF Junior Research Fellowship, and A. M. Shah the recipient of an MRC Clinical Senior Fellowship.

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Received 18 November 1998; accepted in final form 22 March 1999.

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