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Role of non-nitric oxide non-prostaglandin endothelium-derived relaxing factor(s) in bradykinin vasodilation

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Abstract

The most conspicuous effect of bradykinin following its administration into the systemic circulation is a transient hypotension due to vasodilation. In the present study most of the available evidence regarding the mechanisms involved in bradykinin-induced arterial vasodilation is reviewed. It has become firmly established that in most species vasodilation in response to bradykinin is mediated by the release of endothelial relaxing factors following the activation of B_2-receptors. Although in some cases the action of bradykinin is entirely mediated by the endothelial release of nitric oxide (NO) and/or prostacyclin (PGI_2), a large amount of evidence has been accumulated during the last 10 years indicating that a non-NO/PGI_2 factor accounts for bradykinin-induced vasodilation in a wide variety of perfused vascular beds and isolated small arteries from several species including humans. Since the effect of the non-NO/PGI_2 endothelium-derived relaxing factor is practically abolished by disrupting the K^+ electrochemical gradient together with the fact that bradykinin causes endothelium-dependent hyperpolarization of vascular smooth muscle cells, the action of such factor has been attributed to the opening of K^+ channels in these cells. The pharmacological characteristics of these channels are not uniform among the different blood vessels in which they have been examined. Although there is some evidence indicating a role for K_{Ca} or K_V channels, our findings in the mesenteric bed together with other reports indicate that the K^+ channels involved do not correspond exactly to any of those already described. In addition, the chemical identity of such hyperpolarizing factor is still a matter of controversy. The postulated main contenders are epoxyeicosatrienoic acids or endocannabinoid agonists for the CB_1-receptors. Based on the available reports and on data from our laboratory in the rat mesenteric bed, we conclude that the NO/PGI_2-independent endothelium-dependent vasodilation induced by BK is unlikely to involve a cytochrome P450 arachidonic acid metabolite or an endocannabinoid agonist.

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- Prostaglandin
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Fifty years ago Professor M.O. da Rocha e Silva described a factor released from plasma globulins by trypsin or snake venom that caused hypotension in rabbits and cats (1). The purification and later synthesis of the factor now known as bradykinin gave a great impetus to the characterization of the full range of its pharmacological effects (2). Among these, the most conspicuous is the hypotensive effect when bradykinin is administered into the systemic circulation in all species studied. This hypotensive effect results mainly from a decrease in vascular resistance most probably by arteriolar dilation in several organs including heart, kidney, gut, liver and skeletal muscle (3,4).

The mechanism responsible for the arteriolar dilation induced by bradykinin remains to be fully elucidated and it may vary in different arteries of the same species or according to the size of the artery in the same organ or even the same artery in different species. Progress in the understanding of such a mechanism was hampered by the lack of consistent responses of isolated vessels to bradykinin and by the apparent paradoxical observation in most helically cut vascular strips of a vasoconstrictor or absent response in pre-1980 studies (2). It was only after Professor R. Furchgott (5) demonstrated the role of endothelial cells in mediating the acetylcholine-vasodilator effect, which was immediately extended to the action of bradykinin by Altura and Chand (6), that the functional integrity of the endothelium in isolated blood vessel preparations was considered to be crucial to obtain consistent responses. Furthermore, these observations established the notion that endothelial cells produce and release relaxant factors (EDRF) in response to a wide range of vasodilator agents (7). The identification of nitric oxide (NO), or a related compound derived from L-arginine, as the main EDRF together with the observation that bradykinin was capable of inducing the release of NO from endothelial cells in culture (8-10) led to the proposal that the vasodilator and hypotensive effects of bradykinin are mediated by the release of NO by endothelial cells. Although initial observations using L-arginine analogues that inhibit NO synthase showed that these compounds attenuated the hypotensive effect of bradykinin in normal rats (11), results from our laboratory showed that the magnitude of the hypotensive effect of bradykinin in conscious rat was unaltered by N^G-nitromonomethyl L-arginine administration, even at doses that raised mean arterial pressure by 50 mmHg (Figure 1). Similar findings were reported by other groups using other inhibitors of NO synthase (12-14). These observations led us to reinvestigate the role of NO in the endothelium-dependent relaxation induced by bradykinin in isolated resistance vessels of the rat employing the isolated and perfused mesenteric bed (15). This vascular bed was selected because it has been consistently reported that bradykinin infusion either *in vitro* or *in vivo* into the mesenteric circulation causes vasodilatation in rats (16-19), rabbits (20), dogs (21), cats (22), and humans (23). Firstly, we observed that in phenylephrine-preconstricted vessels bradykinin causes a dose-dependent relaxation at

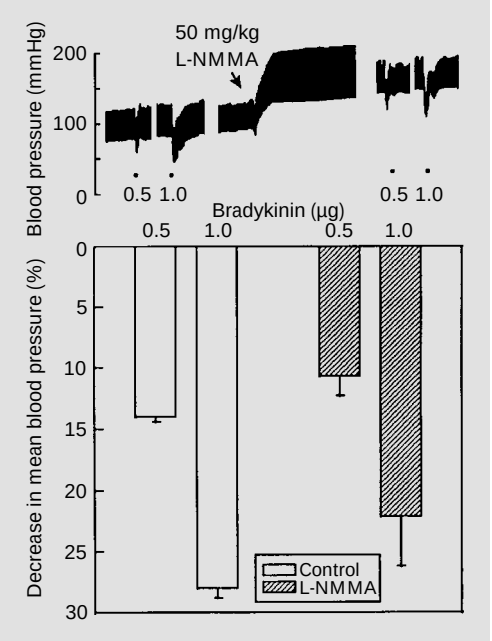

Figure 1 - Hypotensive effect of bradykinin (0.5 and 1.0 µg) before and after intravenous administration of the NO synthase inhibitor L-NMMA (50 mg/kg) in conscious normal male rats. The upper panel shows a representative tracing of directly recorded arterial blood pressure. The lower panel shows the mean ± SEM values (N = 4) of the hypotensive effect expressed as percentage of pre-bradykin injection mean blood pressure.

the lower doses and a biphasic response, relaxation followed by constriction, at the higher doses. The vasodilator effect was abolished by endothelial removal, while the vasoconstrictor one remained unaltered (15). These findings indicate that the vasodilator effect is indeed endothelium-dependent, whereas the vasoconstrictor effect most likely results from a direct action on smooth muscle and involves prostaglandin synthesis since indomethacin, an inhibitor of cyclooxygenase, abolished it and prolonged the vasodilator effect (Figure 2). In addition, we observed that the magnitude of the vasodilator effect was not affected by indomethacin or N^G-nitro L-arginine, an inhibitor of NO synthase, but was abolished by the B_2-receptor antagonist HOE-140, indicating that it depends on the activation of kinin B_2-receptors, but does not involve NO or prostaglandin synthesis.

B_2-receptor activation in cultured endothelial cells has been shown to augment the release of both NO and prostacyclin (24). In addition, earlier reports have shown that cyclooxygenase inhibitors impaired the vasodilator action of bradykinin in the rat skeletal muscle circulation as well as in the isolated rabbit heart (25,26) and in rabbit and cat superior mesenteric arteries (27). On the other hand, bradykinin-induced vasodilation of the mesenteric bed *in vivo* in anesthetized rats has been shown to be attenuated by N^G-nitro L-arginine (19). The results of our experiments (Figure 3) are in apparent conflict with these reports but are entirely consistent with more recent studies showing that in guinea pig and rat isolated heart, in rat isolated kidney, and in the intact dog and human coronary circulation bradykinin-induced vasodilation is partially or totally resistant to NO or prostaglandin synthesis inhibitors (28-32). Furthermore, bradykinin-induced relaxation independent of the release of prostanoid and NO has been described in porcine, bovine and human coronary arteries (33-35) and human small omental arteries (36).

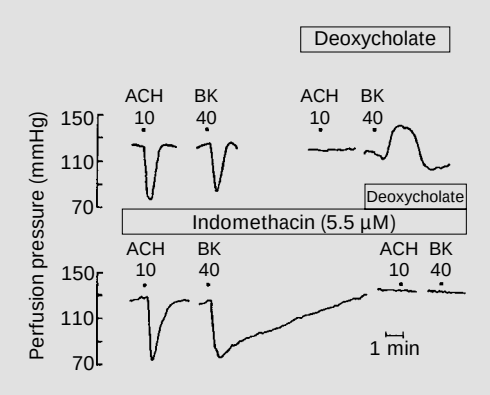

Figure 2 - Typical recordings show examples of the effect of endothelium removal with sodium deoxycholate on the response to acetylcholine (ACH; 10 ng) and bradykinin (BK; 40 ng) in preconstricted rat isolated mesenteric arterial bed perfused with Krebs' solution (top panel) or Krebs' solution containing the cyclooxygenase inhibitor indomethacin (bottom panel).

In rings of pig, canine and human coronary as well as human gastroepiploic arteries, bradykinin elicited transitory hyperpolarization of smooth muscle cells which was endothelium dependent (37-40). Although NO and prostacyclin are capable of causing smooth muscle hyperpolarization in some vessels (41-44), most of the endothelium-dependent smooth muscle hyperpolarization induced by vasodilator agents is unaffected by NO synthase or cyclooxygenase inhibitors (45). Thus, the existence of a still chemically unidentified endothelium-derived hyperpolarizing factor (EDHF) has been postulated. The hyperpolarization of vascular smooth muscle cells induced by EDHF has been attributed to an increase in smooth muscle membrane K^+ conductance since it is impaired by disruption of the K^+ electro-

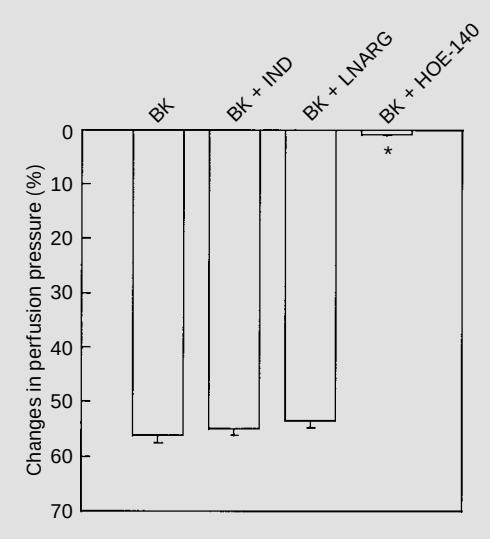

Figure 3 - Vasodilator effect of bradykinin (BK; 40 ng) in the absence or in the presence of the cyclooxygenase inhibitor indomethacin (BK + IND; 5.5 µM), or the NO synthase inhibitor N^G-nitro-L-arginine (BK + LNARG; 200 µM), or the B_2-kinin receptor antagonist HOE-140 (BK + HOE-140; 250 nM). Values (mean ± SEM) represent the percent decrease in mesenteric perfusion pressure (N = 4-6). *P<0.05 *vs* BK (Student *t*-test).

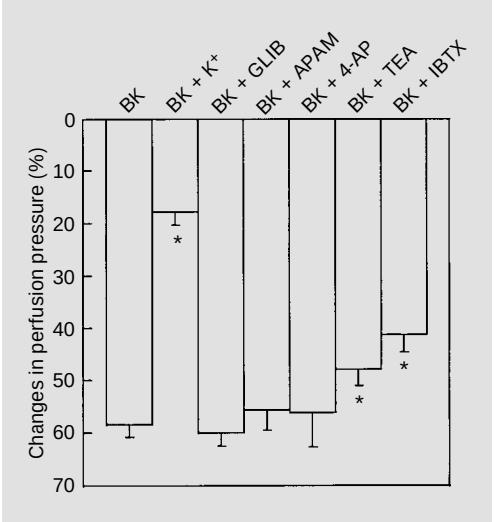

Figure 4 - Vasodilator effect of bradykinin (BK; 40 ng) in the absence or in the presence of the potassium channel blockers glibenclamide (BK + GLIB; 3 µM), apamin (BK + APAM; 1 µM), 4-aminopyridine (BK + 4-AP; 1 mM), tetraethylammonium (BK + TEA; 3 mM), iberiotoxin (BK + IBTX, 100 nM) or high K^+ (BK + K^+; 47 mM). Values (mean ± SEM) represent the percent decrease in mesenteric perfusion pressure (N = 4-6). *P<0.05 *vs* BK (Student *t*-test).

chemical gradient (46). Consequently, we determined the effect of disruption of the K^+ gradient on bradykinin-induced vasodilation in the rat mesenteric arteries. Increasing extracellular K^+ concentration to 40 mM reduced the magnitude of the vasodilatory response by 80% (Figure 4), suggesting that in this vascular bed the NO- and prostaglandin-independent vasodilator effect of bradykinin results from an increase in K^+ conductance. The potassium channels involved in these responses could be located either in smooth muscle or in endothelial cells and their identity remains to be fully defined (47). The magnitude of NO/prostanoid-independent vasodilation induced by acetylcholine in the rabbit abdominal aorta and carotid artery (48) or by bradykinin in rat isolated and perfused heart and kidney (49,50) has been shown to be reduced by charybdotoxin (an inhibitor of large conductance BK_{Ca} and also some K_V channels). On the other hand, apamin (an inhibitor of small conductance K_{Ca} channels) has been shown to inhibit NO-independent vasodilation induced by bradykinin in porcine coronary arteries (51). Glibenclamide (an inhibitor of K_{ATP} channels) has been shown to inhibit NO- and prostaglandin-independent relaxation of rabbit abdominal aorta (48). Other studies, however, have reported conflicting observations (47) suggesting that several pharmacologically distinct K^+ channels are involved in the NO/prostanoid-independent endothelium-dependent vascular smooth muscle hyperpolarization and/or relaxation. In order to characterize the K^+ channel underlying this response we proceeded to test the effect of selective K^+ channel blockers such as glibenclamide, apamin, 4-aminopyridine, and iberiotoxin, as well as the rather nonselective K^+ channel blocker tetraethylammonium. At the concentrations employed, which have been shown to be fully effective in other studies, only iberiotoxin and tetraethylammonium were able to reduce, and only partially, the magnitude of bradykinin-induced vasodilation (Figure 4). These data suggest that part of the vasodilation depends on the activation of BK_{Ca} channels; the remaining vasodilation most probably reflects the activity of other K^+ channels with a unique pharmacology. Since K^+ channels are oligomeric structures, we cannot discard the possibility that a single K^+ channel containing an iberiotoxin-sensitive subunit underlies the vasodilation induced by bradykinin. Indeed, K^+ channels mediating the hyperpolarization induced by acetylcholine-released EDHF in the rat hepatic artery as well as in the guinea pig carotid artery exhibit pharmacological properties distinct from those of the already described K^+ channels (44,52,53).

Regardless of the precise nature of the K^+ channel involved in endothelium-dependent hyperpolarization, it has been postulated that endothelium-dependent relaxation resistant to NO synthase and cyclooxygenase inhibitors might be mediated by epoxyeicosatrienoic acids (EET), metabolites of arachidonic acid formed by the action of cytochrome P450 enzymes, or by endogenous cannabinoid agonists such as anandamide (47,51, 54). Therefore, we determined the effect of two rather nonselective inhibitors of cytochrome P450 monooxygenases, SKF525A (proadifen) and 7-ethoxyresorufin, as well as the alleged selective EET synthesis inhib-

itor clotrimazole. In addition, we also determined the effect of the cannabinoid CB_1-receptor antagonist SR141716A (55) on bradykinin-induced vasodilation of the rat mesenteric bed (Figure 5). As can be observed in the figure, clotrimazole was the only agent capable of reducing the magnitude of bradykinin-induced vasodilation. This effect of clotrimazole is unlikely to have resulted from an inhibition of cytochrome P450 monooxygenases, since the other two inhibitors of these enzymes failed to influence bradykinin response. Although we cannot rule out that a P450 enzyme insensitive to SKF525A or ethoxyresorufin is involved, it is worth noting that in these experiments the two inhibitors were able to reduce the magnitude of acetylcholine-induced vasodilation (15). This interpretation is entirely consistent with recent reports showing that the endothelium-dependent hyperpolarization of the rat mesenteric arteries is not mediated by cytochrome P450 metabolites of arachidonic acid (56,57). Since clotrimazole has been shown to block K^+ channels in different cells, including vascular smooth muscle cells (58), it is likely that the effect of clotrimazole resulted from a blockade of potassium channels.

In conclusion, the reviewed evidence suggests that bradykinin is capable of inducing endothelial cells to release at least three relaxant mediators: prostacyclin, NO and EDHF. The contribution of each of these mediators to the relaxant effect of bradykinin is determined by the species, blood vessel and possibly by the contractile agent employed. Our observations suggest that NO does not appear to be the major EDRF mediating the mesenteric vasodilation and the hypotensive effect of bradykinin in normal rats. In addition, these findings also suggest that the potassium channel involved in the vasodilator effect exhibits pharmacological properties different from the currently described voltage- and calcium-dependent potassium channels. Finally, the EDHF involved in the rat mesenteric bed vasodilation induced by BK appears not to be a cytochrome P450 metabolite or endocannabinoid agonist for the CB_1 receptor.

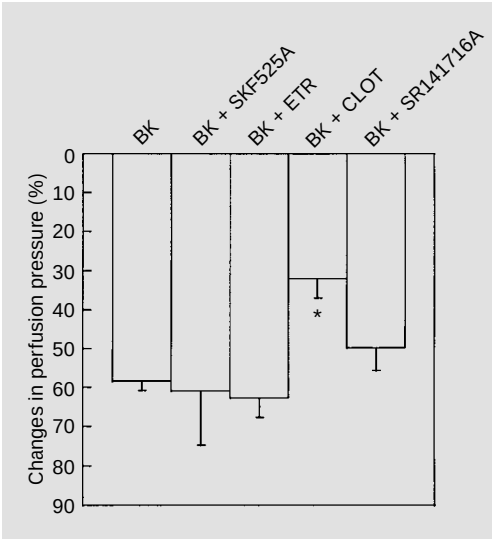

Figure 5 - Vasodilator effect of bradykinin (BK; 40 ng) in the absence or in the presence of the cytochrome P450 enzymes SKF525A (BK + SKF525A; 5 µM), ethoxyresorufin (BK + ETR; 5 µM), clotrimazole (BK + CLOT; 5 µM), or the CB_1-cannabinoid receptor antagonist SR141716A (BK + SR141716A; 1 µM). Values (mean ± SEM) represent the percent decrease in mesenteric perfusion pressure (N = 4-6). *P<0.05 *vs* BK (Student *t*-test).

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