Bradykinin-induced vasoconstriction of rat mesenteric arteries precontracted with noradrenaline

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- 1 Administration of bradykinin caused dose-dependent vasoconstriction in rat isolated perfused mesenteric arteries precontracted with noradrenaline.
- 2 The vasoconstrictor response was not mediated by BK₁-bradykinin receptors.
- 3 Inhibition of cyclo-oxygenase with indomethacin, aspirin or meclofenamate abolished the vasoconstrictor effect of bradykinin, showing that a member of the arachidonic acid cascade may be involved.
- 4 Inhibitors of thromboxane synthesis (imidazole and UK 38485) did not affect or only reduced the bradykinin-induced vasoconstriction.
- 5 The endoperoxide H₂/thromboxane A₂ receptor antagonist SQ 29548 significantly reduced the vasoconstrictor effect of bradykinin, but did not affect the vasoconstrictor response to noradrenaline, adrenaline, vasopressin, 5-hydroxytryptamine or prostaglandins.
- 6 The eicosanoid(s) that mediate bradykinin-induced vasoconstriction appear to be synthesized outside the arterial endothelium.
- 7 The data suggest that the vasoconstrictor effect of bradykinin in the rat isolated mesenteric artery is mediated by vasoconstrictor arachidonic acid metabolites including the cyclic endoperoxides and/or the thromboxanes.

Introduction

Bradykinin is a nonapeptide with potent vascular actions mediating systemic vasodilatation and a fall in blood pressure. A number of studies, however, have shown that bradykinin may also increase arterial pressure or cause a biphasic response. In most arterial vessels bradykinin induces relaxation (see Regoli, 1984). In the rat mesenteric vascular bed bradykinin relaxes the arteries and constricts the veins (Northover & Northover, 1970). In rabbit isolated kidney and ear arteries bradykinin induces vasoconstriction (Barabe *et al.*, 1979). Despite the very well known vasorelaxant effects of kinins, the nature and role of their vasoconstrictor effect in the regulation of vascular tone is far from clear.

In the present study, in the rat isolated perfused mesenteric arterial preparation precontracted with noradrenaline and perfused with a modified Tyrode solution, we found that bradykinin induces vasoconstriction. The bradykinin-induced vasoconstriction was abolished by use of cyclo-oxygenase inhibitors. Because the metabolism of arachidonic acid via the cyclo-oxygenase pathway results in the formation of various eicosanoids that are able to cause vasoconstriction, these are candidates for mediating the vasoconstrictor effect of bradykinin. To test this hypothesis we examined the vasoconstrictor effect of bradykinin in the isolated, precontracted mesenteric vessels of the rat in the presence and in the absence of cyclo-oxygenase inhibitors, thromboxane synthesis inhibitors and a thromboxane A₂/endoperoxide H₂ receptor antagonist.

Methods

The vascular bed of the superior mesenteric artery of the rat was perfused with a modified Tyrode solution by a peristaltic pump as described by McGregor (1965); the mesentery was cut near the intestinal border. Since the output of the pump was constant, changes in perfusion pressure reflect changes in the vascular tone of these vessels. The output of the pump was kept at 3–4 ml per min to maintain a perfusion pressure of about 12 mmHg. The perfusion pressure was measured with a Statham transducer (P2 3AC) and an electronic recorder.

The composition of the perfusing solutions was as follows in mm: NaCl 134, KCl 5, CaCl₂ 0.6, Na₂HPO₄ 5, NaH₂PO₄ 0.5 and glucose 5. Osmolarity was 285 mOsmol l⁻¹ and pH 7.45. The perfusing solution was gassed with oxygen and maintained at 38°C with a thermostatic circulating bath and the vascular preparation was placed on a heated table.

Experiments were carried out after the vessels had been perfused for one h to allow stabilization of the responses. The agonists were dissolved in the solution used for perfusion and a 50 µl bolus injected rapidly with a microsyringe through the cannula inserted in the artery. The injection of 50 µl of the saline solution produced a volume artifact not followed by any vasomotor response. The sensitivity of preparations from different rats differed widely, but in the same preparation sensitivity to any given agonist was usually maintained within fairly constant limits, although after 4 or 5 h of perfusion the reactivity increased. Noradrenaline (0.25 ml min⁻¹ of a 3 × 10⁻⁵ M solution) was added to the perfusing solution with a Harvard pump.

Drugs used were: (–)-noradrenaline D-bitartrate (NA) (Sterling-Winthrop); (–)-adrenaline D-bitartrate (Ad) (Sigma); 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma); arginine vasopressin (Sigma); bradykinin triacetate (BK) (Sigma); prostaglandins E₁, E₂, F_{2α} (Sigma); imidazole (Sigma); indomethacin (Sigma); sodium meclofenamate (Parke-Davis); UK 38485 (3-1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid, Pfizer); Aspirin (Bayer); SQ 29548 (1S-[1α, 2β(5Z), 3β, 4α]]-7-[3-[2-[(phenylamino)-carbonyl]hydrazinomethyl] - 7 - oxabicyclo(2.2.1)hept - 2 - yl] - 5 - heptenoic acid, Squibb). The reagents used for preparing the solutions were of analytical grade. The bradykinin BK₁-receptor antagonist was kindly provided by Dr Regoli.

Analysis of variance and test of multiple comparison were done according to Scheffe (1963).

Results

Bradykinin (10⁻⁶–10⁻⁴ M), injected in the cannula inserted in the mesenteric artery was without vascular effects when the preparation was perfused with the modified Tyrode solution. After addition of noradrenaline (10⁻⁷ M) to the Tyrode solution, which increased the perfusion pressure from 12–15 mmHg to 40–80 mmHg, bradykinin induced

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vasoconstrictor responses that were dose-dependent from 10^{-6} M to 10^{-4} M (Figure 1). Although the vasoconstrictor responses were always present, the magnitude of the response varied with different preparations. Successive injections usually produced decreasing responses. The perfusion pressure increased slowly to reach a peak about 2 min after the injection and remained elevated for 4 to 6 min (Figure 1). Vasoconstrictor responses were usually preceded by a small vasodilatation.

The vasoconstriction did not seem to be mediated through BK_1 -receptors since the BK_1 -receptor antagonist [Leu⁸], des Arg-bradykinin, did not affect the bradykinin-induced vasoconstriction. In five experiments bradykinin 10^{-5} M increased the perfusion pressure of the mesenteric arteries by 13.2 ± 2.1 mmHg, while after injection of the BK_1 -receptor antagonist in doses as high as $200 \mu\text{g ml}^{-1}$ the increase was 13.8 ± 4.1 mmHg.

Inhibition of cyclo-oxygenase

The delay and the slow rise of the vasoconstrictor response to bradykinin suggested that a locally generated mediator might

Figure 1 Effect of noradrenaline (NA) on bradykinin (BK)-induced vasoconstriction. (a) Mesenteric arteries perfused with Tyrode solution. Vessels were relaxed and the perfusion pressure was 19 mmHg. The spikes were artifacts caused by the volume of fluid injected. (b) Effect of BK after the addition of NA to the perfusing solution (increases the perfusion pressure to 80 mmHg).

be involved. Bradykinin is known to release prostaglandins in blood vessels, so we tested the effects of cyclo-oxygenase inhibitors on the response to bradykinin. After the mesenteric arteries had been precontracted with noradrenaline, bradykinin and noradrenaline were injected before and after the vessels had been perfused with indomethacin (10^{-4} M) over 15 min.

Figure 2 shows that indomethacin abolished the bradykinin-induced vasoconstriction without a significant reduction in the noradrenaline-induced vasoconstriction, thus indicating that vascular reactivity had been preserved. No recovery of the bradykinin-induced vasoconstriction was observed 1 h after the indomethacin perfusion had ceased. Meclofenamate (10^{-6} M) perfused over 15 min was also able to inhibit bradykinin-induced vasoconstriction without affecting the vasoconstriction to noradrenaline (Figure 2). Aspirin (3×10^{-3} M) was also able to inhibit bradykinin-induced vasoconstriction. The responses partially recovered one h after the aspirin perfusion had finished.

Role of endothelium

To find out whether the endothelium was responsible for the release of the vasoconstrictor eicosanoid, the endothelial lining of the mesenteric artery was removed either by using distilled water or alcohol. Distilled water was perfused through the mesenteric arteries for five min and the vasodilator effect of acetylcholine (10^{-4} M) compared before and after treatment. The disruption of the endothelium was considered complete when acetylcholine no longer induced vasodilatation and the vasoconstrictor responses to noradrenaline and other agonists were maintained without significant reductions (Figure 3). Under these conditions bradykinin-induced vasoconstriction was not significantly reduced. Similar results were obtained after the endothelial layer had been destroyed by one or two injections of $250 \mu\text{l}$ of 96% alcohol into the cannula inserted in the mesenteric artery (Figure 3).

Effects of different eicosanoids

To see whether products of the arachidonic acid cascade could induce vasoconstrictor responses in the mesenteric vessels, bolus doses of prostaglandin E_1 (PGE_1), PGE_2 and $\text{PGF}_{2\alpha}$ were injected in concentrations ranging from 10^{-7} M to 10^{-4} M. No vasoconstrictor responses were elicited when the mesenteric vessels were perfused with Tyrode solution, but

Figure 2 Inhibition of the bradykinin (BK)-induced vasoconstriction by indomethacin (b), meclofenamate (c) and aspirin (d). (a) A record of one experiment showing that bradykinin vasoconstriction was abolished by indomethacin while that to noradrenaline (NA) was unaffected. Shaded columns, responses to NA; stippled columns, responses to BK. ** $P < 0.001$, * $P < 0.01$.

Figure 3 Effect of disruption of the endothelial layer on responses of mesenteric arteries to various substances. After the injection of alcohol (a and c) acetylcholine vasodilation was abolished while the response to bradykinin was little affected. Similar results after distilled water (b and d). ACh = acetylcholine 10^{-4} M, BK = bradykinin 4×10^{-5} M and NA = noradrenaline. Shaded columns, responses to NA; hatched columns, responses to ACh; stippled columns, responses to BK. ** $P < 0.001$.

after the addition of noradrenaline to the perfusion fluid, these agents induced a dose-dependent vasoconstriction (Figure 4).

Effect of thromboxane A_2 /endoperoxide H_2 receptor blockade

To test whether thromboxane A_2 and/or endoperoxide H_2 were involved in the vasoconstriction induced by bradykinin, SQ 29548 a potent blocker of their receptors was tested in 15

experiments (Figure 5). SQ 29548 ($0.4 \text{ mg } 100 \text{ ml}^{-1}$) significantly ($P < 0.001$) reduced bradykinin-induced vasoconstriction. As previously shown (Figure 4) PGE_1 , PGE_2 and $\text{PGF}_{2\alpha}$ were able to constrict the mesenteric arteries precontracted with noradrenaline, thus it was possible that they were mediators of bradykinin vasoconstriction. To examine this, the effect of SQ 29548 was tested. As shown in Figure 6 a bolus dose of $0.04 \text{ mg } 100 \text{ ml}^{-1}$ that abolished bradykinin-induced vasoconstriction did not reduce the vasoconstriction induced by these prostaglandins, showing that different receptors are involved.

Effect of thromboxane A_2 synthesis inhibitors

Figure 7 shows the effect on bradykinin vasoconstriction of UK 38485 and imidazole, two well known inhibitors of thromboxane A_2 (TXA_2) synthesis. A solution of UK 38485

Figure 4 Vasoconstriction induced by (a) bradykinin (BK) and (b, c and d) prostaglandins. These drugs were without a vasoconstrictor effect before the mesenteric arteries were perfused with noradrenaline (NA). When the vessels were precontracted with NA dose-dependent vasoconstriction was induced by prostaglandin E_1 (PGE_1), PGE_2 , $\text{PGF}_{2\alpha}$ and bradykinin. Arrows show lack of response before the addition of NA and stippled columns, responses after the addition of NA to the perfusing solution.

Figure 5 Effect of thromboxane A_2 /endoperoxide H_2 receptor blockade. SQ 29548 significantly reduced bradykinin-induced vasoconstriction (stippled columns) while that to noradrenaline (shaded columns) was unaffected. ** $P < 0.001$.

Figure 6 Effect of thromboxane A_2 /endoperoxide H_2 receptor blockade on prostaglandin vasoconstriction. By perfusing with SQ 29548 solution, bradykinin (BK) vasoconstriction was almost completely abolished, while noradrenaline (NA); prostaglandin E_1 (PGE_1), PGE_2 - and $PGF_{2\alpha}$ -induced vasoconstrictor responses were not significantly reduced. Open columns, responses before the addition of SQ 29548; stippled columns, responses after the addition of SQ 29548; $n = 6$. * $P < 0.01$.

(0.8 mg 100 ml⁻¹) perfused through the mesenteric vessels for 15 min reduced bradykinin vasoconstriction, which partially recovered 30 min after the administration of the drug had ceased. On the other hand, imidazole (5×10^{-4} M) after 1 h of perfusion did not significantly reduce bradykinin-induced vasoconstriction.

Discussion

In the present study we investigated the mechanism of the vasoconstrictor effect of bradykinin on the mesenteric arteries of the rat precontracted with noradrenaline. Bradykinin is known to dilate most arteries (Regoli, 1984), although in rabbit isolated kidney and ear arteries it causes vasoconstriction (Barabé *et al.*, 1979).

The mechanism by which bradykinin induces vasodilatation or vasoconstriction on different vascular beds is unknown. The first step in the action of bradykinin involves the binding of bradykinin to specific receptors. Two classes of bradykinin receptors have been identified. BK_1 -receptors mediate contraction of large arteries and veins and relaxation of peripheral resistance vessels. BK_2 -receptors mediate relaxing effects on isolated arteries of the dog and others species, and contraction of veins and resistance arteries (Regoli & Barabé, 1980). In our experiments the use of a BK_1 -receptor antagonist did not change the bradykinin-induced vasoconstriction in the mesenteric arteries, suggesting that BK_1 -receptors are not involved.

Bradykinin is known to release prostaglandins in various tissues including veins and arteries. Bradykinin has been shown to release arachidonic acid in isolated tissues from

rabbit (Needleman *et al.*, 1978). The possibility that bradykinin-induced vasoconstriction was mediated by products of the arachidonic cascade was investigated here. Inhibition of cyclo-oxygenase by indomethacin, meclofenamate and aspirin abolished bradykinin-induced vasoconstriction, strongly suggesting that it was mediated by prostaglandins.

The metabolism of arachidonic acid via the cyclo-oxygenase pathway results in the formation of various eicosanoids among them PGE_1 , PGE_2 , $PGF_{2\alpha}$, thromboxane A_2 and endoperoxide H_2 which cause vasoconstriction in various arteries (Toda & Miyazaki, 1978; van Holst *et al.*, 1982; Hayashi *et al.*, 1986; Lüscher & Vanhoutte, 1986).

In order to characterize the eicosanoid that mediates bradykinin-induced vasoconstriction we tested the possibility that PGE_1 , PGE_2 and $PGF_{2\alpha}$, which produced vasoconstriction in our preparation, contributed to the bradykinin response. In one series of experiments we determined that SQ 29548, a potent antagonist of thromboxane A_2 /endoperoxide H_2 receptors, greatly reduced the bradykinin-induced vasoconstriction while the pressor effect of PGE_1 , PGE_2 and $PGF_{2\alpha}$ was only slightly affected. In other experiments, treatment with UK 38485 an inhibitor of thromboxane synthetase, reduced but did not abolish the bradykinin vasoconstriction and imidazole, another inhibitor of thromboxane synthesis, was without effect on bradykinin-induced vasoconstriction. One interpretation of these findings is that in mesenteric arteries the vasoconstrictor effect of bradykinin is mainly mediated by endoperoxides. The application of exogenous prostaglandin endoperoxides causes contraction of isolated vascular smooth muscle via an interaction with receptors which are shared with thromboxane A_2 (Coleman *et al.*, 1981; Saussy *et al.*, 1985). As suggested by the results of the present study, prostaglandin endoperoxides formed endogenously by bradykinin may stimulate the contraction of vascular smooth muscle. However, we cannot exclude the participation of thromboxane A_2 and other prostaglandins. Pertaining to this point, the results of a recent study suggest that prostaglandin endoperoxides contribute to the high blood pressure in rats made hypertensive by high salt intake and chronic infusion of angiotensin II (Mistry & Nasjletti, 1988).

In our experiments bradykinin was found to be without vascular effects on the relaxed branches of the mesentery artery, while it induced a dose-dependent vasoconstriction when these vessels were precontracted with noradrenaline. The question arises as to whether bradykinin releases the prostanoid only when the arteries are contracted with noradrenaline, or whether the prostanoid released is vasoconstrictor only in the presence of noradrenaline. Our data show that PGE_1 , PGE_2 and $PGF_{2\alpha}$ constrict the mesenteric arteries only after they have been precontracted with noradrenaline, suggesting that the second alternative may be correct.

We tried to find out whether the synthesis of the eicosanoid mediating bradykinin-induced vasoconstriction occurs in the endothelium of these arteries. We compared the bradykinin-induced vasoconstriction in rat mesenteric arteries with intact endothelium to that in arteries after endothelium removal with distilled water or alcohol. The endothelium is not involved since bradykinin-induced vasoconstriction was not abolished after the endothelium was removed. Although we do not know in what cells synthesis of the vasoconstrictor prostanoids occurs, it seems that smooth muscle cells may be the target of bradykinin. In dog isolated arteries (coronary, coeliac, femoral, splenic, mesenteric, gastric) precontracted with noradrenaline, bradykinin induces a relaxation response not blocked by indomethacin which appears to be endothelium-mediated, while in cats and rabbits the relaxation does not depend on the endothelium and is blocked by cyclo-oxygenase inhibitors (Cherry *et al.*, 1982). On the other hand, in rabbit arteries, the relaxation induced by bradykinin does not depend on the endothelium and is blocked by cyclo-oxygenase inhibitors (Toda, 1974; Regoli *et al.*, 1982). This appears to indicate that in rabbit arteries the vasodilator prostanoid released by bradykinin may be formed outside the

Figure 7 Effect of thromboxane A_2 synthesis inhibitors. (a) UK 38485 significantly reduced bradykinin (BK)-induced vasoconstriction. (b) Imidazole did not change significantly bradykinin-induced vasoconstriction. Shaded columns, responses to noradrenaline; stippled columns, responses to BK. ** $P < 0.001$.

endothelium, probably in smooth muscle. In cultured mesenteric smooth muscle cells bradykinin increases the synthesis of 6-keto PGE₁, a metabolite of PGI₂, and the effect is inhibited by acetylsalicylic acid (Takeuchi *et al.*, 1988).

In summary, this study demonstrates that bradykinin causes dose-dependent constriction of the rat mesenteric arteries precontracted with noradrenaline. The vasoconstrictor responses are not mediated by BK₁-receptors. Inhibition of cyclo-oxygenase abolishes the vasoconstrictor effect of bradykinin. The eicosanoid(s) responsible is formed outside

the endothelium. We conclude that cyclic endoperoxides and/or thromboxanes appear to mediate bradykinin-induced vasoconstriction, although the participation of other eicosanoids cannot be excluded.

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References

- BARABE, J., MARCEAU, F., THERIAULT, B., DROUIN, J.N. & REGOLI, D. (1979). Cardiovascular actions of kinins in the rabbit. *Can. J. Physiol. Pharmacol.*, **57**, 78–91.
- CHERRY, P.P., FURCHGOTT, R.F., ZAWADZKI, J.V. & JOTHIANANDAS, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Physiological Sciences*, **79**, 2106–2110.
- COLEMAN, R., HUMPHREY, P., KENNEDY, I., LEVY, G. & LUMLEY, P. (1981). Comparison of the actions of UK 6619, a prostaglandin H₂ analogue with those of prostaglandin H₂ and thromboxane H₂ on some isolated smooth muscle preparations. *Br. J. Pharmacol.*, **73**, 773–778.
- HAYASHI, S., PARK, M.K. & KUEHL, J.J. (1986). Effect of prostaglandins and arachidonic acid on baboon cerebral and mesenteric arteries. *Prostaglandins*, **32**, 587–595.
- LÜSCHER, T.T. & VANHOUTTE, P.M. (1986). Endothelium dependent contraction to acetylcholine in the aorta of spontaneously hypertensive rats. *Hypertension*, **8**, 344–348.
- MISTRY, M. & NASJLETTI, A. (1988). Role of pressor prostanoids in rats with angiotensin II-salt-induced hypertension. *Hypertension*, **11**, 758–762.
- MCGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177**, 21–30.
- NEEDLEMAN, P., BRONSON, S.D., SYCHE, A., SIVAKOFF, M. & NICOLAU, K.C. (1978). Cardiac and renal prostaglandin I₂ biosynthesis and biological effect on isolated perfused rabbit tissue. *J. Clin. Invest.*, **61**, 839–849.
- NORTHOVER, R.M. & NORTHOVER, B.J. (1970). The effect of vasoactive substances on rat mesenteric blood vessels. *J. Pathol.*, **101**, 99–108.
- REGOLI, D. (1984). Neurohumoral regulation of precapillary vessels. The kallikrein-kinins systems. *J. Cardiovasc. Pharmacol.*, **6**, (Supp. 2), S401–S412.
- REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.
- REGOLI, D., MIZRAHI, J., D'ORLEANS-JUSTE, P. & CARANIKAS, S. (1982). Effects of kinins on isolated blood vessels. Role of endothelium. *Can. J. Physiol. Pharmacol.*, **60**, 1580–1583.
- SAUSSY, D., MAIS, D., KNAPP, D. & HALUSHKA, P.V. (1985). Thromboxane A₂ and prostaglandin endoperoxide receptors in platelets and vascular smooth muscle. *Circulation*, **72**, 1202–1207.
- SCHEFFE, H. (1963). *The Analysis of Variance*. New York: John Wiley.
- TAKEUCHI, K., ABE, K., YASUJAMA, H., SATO, M., OMATA, K., KASAI, Y., KOMAZAHUA, M. & YOSHINAGA, K. (1988). The cellular action of bradykinin in vascular smooth muscle. *Am. J. Hypertens.*, **2**, 105A.
- TODA, N. (1974). The action of vasodilating drugs on isolated basilar coronary arteries of the dog. *J. Pharmacol. Exp. Ther.*, **191**, 139–146.
- TODA, N. & MIYAZAKI, M. (1978). Role of isolated dog cerebral and peripheral arteries to prostaglandins after application of aspirin and polyphoretin phosphate. *Stroke*, **9**, 490–497.
- VAN HOLST, H., GRANSTROM, E., HAMARSTROM, S., SAMUELSON, B. & STEINER, L. (1982). Effect of Leucotrienes C₄D₄, prostaglandins and thromboxane A₂ on isolated human cerebral arteries. *Acta Neurochir.*, **62**, 177–185.

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