Vascular effects of arachidonic acid in the rat perfused heart: role of the endothelium, cyclooxygenase, cytochrome P450, and K^+ channels

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Abstract The vascular effects of arachidonic acid (AA) were addressed in the rat perfused heart in terms of metabolic pathways and effector mechanisms. Under basal perfusion pressure, AA elicited dilator responses. However, in hearts treated with nitroarginine to eliminate nitric oxide and to elevate perfusion pressure, the predominant effect of AA was vasoconstriction which was converted to a vasodilator effect by inhibition of cyclooxygenase or antagonism of TP receptors. The vasodilator effect of AA in nitroarginine- and indomethacin-treated hearts was greatly attenuated by clotrimazole, an inhibitor of cytochrome P450, and by inhibition of K^+ channels with tetraethylammonium; in the absence of indomethacin, clotrimazole enhanced the vasoconstrictor effect of AA. When endothelin was used to constrict the coronary vasculature, AA also produced cyclooxygenase-dependent vasoconstriction. In hearts constricted with the endoperoxide analogue, U46619, only endothelium-dependent vasodilator effects of AA were observed that were reduced by indomethacin or clotrimazole. These results indicate that the coronary vasoconstrictor effect of AA which is expressed with elevated tone, results from its conversion by cyclooxygenase to a product(s) that activates TP receptors. The vasodilator effect exhibits two endothelium-dependent components, one mediated by cyclooxygenase products and the other by a cytochrome P450-derived product that activates K^+ channels.—Qiu, Y., and J. Quilley. **Vascular effects of arachidonic acid in the rat perfused heart: role of the endothelium, cyclooxygenase, cytochrome P450, and K^+ channels.** ***J. Lipid Res.*** **1999.** 40: **2177–2184.**

Supplementary key words constrictor and dilator components • basal versus elevated perfusion pressure • indomethacin • TP receptor antagonism • clotrimazole • tetraethylammonium

Arachidonic acid (AA) is transformed by three enzymic pathways (cyclooxygenase, lipoxygenase, and cytochrome P450) to produce a myriad of biologically active metabolites (1). The vascular effects of AA are determined by the vasoactivity of the products generated which are species- and tissue-dependent and vary both longitudinally and transversely within the vasculature (2). Thus, not all the vascular effects of AA result from its transformation to PGI_2, the principal cyclooxygenase metabolite of the endothelium (3). Many studies have now shown that the cytochrome P450 pathway may also contribute to the vasodilator effects of AA (4–6). Indeed, a cytochrome P450 product, namely an epoxide (EET), has been proposed as an endothelium-derived hyperpolarizing factor that contributes to the action of some dilator agents such as bradykinin (7–11). However, responses to AA vary according to the vascular bed and the experimental conditions. For example, in the rat kidney the predominant action of AA is a vasoconstrictor effect that is mediated by PGH_2 (12); when this is prevented and the tone is elevated, a cytochrome P450-dependent vasodilator effect can be uncovered (5). In contrast, in the perfused mesenteric vascular bed of the rat, the vasodilator effect of AA that was also observed in the presence of indomethacin was reported to be independent of cytochrome P450 but dependent on activation of K^+ channels sensitive to apamin and glyburide (13). Similarly, in the rat heart treated with indomethacin and nitroarginine, we have reported that the vasodilator effect of AA is attenuated by charybdotoxin (14); however, a role for cytochrome P450 was not addressed nor was the role of cytochrome P450, the endothelium, or cyclooxygenase to the constrictor and dilator components of the AA response.

Although cyclooxygenase- and cytochrome P450-dependent vasorelaxant actions of AA have been described for canine and bovine coronary arteries, the actions of AA in the rat heart, which also reflects responses of the microvasculature, have not been fully characterized. Studies from almost two decades ago, before the importance of cytochrome P450 and K^+ channels to vascular mechanisms was appreciated, addressed only the cyclooxygenase pathway in the vasoconstrictor/vasodilator effect of AA

Abbreviations: AA, arachidonic acid; NO, nitric oxide; PGI_2, prostacyclin; PGH_2, prostaglandin endoperoxide; TxA_2, thromboxane; EET, epoxyeicosatrienoic acid; TEA, tetraethylammonium.

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(15–18) without any definition of the contribution of the endothelium, nitric oxide (NO), cytochrome P450, or K^+ channels to the vasodilator component that is resistant to inhibition of cyclooxygenase (17). Moreover, in those studies, conflicting results were obtained which may reflect different experimental conditions. Consequently, in the present study, we used the rat perfused heart to analyze the actions of AA in terms of metabolic pathways and effector mechanisms under a variety of experimental conditions. The results show that in the rat heart under basal conditions, AA causes vasodilation whereas in hearts with elevated tone, AA produces a cyclooxygenase-dependent vasoconstrictor effect that is mediated via activation of TP receptors. When the constrictor effect of AA is prevented, an endothelium-dependent vasodilator action is uncovered which is partially dependent on cycloxygenase but also reflects a significant contribution from a cytochrome P450-dependent mechanism that involves activation of K^+ channels.

METHODS

Male Wistar rats, weight 300–430 g, were anesthetized with pentobarbitone (65 mg/kg ip) and given heparin (1000 U/kg, iv). After thoracotomy, the heart, with attached aorta, was excised and placed in ice-cold Krebs' buffer. The aorta was cannulated and the heart was perfused, according to the method of Langendorff, with oxygenated Krebs' buffer at 37°C at a flow rate of 8–10 ml/min to obtain an initial basal perfusion pressure of 30–40 mm Hg.

Vascular responses in nitroarginine-treated hearts

Nitroarginine (50 μm) was added to the coronary perfusate to inhibit NO synthesis and elevate perfusion pressure to approximately 120–140 mm Hg. Once a stable elevated perfusion pressure was obtained, vascular responses to AA (1, 3, and 10 μg) were determined in the absence and presence of indomethacin (2.8 μM), SQ29548 (1 μM), clotrimazole (1 μm), and TEA (10 mm).The experiments with clotrimazole and TEA were conducted in the presence of indomethacin to investigate the role of cytochrome P450 and K^+ channels, respectively, to the vasodilator component of the AA response which was uncovered when the vasoconstrictor component was eliminated by inhibition of cyclooxygenase. In these experiments, indomethacin was added to the perfusate at the start of the perfusion, whereas SQ29548, clotrimazole, and TEA were added to the perfusate once a stable elevated perfusion pressure was attained in response to nitroarginine. The concentration of clotrimazole that was used (1 μM) has been previously shown to attenuate the renal vasodilator component of the response to AA (5) as well as the renal and coronary NO-independent vasodilator effects of bradykinin (8, 19). Although clotrimazole has been reported to inhibit K^+ channels (20, 21) our studies in the heart and kidney indicate that this is not the case as vasodilator responses to SCA 40, cromakalim, and EETs were unaffected in the presence of 1 μM clotrimazole (7, 8, 19, 22). The concentration of SQ29548 (1 μm) that was used has been previously shown to inhibit renal vasoconstrictor responses to the endoperoxide analogue, U46619, and AA (12). TEA, at a concentration of 10 mM, inhibits all types of K^+ channels (23) and we have shown that in the rat isolated heart it attenuates vasodilator responses to cromakalim and bradykinin (7). Responses to bradykinin (100 ng) were determined in these experiments to confirm that clotrimazole and TEA were effective. Two–three preparations/day were completed where at least one was assigned to the control group (untreated) or the indomethacin-treated group. Consequently, the control group or the indomethacin-treated group was the same for all the interventions: SQ29548, TEA with indomethacin, and clotrimazole with indomethacin. Responses to nitroprusside were also determined to assess any non-specific effects of TEA and clotrimazole on vasodilator responsiveness of the heart.

In additional experiments where nitroarginine was used to inhibit NO synthesis and elevate perfusion pressure, we also addressed the effects of clotrimazole as well as endothelial denudation on responses to AA in the absence of indomethacin. The endothelium was removed by perfusing the heart with 2 ml distilled water and 2 ml air. Responses to bradykinin were determined to confirm removal of the endothelium and the effectiveness of clotrimazole whereas reponses to nitroprusside were determined to ensure that responsiveness to an endothelium- and cytochrome P450-independent vasodilator agent remained intact.

Vascular responses in hearts with intact NO synthesis constricted with U46619

Because NO constitutes a major vasodilator system that contributes to coronary vascular tone and may influence vascular responses, we conducted additional experiments where the NO system remained intact to determine whether AA produced similar effects. Thus, the endoperoxide analogue, U46619 (10 ng/ml) was used to elevate perfusion pressure and responses to AA, bradykinin, and nitroprusside determined in the absence and presence of indomethacin, nitroarginine, and a combination of indomethacin and nitroarginine.

In a separate series of experiments with hearts constricted with U46619, vasodilator responses to AA, bradykinin, and nitroprusside were compared in the absence (control) and presence of clotrimazole and after removal of the endothelium to address the contribution of cytochrome P450 and the endothelium, respectively, to the vasodilator effect of AA that is observed in the presence of U46619.

Vascular responses in hearts constricted with endothelin

As U46619 activates TP receptors, its presence could mask the constrictor effect of endoperoxides or TxA_2 generated from AA administered to the heart. Consequently, we also used endothelin (2–3 ng/ml) to elevate coronary perfusion pressure before testing responses to AA in the absence and presence of indomethacin.

Vascular responses under basal perfusion pressure

Finally, we determined vascular responses to AA (1, 3, and 10 μg) and bradykinin (30 ng) under basal conditions, i.e., in the absence of any interventions to elevate perfusion pressure. Under these conditions, perfusion pressure increased with time from an initial value of 30–40 mm Hg to 80–100 mm Hg, whereupon responses to AA and bradykinin were examined.

Materials

Sodium arachidonate was purchased from NuChek, Elysian, MN, dissolved in distilled water, and divided into aliquots that were sealed under nitrogen and stored at −20°C. SQ29548 was a gift from Bristol-Myers Squibb, Princeton, NJ, and was dissolved in ethanol, diluted in 2 mm sodium carbonate, and divided into aliquots that were stored at −20°C. Bradykinin, TEA, nitroprusside, indomethacin, nitroarginine, and clotrimazole were all obtained from the Sigma Chemical Co., St. Louis, MO. Bradykinin, nitroarginine, nitroprusside, and TEA were dissolved in distilled water whereas indomethacin was dissolved in 4.2% sodium bicarbonate and clotrimazole in ethanol.

Data analysis

Data are presented as the mean ± SEM and were compared by analysis of variance. Where significance was indicated, individual points were compared using a Student's *t*-test. Differences were considered significant when $P < 0.05$.

RESULTS

Vascular responses in hearts treated with nitroarginine

In the control group (n = 8), elevated perfusion pressure after nitroarginine was 139 ± 3 mm Hg compared to 135 ± 5 mm Hg for the indomethacin group (n = 10), 134 ± 2 mm Hg for the SQ29548 group (n = 8), and 124 ± 7 mm Hg for the clotrimazole/indomethacin group (n = 7). In the group treated with indomethacin plus TEA (n = 6), perfusion pressure was 129 ± 2 mm Hg before and 160 ± 6 mm Hg after the addition of TEA to the coronary perfusate. Thus, blockade of K^+ channels increased coronary perfusion pressure by approximately 30 mm Hg. In the group in which the endothelium was removed, perfusion pressure was less at 118 ± 6 mm Hg; after perfusion with air/water, perfusion pressure was briefly elevated by approx. 20 mm Hg above the pretreatment value and then declined followed by a slow increase towards the pretreatment level. Similarly, clotrimazole resulted in a fall in perfusion pressure that slowly returned towards the pretreatment level and elevated perfusion pressure in this group was 115 ± 4 mm Hg.

Under control conditions, AA produced dose-dependent increases in perfusion pressure, i.e., vasoconstriction, which was sometimes preceded by vasodilation (**Fig. 1**) although the vasodilator component was not seen in all preparations or to all doses of AA. In the presence of indomethacin to prevent the conversion of AA by cyclooxygenase to endoperoxides, prostaglandins, and thromboxane, the vasoconstrictor component of the AA response was almost abolished and dose-dependent vasodilation was uncovered in all cases (Fig. 1 and **Fig. 2**). The vasodilator response to bradykinin was unaffected by indomethacin (Fig. 2).

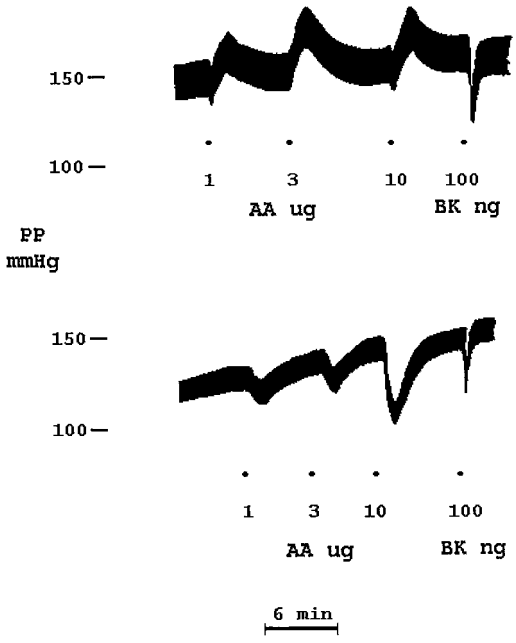

Fig. 1. Recordings of perfusion pressure (PP) in response to AA and bradykinin in the absence (upper panel) and presence (lower panel) of indomethacin (2.8 μm) in isolated hearts treated with nitroarginine (50 μm) to inhibit NO synthesis and elevate PP to approx. 120–140 mm Hg.

Antagonism of TP receptors with SQ29548 produced findings essentially similar to those observed with indomethacin. Thus, the vasoconstrictor effect of AA was abolished and dose-dependent vasodilation was uncovered (**Fig. 3**). The vasodilator response to bradykinin in the presence of SQ29548 was slightly less than that obtained in the control group or in the group treated with indomethacin.

To determine the contribution of K^+ channels in the vasodilator component of the coronary AA response and to confirm the results we had previously obtained with charybdotoxin (14), we tested the effects of TEA in the presence of indomethacin to eliminate the constrictor component. In the presence of TEA, the vasodilator effect of AA was almost abolished (Fig. 3). As expected, TEA also significantly reduced the vasodilator effect to bradykinin but did not affect the vasodilator response to nitroprusside (Fig. 3).

To investigate a role of cytochrome P450 in the vasodilator component of the vascular response to AA which was uncovered when cyclooxygenase was inhibited, we used clotrimazole to inhibit cytochrome P450, based on several of our previous studies (5, 8, 19). Clotrimazole caused a transient decrease in the nitroarginine-induced elevation of perfusion pressure but this returned to the pretreatment level (124 ± 7 mm Hg) which was not different from that obtained in the comparative group (135 ± 5 mm Hg), that treated with indomethacin. In the presence of clotrimazole, however, vasodilator responses to AA were greatly attenuated (Fig. 3). Similarly, the coronary vasodilator response to bradykinin was almost abolished by clotrimazole which had a lesser effect on the vasodilator response to nitroprusside.

In nitroarginine-treated hearts subjected to air/water, the vasodilator effect of bradykinin was almost abolished, 1 ± 1 mm Hg, confirming removal of the endothelium. In contrast, the vasodilator effect of nitroprusside was unchanged, 21 ± 2 mm Hg. In these preparations, 1, 3, and 10 μg AA elicited vasoconstrictor responses of 3 ± 1, 9 ± 2, and 8 ± 1 mm Hg, respectively, that were less than that observed in endothelium-intact preparations, 8 ± 1, 17 ± 2, and 14 ± 3 mm Hg, respectively. In both cases there was evidence for tachyphylaxis, insomuch that the third and highest dose of AA did not increase perfusion pressure over that seen with the intermediate dose of AA, 3 μg. As with endothelial denudation, treatment of hearts with clotrimazole in the absence of indomethacin almost abolished the vasodilator effect of bradykinin, 3 ± 0 mm Hg, confirming its effectiveness, whereas responses to nitroprusside were unaffected, 21 ± 4 mm Hg. Under these

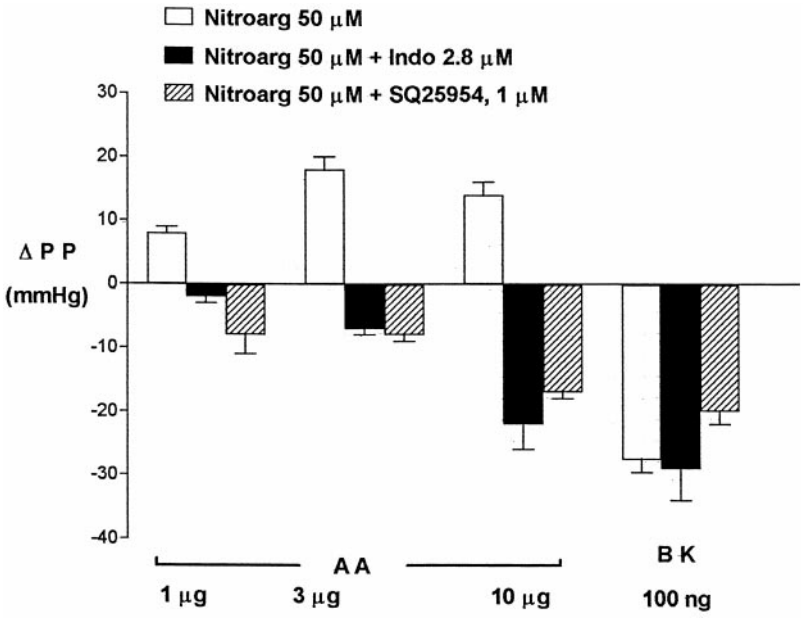

Fig. 2. Changes in perfusion pressure (PP) in nitroarginine-treated hearts in response to AA and bradykinin (BK) under control conditions (open bars) and after treatment with indomethacin (2.8 μm) to inhibit cyclooxygenase (solid bars) or SQ29548 (1 μm) to antagonize TP receptors (hatched bars).

conditions, 1, 3, and 10 μg AA increased perfusion pressure by 23 ± 8, 24 ± 7, and 10 ± 1 mm Hg, respectively. As before, there was evidence of tachphylaxis to the vasoconstrictor effect of AA.

Vascular responses in hearts constricted with endothelin

In hearts in which perfusion pressure was elevated with endothelin to 123 ± 3 mm Hg (n = 3), AA dose-dependently increased perfusion pressure (**Fig. 4**). In 2 out of 3 cases, the constrictor response to 10 μg AA was followed by a dilator response, mean 19 mm Hg. In the presence of indomethacin (n = 3), where perfusion pressure was elevated to 128 ± 4 mm Hg by endothelin, the vasoconstrictor response to AA was prevented and dose-dependent vasodilator responses were obtained (Fig. 4).

Vascular responses in hearts constricted with U46619

When vascular tone was elevated with U46619 to raise perfusion pressure to 131 ± 1 mm Hg (n = 5), AA did not cause vasoconstriction (**Fig. 5**) but dose-dependently reduced perfusion pressure (Fig. 5 and **Fig. 6**); in the presence of indomethacin (n = 5) where perfusion pressure was 127 ± 3 mm Hg, the vasodilator effect of AA was reduced, an effect that was slightly enhanced when nitro-

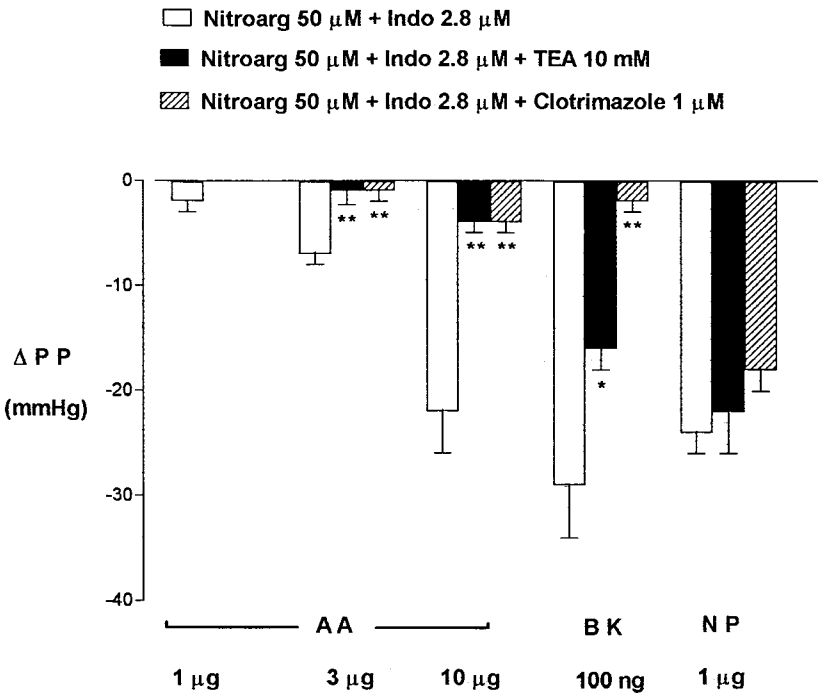

Fig. 3. Changes in perfusion pressure (PP) in nitroarginine-treated hearts in response to AA, bradykinin (BK), and nitroprusside (NP) in the presence of indomethacin (open bars), indomethacin plus TEA (10 mM; solid bars), and indomethacin plus clotrimazole (1 μM; hatched bars); * $P < 0.05$; ** $P < 0.01$.

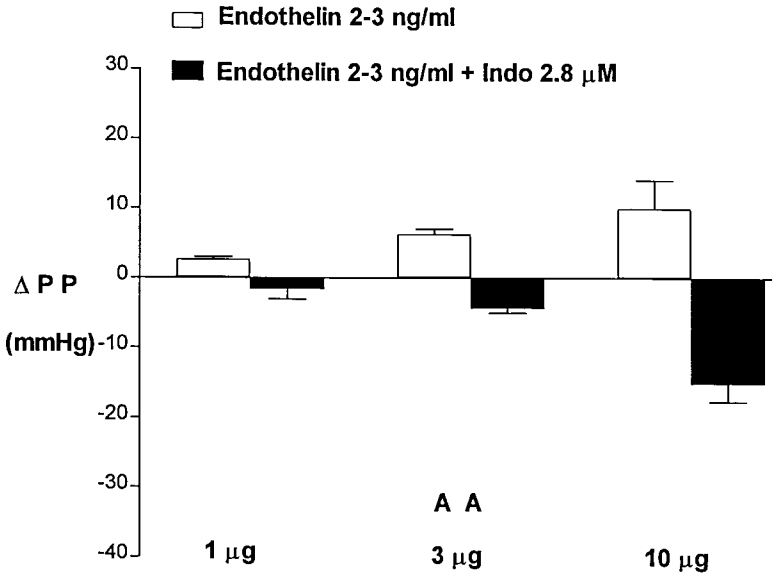

Fig. 4. Changes in perfusion pressure (PP) in response to arachidonic acid (AA) in the absence (open bars) and presence (solid bars) of indomethacin in hearts in which perfusion pressure was elevated with endothelin (2–3 ng/ml).

arginine was combined with indomethacin (n = 4; Fig. 6); perfusion pressure in this group was 145 ± 2 mm Hg. In the presence of nitroarginine alone (n = 5) where perfusion pressure was 130 ± 4 mm Hg, AA also dose-dependently lowered perfusion pressure (Fig. 6).

In the other series of experiments with hearts constricted with U46619, perfusion pressure in the control group (n = 5) was 137 ± 5 mm Hg compared to 127 ± 3 mm Hg in the clotrimazole-treated group (n = 4) and 141 ± 3 mm Hg in the endothelium-denuded group (n = 3) in which the concentration of U46619 added to the perfusate was increased from 10 to 20 ng/ml in two out of the three preparations to restore perfusion pressure to a similar level observed in the control group. Clotrimazole and removal of the endothelium almost abolished the vasodilator effect of bradykinin, showing the effectiveness of these interventions, whereas responses to nitroprusside were unaffected. However, clotrimazole reduced the dilator responses to AA by greater than 50% and removal of the endothelium almost abolished them (**Fig. 7**).

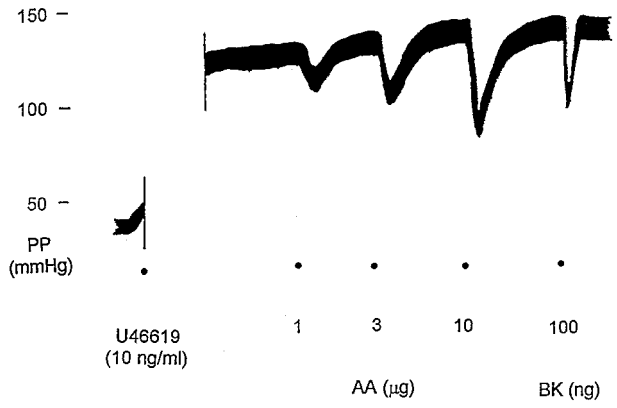

Fig. 5. Recording of perfusion pressure (PP) showing vasodilator responses to arachidonic acid (AA) and bradykinin (BK) in a heart in which perfusion pressure was elevated with U46619 (10 ng/ml).

Vascular responses under basal conditons

In hearts in which perfusion pressure was not pharmacologically elevated (n = 4), perfusion pressure was 94 ± 3 mm Hg and AA elicited vasodilator responses which were sometimes preceded by small, rapid vasoconstrictor responses. 1, 3, and 10 μg AA reduced perfusion pressure by 15 ± 4, 22 ± 10, and 41 ± 5 mm Hg, respectively, and bradykinin (30 ng) decreased perfusion pressure by 28 ± 4 mm Hg.

DISCUSSION

Several conclusions can be reached from this study. First, the coronary vascular response to AA, either dilation or constriction, is dependent on the prevailing tone. This is based on the observations showing that, under basal conditions, AA resulted in an initial vasoconstrictor effect followed by a more prolonged vasodilator effect similar to that previously reported by Shaffer, Cagen, and Malik (15) and Shaffer and Malik (17). However, when tone was elevated with either nitroarginine or endothelin, we observed a predominant vasoconstrictor effect that was sometimes preceded or followed by a vasodilator effect. Under these conditions, our results were similar to those reported by Belo and Talesnik (16) and Talesnik and Tsoporis (18). Thus, the results of this study provide some insight into the apparently discrepant results reported by Shaffer and Malik (17) and Belo and Talesnik (16) and suggest that the level of tone dictates the formation or activity of vasoconstrictor/vasoconstrictor prostanoids.

The second conclusion, that in hearts with elevated vascular tone the vasoconstrictor effect of AA is cyclooxygenase-dependent and mediated via activation of TP receptors, is based on results showing that the predominant vasoconstrictor effect observed in hearts with tone elevated by either nitroarginine or endothelin is abolished and reversed to a dilator effect by inhibition of cyclooxygenase

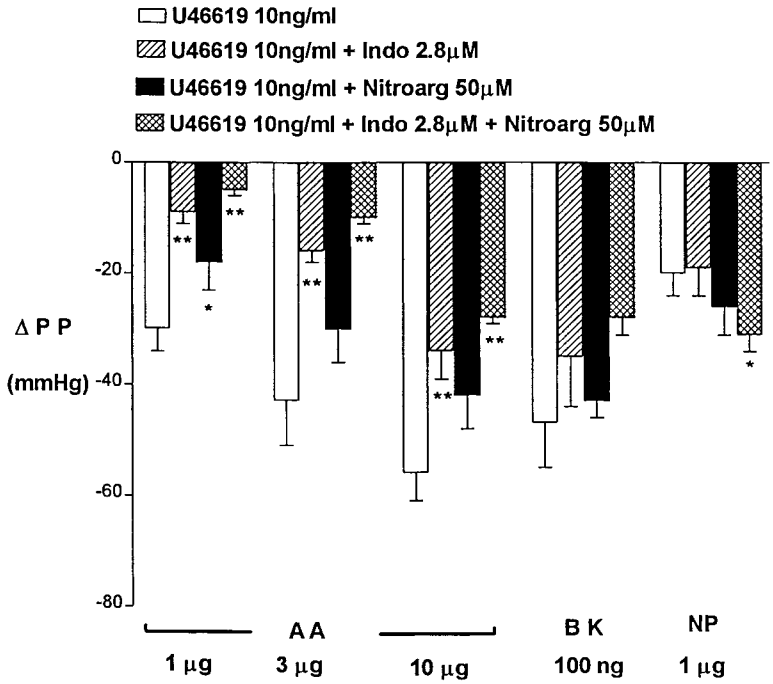

Fig. 6. Changes in perfusion pressure (PP) in response to arachidonic acid (AA), bradykinin (BK), and nitroprusside (NP) in the absence (open bars) and presence of indomethacin (hatched bars), nitroarginine (solid bars), and a combination of indomethacin and nitroarginine (cross-hatched bars) in hearts in which vascular tone was elevated with U46619; * $P < 0.05$; ** $P < 0.01$.

with indomethacin or antagonism of TP receptors with SQ 29548. These results are similar to those that we obtained in the isolated kidney (12) where the primary effect of AA is vasoconstriction regardless of the degree of tone.

The role of the endothelium in the coronary vasoconstrictor effect of AA in nitroarginine-treated hearts was not conclusively defined in these experiments. Thus, removal of the endothelium, which abolished dilator responses to bradykinin while leaving those to nitroprusside intact, reduced, but did not abolish, the vasoconstrictor effect of AA, suggesting that cyclooxygenase-dependent transformation of AA by the vascular smooth muscle may also contribute to the formation of constrictor prostanoids. This is a distinct possibility given the relative size of vascular smooth muscle compared to the endothelium and its ability to metabolize AA via cyclooxgenase. However, any inhibition of the vasoconstrictor effect of AA may be masked, to some degree, by the apparent development of tachyphylaxis which we also reported for the rat kidney (24) and which likely results from autoinactivation of cyclooxgenase upon repeated exposure to AA. Although

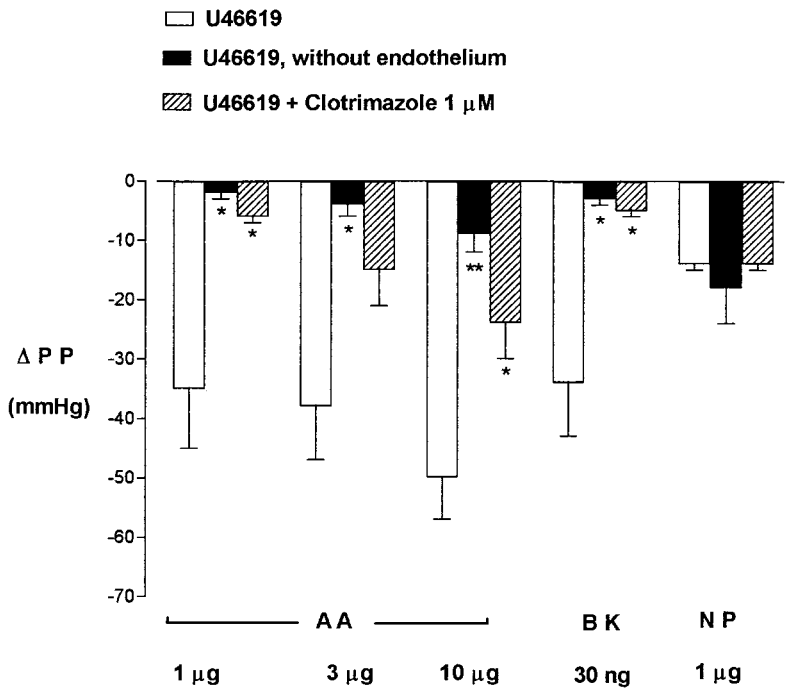

Fig. 7. Changes in perfusion pressure (PP) in response to AA, bradykinin (BK), and nitroprusside (NP) in hearts constricted with U46619 under control conditions (open bars) and after endothelial denudation (solid bars) or treatment with clotrimazole (1 μm; hatched bars); * $P < 0.05$; ** $P < 0.01$.

part of the coronary vasodilator effect of AA is also cyclooxygenase-dependent and may exhibit tachyphylaxis, this may be negated by the expression of a cytochrome P450-dependent component.

The cyclooxygenase-dependent vasoconstrictor component of the vascular effect of AA that is observed in hearts treated with nitroarginine may also be offset or moderated by a cytochrome P450-dependent mechanism. This premise is based on the results obtained with clotrimazole to inhibit cytochrome P450 and which enhanced the vasoconstrictor effect of the two lower doses of AA; no enhancement of the response to the highest dose of AA was observed because of the development of tachyphylaxis. However, this interpretation of these results requires caution as the greater vasoconstrictor effect of AA in nitroarginine-treated hearts in the presence of clotrimazole could also reflect a greater capacity for vasoconstriction as the perfusion pressure in this group was lower than that treated with nitroarginine alone.

The third conclusion is that the vasodilator effect of AA in nitroarginine-treated hearts that is uncovered by inhibition of cyclooxygenase is dependent on cytochrome P450. Thus, the cyclooxygenase- and NO-independent vasodilator effect of AA is virtually abolished by the cytochrome P450 inhibitor, clotrimazole, which also abolished the vasodilator effect of bradykinin that is attributed to the release of an EDHF. Furthermore, in hearts constricted with U46619 where only vasodilator responses to AA are apparent, clotrimazole reduces the response to AA. Although clotrimazole has been reported to inhibit K^+ channels, our earlier studies in the heart found no evidence of this. Thus, clotrimazole, at the concentration used here, was without effect on vasodilator responses to various agents that activate K^+ channels including cromakalim, SCA 40, NS 1619, and 5,6 EET (8, 19, 22, 25). Moreover, clotrimazole was without effect on vasodilator mechanisms in general as vasodilator responses to nitroprusside were unaffected. Our results showing a cytochrome P450-dependent component to the vasodilator effect of AA in the rat heart are in contrast to those reported by Adeagbo and Malik (13) for the rat mesentery and by Weintraub et al. (26) for porcine coronary arteries where no effect of inhibitors of cyclooxygenase, lipoxygenase, or cytochrome P450 was observed.

The fourth conclusion, that the cytochrome P450-dependent component of the vasodilator response to AA that is isolated by inhibition of cyclooxygenase and NO synthesis is dependent on activation of K^+ channels, is based on the results with TEA which is an inhibitor of all types of K^+ channels at the concentration used in this study (10 mM). The inhibitory effect of TEA confirms the results of an earlier study where we showed that charybdotoxin attenuates the vasodilator effect of AA in hearts treated with nitroarginine and indomethacin (14). As TEA results in a degree of inhibition of the vasodilator response to AA similar to clotrimazole, indicating that both agents are acting on the same pathway but at different levels, it is logical to conclude that a cytochrome P450-dependent product of AA elicits vasodilation by activating K^+ channels. Although a similar conclusion can be reached regarding the coronary vasodilator effect of bradykinin, we have no explanation as to why clotrimazole is a more effective inhibitor than TEA in this study; in an earlier study clotrimazole and TEA were equally effective as inhibitors of the coronary vasodilator effect of bradykinin (7). The type of K^+ channel involved in the vasodilator effect of AA was not determined in this study as we had previously shown that charybdotoxin attenuates the response to AA (14), suggesting a voltage-dependent or calcium-activated K^+ channel in contrast to an apamin- and glyburide-sensitive K^+ channel in the rat mesentery (13).

Last, we conclude that the vasodilator effect of AA is endothelium-dependent and exhibits at least two components that are cyclooxygenase- and cytochrome P450-dependent. Thus, removal of the endothelium from hearts constricted with U46619 almost abolished the vasodilator effect of both AA and bradykinin while not affecting that of nitroprusside, indicating that this intervention did not affect endothlium-independent vasodilator responses. Although the vasodilator component of the response to AA remaining after inhibition of cyclooxygenase and NOS is clearly dependent on cytochrome P450 and K^+ channels, the experiments where perfusion pressure was elevated with U46619 show that a substantial part of the vasodilator effect of AA is also dependent on cyclooxygenase activity as indomethacin reduced the response by approximately 50%. However, we confirmed the role of cytochrome P450 by showing that clotrimazole, in the absence of indomethacin, also reduced the response to AA. Thus, we would anticipate that the combination of indomethacin and clotrimazole or TEA would abolish the vasodilator effect of AA in U46619-constricted hearts. Moreover, under these conditions, the vasodilator activity of AA was greater than that observed in the experiments where both indomethacin and nitroarginine were included in the perfusate, a logical expectation if part of the dilator component is cyclooxygenase-dependent. However, it should be noted that in the series of experiments conducted with U46619 as the constricting agent, responses to bradykinin, but not those to nitroprusside, were also greater than those obtained in the experiments where nitroarginine was used to elevate perfusion pressure. The cyclooxygenase metabolite of AA that is responsible for part of the vasodilator activity of AA was not determined in this study although both PGI_2 and PGE_2 are potent coronary vasodilator agents with PGI_2 being the more potent (17). However, Gerritsen and Cheli (2) have shown that PGE_2 is the major prostaglandin formed by rabbit coronary microvessels although Shaffer and Malik (17) found higher levels of 6-keto $PGF_{1\alpha}$ in the coronary perfusate from the rat heart. Finally, although coronary vascular tone was elevated, a condition that seems to predispose to AA-induced vasoconstriction, only vasodilator responses were observed in the presence of U46619 as the constricting agent. This can be explained by U46619 occupying the TP receptor such that formation of PGH_2/TxA_2 from exogenous AA is insufficient to further elevate tone.

In summary, in the rat perfused heart, AA elicits vasodi-

lation or vasoconstriction depending on whether tone is elevated. The cyclooxygenase-dependent vasoconstriction is mediated via stimulation of TP receptors and can be reversed to a vasodilator effect by indomethacin. The vasodilator effect of AA which is endothelium-dependent, is partially dependent on cyclooxygenase activity but also exhibits a cytochrome P450-dependent component which is mediated via activation of K^+ channels.

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