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Cytochrome P450-dependent effects of bradykinin in the rat heart

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1 Vasodilator responses to bradykinin (BK) in the rat heart are reported to be independent of NO and cyclo-oxygenase/lipoxygenase products of arachidonic acid (AA).

2 We verified that inhibition of NO synthase with L-nitroarginine (50 μM) and cyclo-oxygenase with indomethacin (2.8 μM) were without effect on vasodilator responses to BK (10–1000 ng) in the Langendorff rat heart preparation.

3 L-Nitroarginine elevated perfusion pressure, signifying a crucial role of NO in the maintenance of basal vasculature tone.

4 In hearts treated with L-nitroarginine to eliminate NO and elevate perfusion pressure, vasodilator responses were reduced by inhibitors of cytochrome P450 (P450), clotrimazole (1 μM) and 7-ethoxyresorufin (1 μM). 17-Octadecynoic acid (17-ODYA 2 μM), a mechanism based inhibitor of P450-dependent metabolism of fatty acids, also reduced vasodilator responses to BK.

5 These results confirm that NO and prostaglandins do not mediate vasodilator responses to BK in the rat heart but suggest a major role for a P450-dependent mechanism via AA metabolism.

Keywords: Rat perfused heart; vasodilatation to bradykinin; nitric oxide; prostaglandins; cytochrome P450

Introduction

Bradykinin (BK) is a nonapeptide that elicits endothelium-dependent vasodilatation, an effect generally attributed to its ability to stimulate nitric oxide (NO) synthesis (Vane *et al.*, 1990). However, there are also numerous reports of NO-independent vasodilator responses to BK, particularly in coronary vessels (Richard *et al.*, 1990; Cocks & Angus, 1991; Tschudi *et al.*, 1991). Thus, there is evidence that BK can stimulate the formation/release of, a yet to be characterized, hyperpolarizing factor (Beny & Brunet, 1988; Flavahan *et al.*, 1989; Nagao *et al.*, 1992). Moreover, BK can also stimulate phospholipases to release arachidonic acid (AA) which can be metabolized by cyclo-oxygenase, lipoxygenase and cytochrome P450 (P450) to products that may contribute to the vascular response. In skeletal muscle the vasodilator effect of BK is mediated by prostaglandins (Messina *et al.*, 1975) whereas in cerebral vessels free radicals, derived from cyclo-oxygenase metabolism of AA, appear to mediate the response (Kontos *et al.*, 1984). Recently, we reported that a major component of the vasodilator response to BK in the isolated, perfused kidney of the rat was dependent on P450 (Fulton *et al.*, 1992). Baydoun & Woodward (1991) also found that the vasodilator response to BK in the isolated heart of the rat was independent of NO and cyclo-oxygenase and lipoxygenase metabolites of AA. However, a role for P450-derived AA metabolites was not addressed. As the coronary vasculature is heavily invested with P450 (Pinto *et al.*, 1987) we used the isolated heart to assess the contribution of P450 to the coronary vasodilator effect of BK.

Methods

Male Wistar rats (350–400 g) were anaesthetized with pentobarbitone (65 mg kg^{-1}, i.p.) and given heparin (1000 u kg^{-1}, i.v.). Following thoracotomy, the heart was excised and perfused, via an aortic cannula, at constant flow with oxygenated Krebs buffer at 37°C according to the method of Langendorff (1895) as modified by Broadley (1979). Flow was adjusted to 8–8.5 ml min^{-1} which resulted in a basal perfusion pressure of 25–40 mmHg which increased and stabilised at approximately 80 mmHg after 15–20 min. To amplify vasodilator responses, vascular tone was further elevated with phenylephrine (0.75–1 μM), U46619 (8–14 nM) or nitroarginine (50 μM).

In the first series of experiments the contribution of NO to the coronary vasodilator response to BK was assessed by comparing changes in perfusion pressure in hearts constricted with either phenylephrine or U46619 and those in which NO synthesis was inhibited with L-nitroarginine which alone was sufficient to raise perfusion pressure. Thus, phenylephrine was relatively ineffective in causing constriction compared to L-nitroarginine, elevating perfusion pressure to 96 mmHg versus 136 mmHg. Therefore, experiments were also conducted in hearts treated with U46619 to achieve a similar perfusion pressure to that obtained with L-nitroarginine. In all subsequent experiments, L-nitroarginine (50 μM) was used to eliminate NO and to elevate vascular tone. Thus, the control group except for those experiments shown in Figures 1 and 2, consisted of L-nitroarginine-treated hearts and BK responses were compared to those obtained in hearts treated with L-nitroarginine and inhibitors of cyclo-oxygenase or cytochrome P450 which were added to the perfusate from the start of the experiment. Indomethacin (2.8 μM) was used to inhibit cyclo-oxygenase whereas clotrimazole (1 μM), 7-ethoxyresorufin (1 μM) or 17-octadecynoic acid (17-ODYA, 2 μM) were used to inhibit cytochrome P450. We have previously shown that these concentrations of P450 inhibitors do not influence renal vasodilator or aortic vasorelaxant responses to acetylcholine or sodium nitroprusside (Oyekan *et al.*, 1991, 1994). After a dose-response curve to BK was obtained, the vasodilator response to 1000 ng nitroprusside was determined to assess any non-specific effects of the inhibitors used.

Data from the various treatment and control groups were compared by analysis of variance and individual data points compared by Fisher's test using the Stat View statistical programme (Brain Power Inc, California, U.S.A.). Differences were considered significant when $P < 0.05$.

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Materials

17-ODYA, U46619 (11, 9 epoxy methano-prostaglandin H_2), was obtained from Biomol, Plymouth Meeting, PA, U.S.A. and stored in ethanol (10 mg ml^{-1}) at −20°C. 7-Ethoxyresorufin was obtained from Pierce Laboratories, Rockford, Il, U.S.A., and stored at −20°C in ethanol (1 mg ml^{-1}). Clotrimazole, L-nitroarginine, phenylephrine and sodium nitroprusside were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Clotrimazole was dissolved in ethanol and L-nitroarginine, phenylephrine and sodium nitroprusside in water. Indomethacin which was dissolved in 4% $NaHCO_3$ was a gift from Merck.

Results

Phenylephrine elevated coronary perfusion pressure to 96 ± 11 mmHg and BK produced dose-dependent reductions in perfusion pressure (Figure 1). Inhibition of NO synthesis with L-nitroarginine, which resulted in a perfusion pressure of 136 ± 4 mmHg, did not reduce the vasodilator responses to BK (Figure 1) but rather enhanced them, attaining significance at the 1000 ng dose (61 ± 6 mmHg vs 38 ± 5 mmHg in the phenylphrine-treated group). The vasodilator response to nitroprusside was slightly, but insignificantly, increased in the presence of L-nitroarginine (41 ± 8 vs 30 ± 5 mmHg). As there were substantial differences in perfusion pressure between the two groups, we also compared responses to BK in hearts preconstricted with U46619 to elevate perfusion pressure to the same level as that attained with L-nitroarginine in the presence of indomethacin. Thus, U46619 resulted in a perfusion pressure of 129 ± 7 mmHg, comparable to that achieved with L-nitroarginine. However, vasodilator responses to BK were not different in L-nitroarginine plus indomethacin and U46619-treated hearts (Figure 2) although responses to nitroprusside were greatly enhanced by the combination of L-nitroarginine and indomethacin.

Inhibition of cyclo-oxygenase with indomethacin or cytochrome P450 with clotrimazole, 7-ethoxyresorufin or 17-ODYA did not affect the perfusion pressure attained with L-nitroarginine. Indomethacin was without effect on the coronary vasodilator responses to all doses of BK in hearts preconstricted with L-nitroarginine (data not shown), confirming the results reported by Baydoun & Woodward (1991). In contrast, clotrimazole markedly reduced the vasodilator responses to BK (Figure 3) in L-nitroarginine-treated hearts but did not modify that to nitroprusside. Thus, responses to BK were inhibited by up to 80% in the presence of clotrimazole ($P < 0.01$). 7-Ethoxyresorufin was less effective than clotrimazole in inhibiting responses to BK, a significant reduction (39%) being obtained only at the highest dose of BK (Figure 4). 7-Ethoxyresorufin, like clotrimazole, was without effect on the coronary vasodilator response to nitroprusside.

As inhibition of cytochrome P450 with clotrimazole or 7-ethoxyresorufin reduced coronary vasodilator responses to

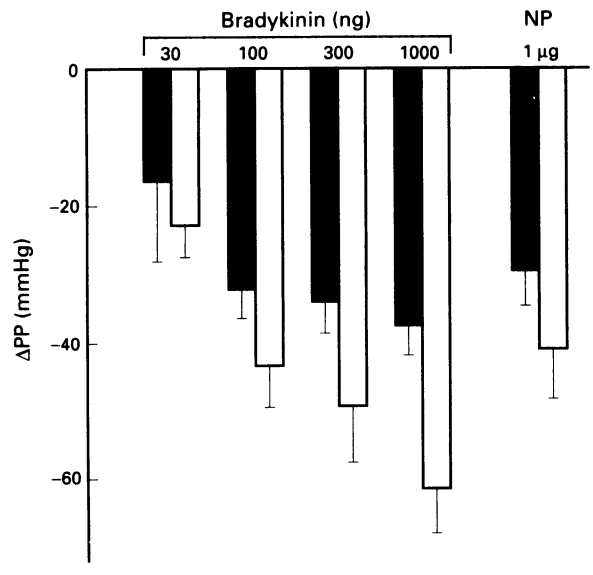

Figure 1 Perfusion pressure responses to bradykinin and nitroprusside (NP) in preconstricted rat hearts: effect of NO synthesis inhibition with L-nitroarginine (50 μM): solid column, phenylephrine 1 μM ($n = 5$); open columns, L-nitroarginine 50 μM ($n = 9$).

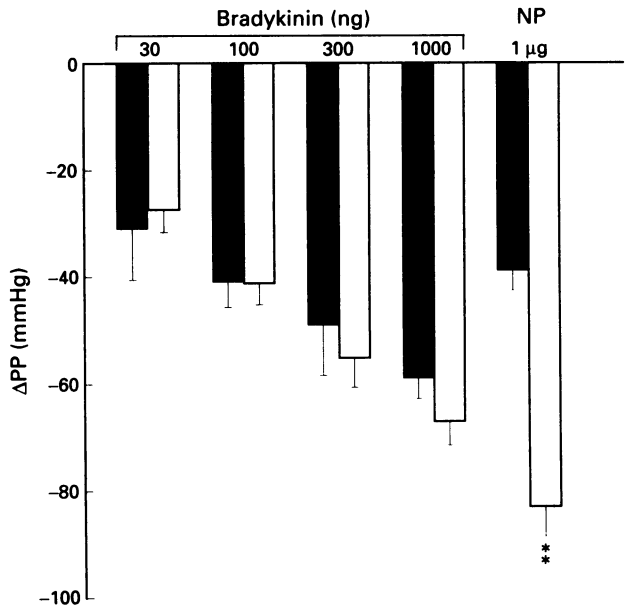

Figure 2 Comparison of perfusion pressure responses to bradykinin in rat hearts constricted with L-nitroarginine to inhibit NO synthesis and those constricted with U46619: solid column, U46619 2–5 ng ml^{-1} ($n = 3$); open columns, L-nitroarginine 50 μM + indomethacin 2.8 μM ($n = 9$). $^{**}P < 0.01$.

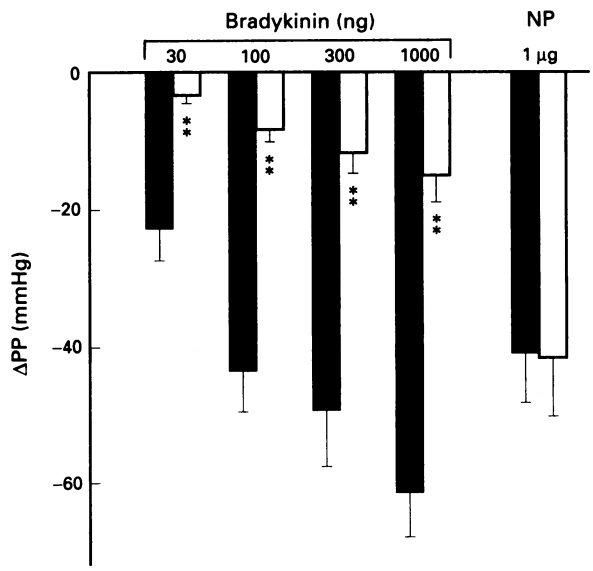

Figure 3 Effect of inhibition of P450 with clotrimazole on vasodilator responses to bradykinin and nitroprusside in rat isolated hearts in which L-nitroarginine was used to eliminate NO synthesis and raise perfusion pressure: solid columns, L-nitroarginine 50 μM ($n = 9$); open columns, L-nitroarginine 50 μM + clotrimazole 1 μM ($n = 9$). $^{**}P < 0.01$.

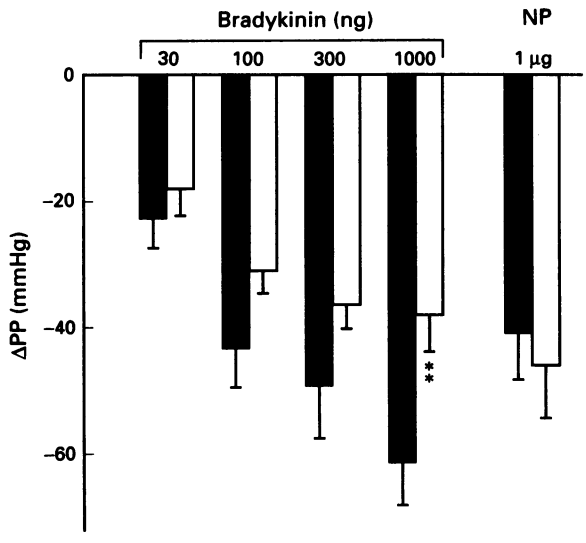

Figure 4 Bradykinin-induced changes in the perfusion pressure of rat isolated hearts preconstricted with L-nitroarginine in the presence and absence of the cytochrome P450 inhibitor, 7-ethoxyresorufin: solid column, L-nitroarginine 50 μM ($n = 9$); open columns, L-nitroarginine 50 μM + 7-ethoxyresorufin 1 μM ($n = 5$). $^{**}P < 0.01$.

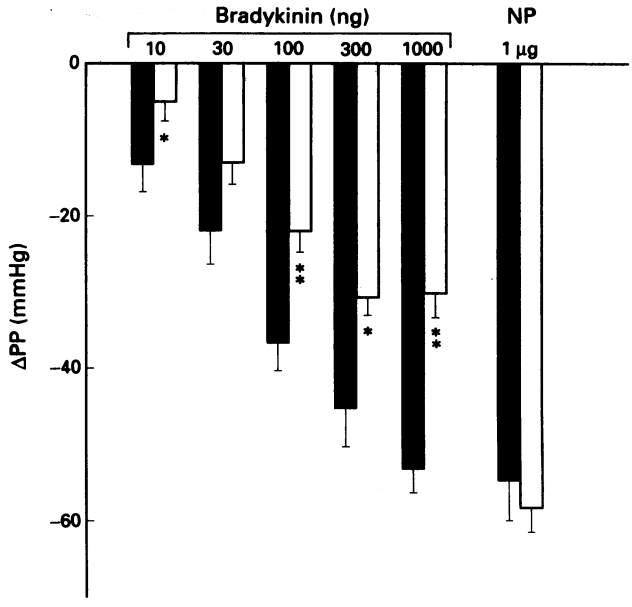

Figure 5 Vasodilator responses to bradykinin and nitroprusside in rat isolated perfused hearts preconstricted with L-nitroarginine (50 μM) in the absence and presence of 17-ODYA to inhibit cytochrome P450 metabolism of fatty acids: solid columns, L-nitroarginine 50 μM ($n = 5$); open columns, L-nitroarginine 50 μM + 17-ODYA 2 μM ($n = 5$). $^{*}P < 0.05$; $^{**}P < 0.01$.

BK, we next tested the effects of 17-ODYA in order to investigate the possibility that metabolites of AA, generated via cytochrome P450, were involved in the response to BK. As 17-ODYA is a suicide-substrate inhibitor of cytochrome P450 isozymes that metabolize long-chain fatty acids (Zou *et al.*, 1994b), it possesses a specificity for mono-oxygenases that metabolize AA that is not demonstrated by other inhibitors. 17-ODYA reduced responses to all doses of BK in L-nitroarginine-treated hearts, the greatest inhibition (43%) occurring with the highest dose of BK (Figure 5), but did not modify the vasodilator response to nitroprusside.

Discussion

The results of this study demonstrate that, in the isolated perfused heart of the rat, vasodilator responses to BK are not mediated by NO as inhibition of synthesis with L-nitroarginine did not reduce the responses, confirming the findings of Baydoun & Woodward (1991). Although we did not verify that the concentration of L-nitroarginine (50 μM) used by us abolished synthesis of NO, there are reasons for believing this to be the case. This concentration was shown by Cachofeiro & Nasjletti (1991) to prevent stimulated increases in urinary cyclic GMP from the rat kidney; L-nitroarginine produced a marked increase in coronary perfusion pressure, greater than could be achieved with phenylephrine, suggesting removal of an endogenous vasodilator system that is crucial to the maintenance of basal vascular tone; responses to nitroprusside were enhanced, albeit insignificantly, in the presence of L-nitroarginine consistent with reduction of background levels of NO. However, the two groups used to test the contribution of NO, i.e., those treated with phenylephrine vs those treated with L-nitroarginine, exhibited different perfusion pressures, 96 vs 135 mmHg and, therefore, the maximum responses to BK in the control group may have been limited by the level of tone. Consequently, we also compared responses in hearts constricted with U46619 to attain a similar perfusion pressure to that achieved with L-nitroarginine. As no differences were observed in the vasodilator response to BK we can exclude a role of NO. Similarly, in porcine and canine isolated coronary arteries vasorelaxant responses to BK were minimally affected or unaffected by interventions aimed at reducing the synthesis or action of NO (Richard *et al.*, 1990; Cocks & Angus, 1991; Tschudi *et al.*, 1991). Consequently, mediators other than NO, are required to explain the coronary vasodilator effect of BK. The results of this study and those of a previous study of the rat heart (Baydoun & Woodward, 1991) provide evidence that BK utilizes a P450-dependent mechanism in certain vascular tissues. Thus, clotrimazole and 7-ethoxyresorufin attenuated responses to BK in the rat heart but did not affect dilator responses to nitroprusside indicating that these agents were specific for P450. These results are qualitatively similar to those reported by Pinto *et al.* (1987) for canine coronary arteries in which a role for cyclo-oxygenase and P450 in the vasorelaxant response to BK was suggested, based on the use of indomethacin and SKF525A. In the present study we also showed that 17-ODYA, an inhibitor of P450 metabolism of fatty acids, attenuated the coronary vasodilator response to BK, providing evidence for P450-dependent metabolism of AA which is released in response to BK. The observations that three structurally distinct inhibitors of P450 reduce BK responses provide strong evidence for a role of this system. Furthermore, as these agents differ in their capacity to inhibit the various P450 isozymes that metabolize AA, epoxygenase versus ω and ω-1 hydroxylase, their effects provide insights as to the identity of the putative mediator. Thus, clotrimazole, which was the most effective inhibitor of the BK response, is more specific for the epoxygenase pathway of P450 metabolism of AA whereas 17-ODYA and 7-ethoxyresorufin, which produced less attenuation of the response, exhibit no differential inhibitory activity towards epoxygenase vs ω-hydroxylase (Zou *et al.*, 1994a). Therefore, these results are consistent with an epoxygenase product as the mediator of P450-dependent coronary vasodilator responses to BK. Moreover, preliminary results of GC-MS analysis of coronary perfusates indicate that the ω-hydroxylase pathway of AA metabolism is negligible in the rat heart whereas epoxide released can be demonstrated.

In summary, this study suggests that a P450-dependent mechanism via metabolism of AA is the major mediator of the coronary vasodilator effect of BK in the rat and that the contribution of NO is minimal or absent. These results are consistent with the ability of BK to release AA and its

subsequent conversion by vascular P450, the highest levels of which are found in the coronary vasculature (Pinto *et al.*, 1987). However, the identity of the mediator and its mechanism of vasodilatation remain to be elucidated.

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