Responses to arachidonic acid and other dilator agonists and their modification by inhibition of prostaglandin synthesis in the canine hindlimb

E. MORCILLO

Department of Pharmacology, Faculty of Medicine, Valencia, Spain

The influence of indomethacin, in doses that completely inhibit the response to arachidonic acid, has been examined on canine hindlimb vascular responses to intra-arterial administration of bradykinin, histamine, nitroglycerin, isoprenaline and papaverine. The hindlimb was perfused at constant flow. Dose-response curves to intra-arterial administration of arachidonic acid (12 to 200 μg kg^{-1}), bradykinin (0·4 to 100 ng kg^{-1}), histamine (1·8 to 120 ng kg^{-1}), nitroglycerin (15 to 100 ng kg^{-1}), isoprenaline (12 to 100 ng kg^{-1}) and papaverine (1·2 to 160 μg kg^{-1}) (all n = 4) were compared before and 30 min after indomethacin (5 mg kg^{-1} i.v.). All the drugs produced dose-related decreases in hindlimb perfusion pressure. After indomethacin, responses for all dilator agonists except arachidonic acid, were significantly greater than control ($P < 0·05$), both in terms of absolute (mmHg) or percent change. Dose-response curves after indomethacin had a left upward shift compared with control. Arachidonic acid responses were completely blocked by indomethacin. These findings suggest that indomethacin produces a non-specific increase in responsiveness of the hindlimb vascular bed to dilator substances, except arachidonic acid. The data presented do not support the hypothesis that the peripheral vasodilatation produced by bradykinin, nitroglycerin and histamine could be mediated by endogenous prostaglandin release.

Several dilator agonists are able to stimulate biosynthesis and release of prostaglandins in both in vivo and in vitro preparations: histamine and sodium nitroprusside (Pitlick et al 1977), bradykinin (McGiff et al 1972; Needleman et al 1975; Vane & Ferreira 1975; Juan 1977) and nitroglycerin (Morcillo et al 1977). Inhibitors of prostaglandin synthesis suppressed this evoked release of prostaglandins and attenuate the systemic and local haemodynamic responses elicited by these vasodilator drugs —bradykinin (Collier & Shorley 1960; Vargaftig 1966; Needleman et al 1975), nitroglycerin (Morcillo et al 1978)—thereby suggesting that endogenous prostaglandins are at least partially involved in their mechanism of vasodilatation.

The present study was undertaken to evaluate the influence of indomethacin, in doses that inhibited the response to arachidonic acid, on canine hindlimb vascular responses to intra-arterial injections of bradykinin, histamine, nitroglycerin, isoprenaline and papaverine.

METHODS

Mongrel dogs of either sex, 8 to 12 kg, were anaesthetized with 0·6 ml kg^{-1} i.v. of a dial-urethane solution containing allobarbitone 100, urethane 400 and monoethylurea 400 g litre^{-1}, intubated and maintained on positive-pressure ventilation (room air) with a Harvard pump. Ventilation rate was 10 to 12 min^{-1} and tidal volume 300 to 350 ml. The right jugular vein was cannulated. Heparin sodium 1000 U.S.P. units was given i.v. every hour throughout the experiment. Systemic blood pressure was measured from a cannula in the right common carotid artery connected to a Statham P23Db pressure transducer. Heart rate was measured from the e.c.g. (lead II) using a Grass tachograph.

The right hindlimb was acutely denervated by severing sciatic and femoral nerves. A few experiments were performed on normally innervated preparations. The left femoral artery was cannulated and blood pumped (Holter P1110 pump) through heparinized plastic tubing (Tygon) at a constant flow rate into the right femoral artery. The perfusion pressure was measured by a Statham P23Db pressure transducer from a side arm in the perfusion circuit between the pump and the hindlimb. At the beginning of the experiment the rate of flow was adjusted to make perfusion pressure approximately equal to systemic arterial pressure and kept constant at this rate for the total duration of the experiment. Average flow rate was 2·5 ± 0·3 ml kg^{-1} min^{-1}.

E.c.g., heart rate, systemic arterial blood pressure and perfusion pressure were continuously monitored using a Grass polygraph. Measurements of P_{O_2}, P_{CO_2} and pH were made at intervals to test the adequacy

of ventilation and perfusion. The values observed were: Po_2 100–130 mmHg, Pco_2 22–25 mmHg and pH 7·40–7·46.

Drugs. Arachidonic acid, grade I (approx. 99%) (Sigma), bradykinin triacetate (Sigma), histamine dihydrochloride (Fisher), indomethacin (Merck Sharp & Dohme), isoprenaline (Isuprel, Winthrop Lab.), nitroglycerin (Nitrostat, Parke Davis) and papaverine hydrochloride (Marion). Arachidonic acid was dissolved in 100 mM sodium carbonate with stirring under nitrogen in the absence of light. Indomethacin solution was prepared by adding a warm solution (50° C) of 60 mg sodium bicarbonate in 6 ml 0·9% NaCl (saline) to 60 mg drug dissolved in 2 ml 90% ethanol. The remaining drugs were diluted in saline. All the solutions were prepared daily and used only on the day of preparation.

Experimental design and data analysis. Control dose-response curves for decreases in perfusion pressure were made for every agonist and then repeated 30 min after indomethacin (5 mg kg^{-1}i.v.). The ranges for each agonist are as follows: arachidonic acid, 12 to 200 μg kg^{-1}, bradykinin 0·4 to 100 ng kg^{-1}, histamine 1·8 to 120 ng kg^{-1}, nitroglycerin 15 to 100 ng kg^{-1}, isoprenaline 12 to 100 ng kg^{-1} and papaverine 1·2 to 160 μg kg^{-1} (n = 4 with all drugs). Concentrations of drugs are expressed in terms of the base or acid. At least one additional experiment was done for each agonist following the same protocol but maintaining intact innervation to test if any difference was present in comparison with

the results obtained on the denervated preparation. Results are expressed as mean ± s.e.m. Statistical significance was evaluated using the paired Student t-test and accepted when $P < 0.05$.

RESULTS

Administration of arachidonic acid, bradykinin, histamine, nitroglycerin, isoprenaline and papaverine into the perfusion circuit produced dose-related decreases in hindlimb perfusion pressure (Figs 1, 2 and 3) without simultaneous modification in heart rate or systemic arterial pressure. Only the largest doses produced occasionally small decreases in systemic pressure and reflex tachycardia after the peak local response due to recirculation of the drug. Experiments performed on intact innervated preparations gave identical results for all the dilator agonists tested. Arachidonic acid had a significantly longer time to peak response (t) and duration (T1/2) compared with the rest of vasodilators in both denervated and intact preparations (Table 1, Fig. 3).

Indomethacin (5 mg kg^{-1} i.v.) produced a significant sustained increase in systemic and perfusion pressure with no significant change in heart rate (Fig. 4).

The decrease in hindlimb arterial perfusion pressure in response to intra-arterial administration of bradykinin, histamine, nitroglycerin, isoprenaline and papaverine were equal or significantly greater —both in terms of absolute (mmHg) or percent change—than control after administration of indo-
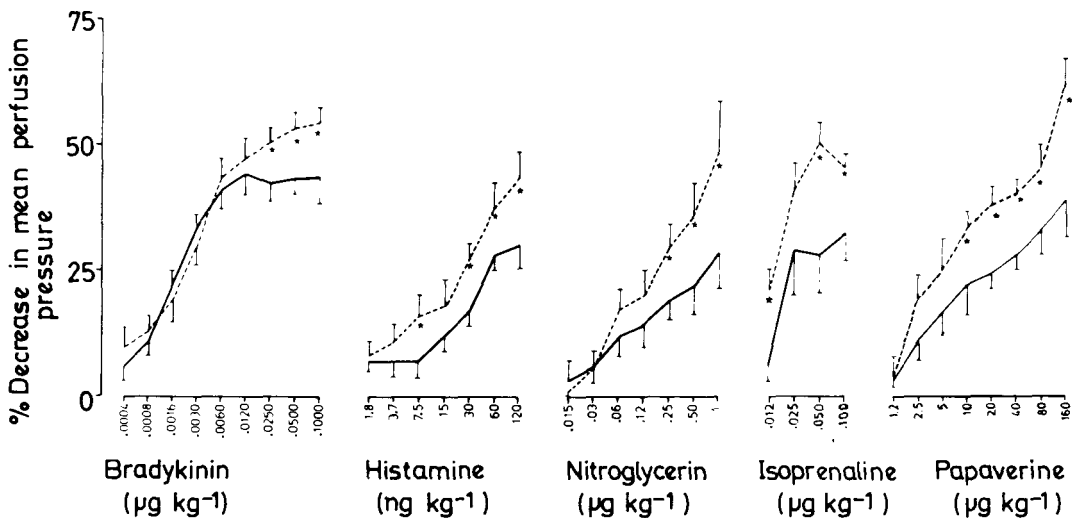

FIG. 1. Effect of indomethacin (5 mg kg^{-1} i.v.) on hindlimb vasodilator responses to bradykinin, histamine, nitroglycerin, isoprenaline and papaverine (all n = 4). Solid line is control dose-response curve. Dotted line is after indomethacin. Responses are expressed as percent decrease in perfusion pressure. Values are mean ± s.e.m. *$P < 0.05$ compared with control response for the same dose.
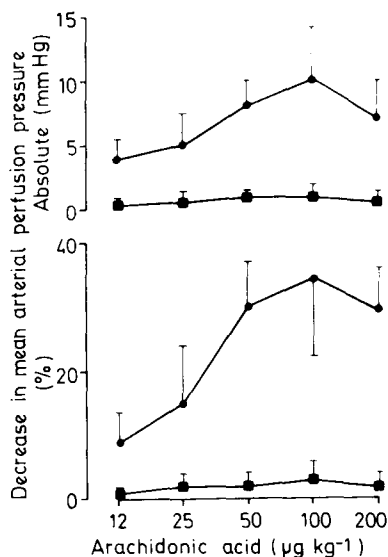
FIG. 2. Dose-related decrease in hindlimb perfusion pressure by arachidonic acid (●) intra-arterial (n = 4). Inhibition by indomethacin (■) 5 mg kg⁻¹ i.v. was significant at all dose levels. Values are mean ± s.e.m.

methacin. This greater response was particularly evident in the upper part of the dose-response curve. In consequence, dose-response curves to the vasodilators, after indomethacin, had a left upward shift compared with the control (Fig. 1).

Time to peak response and duration were not significantly different for these drugs after indomethacin compared with control values (Table 1). In contrast, indomethacin consistently reduced the decrease in perfusion pressure, duration and time to peak response of arachidonic acid (Fig. 2, Table 1).

DISCUSSION

Results show that decreases in hindlimb arterial perfusion pressure in response to intra-arterial administration of bradykinin, histamine, nitroglycerin, isoprenaline and papaverine are equal or
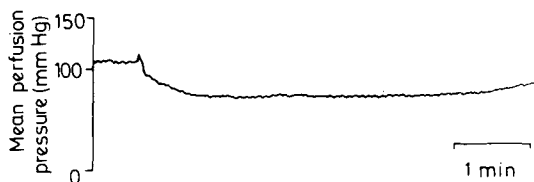

FIG. 3. Effect of administration of arachidonic acid 100 μg kg⁻¹ into the perfusion circuit on mean hindlimb perfusion pressure. Injection during constant flow causes a spike artifact.

Table 1. $t\dagger$ and $T\frac{1}{2}\ddagger$ values for dilator agonist responses in hindlimb and their modification by indomethacin (5 mg kg⁻¹ i.v.).

	Before indomethacin		After indomethacin	
	t	$T\frac{1}{2}$	t	$T\frac{1}{2}$
Arachidonic acid (100 μg kg⁻¹)	114 ± 13*	456 ± 15*	25 ± 7**	56 ± 9**
Bradykinin (100 ng kg⁻¹)	20 ± 2	57 ± 11	20 ± 3	50 ± 8
Histamine (120 ng kg⁻¹)	20 ± 3	49 ± 5	21 ± 5	61 ± 8
Nitroglycerin (1 μg kg⁻¹)	22 ± 4	54 ± 3	23 ± 5	64 ± 9
Isoprenaline (100 ng kg⁻¹)	30 ± 7	60 ± 8	35 ± 6	71 ± 9
Papaverine (80 μg kg⁻¹)	22 ± 1	47 ± 4	18 ± 3	43 ± 6

† t is time(s) from the moment of the injection until the maximum decrease in perfusion pressure is reached.

‡ $T\frac{1}{2}$ is the time(s) from the moment of the injection until the point at which the perfusion pressure has returned half-way from maximum response to control level.

Values are mean ± s.e.m. Administration of dilator agonists was intra-arterial. * $P < 0.05$ compared with values for other agonist. **$P < 0.05$ compared with values before indomethacin.

significantly greater—both in terms of absolute (mmHg) and percent change—than control after the pretreatment with indomethacin in a concentration that completely inhibited the response to arachidonic acid.

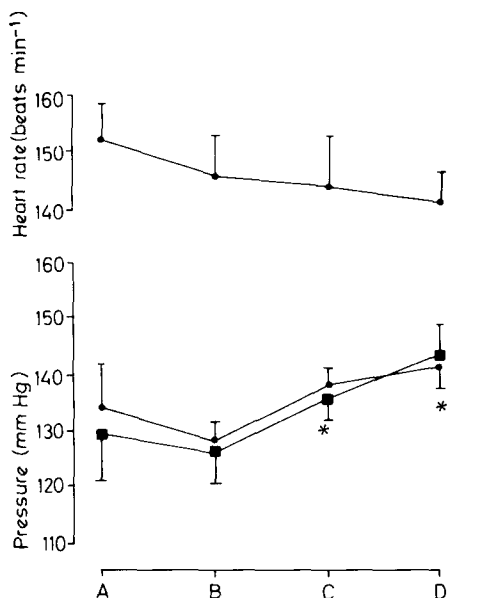The vascular actions of bradykinin on systemic and local circulation have been consistently related

FIG. 4. Heart rate, mean systemic arterial pressure (●) and mean hindlimb perfusion pressure (■) values throughout the experiment. A: Values at the beginning of the experiment, B: Values before indomethacin (approx. 2 h after A). C: Values 30 min after B, D: Values at the end of the experiment (approx. 2 h after C). * $P < 0.05$ compared with values in B (n = 27).

with the release of endogenous prostaglandins by several investigators (McGiff et al 1972; Needleman et al 1975; Vane & Ferreira 1975; Juan 1977). In consequence, the vasodilator response to bradykinin should be attenuated by inhibitors of prostaglandin synthesis.

In the present study, the dilator responses to bradykinin in the canine hindlimb vascular bed were not decreased after indomethacin. Instead they remained equal or were significantly increased. A similar trend was observed with histamine and nitroglycerin, apparently also able to release prostaglandins from certain tissues (Pitlick et al 1977; Morcillo et al 1977), and with isoprenaline and papaverine. Dusting et al (1978) also reported potentiation of vasodilator responses to prostaglandin E_2 and prostacyclin after indomethacin in canine mesenteric and femoral vascular beds.

These findings do not support the hypothesis that peripheral vasodilator responses to bradykinin and other dilator agonists may be mediated by endogenous prostaglandins. These results are in agreement with those obtained by Fink et al (1977) and Chapnick et al (1977) for decreases in renal perfusion pressure by nitroglycerin and bradykinin in the in situ rabbit and cat kidney, and with those of Goldberg et al (1976) for venoconstrictor responses to bradykinin.

However, the possibility that this non-specific increase in responsiveness to dilator substances produced by indomethacin could actually mask or counteract a real inhibitory effect, as suggested by Fink et al (1977), cannot be excluded. From Fig. 1 the dose-response curve for bradykinin after indomethacin can be seen to be somewhat less shifted to the left compared with leftward displacements obtained for the other dilator agonists. But, it appears unlikely that the sustained indomethacin-induced increase in resistance did influence the present results because both absolute (mmHg) and percent changes from control values were equally modified and the use of percentages minimizes the influence of the initial resistance level on the vascular response.

Intra-arterial administration of arachidonic acid consistently produced a slow longlasting decrease in perfusion pressure in both denervated and intact preparations. The vasodilatation by arachidonate is most probably due to prostaglandin H_2, E_2 and I_2 formation although their relative contribution has

not yet been precisely determined (Dusting et al 1978). The present results are in agreement with those of Ryan et al (1977) but not with those of Fitzpatrick et al (1977) who found a marked vasoconstriction over the same dose-range in innervated isolated hindlimb. The technique employed by those investigators was different from that used here. In addition they found a transient vasodilatation after indomethacin while a small but sustained increase in perfusion pressure after indomethacin was seen in the present work. A recent study by Dusting et al (1978) also report vasodilatation following arachidonic acid and vasoconstriction with indomethacin in the hindlimb circulation of the dog.

In summary, the present results show that in spite of the role that endogenous prostaglandins seem to have in maintaining the vascular tone in the hindlimb, they do not mediate the response to the dilator agonists tested except arachidonic acid.

REFERENCES

Chapnick, B. M., Paustian, P. W., Feigen, L. P., Joiner, P. D., Hyman, A. L., Kadowitz, P. J. (1977) Circ. Res. 40: 348–354

Collier, H. O., Shorley, P. G. (1960) Br. J. Pharmacol. 15: 601–610

Dusting, G. J., Moncada, S., Vane, J. R. (1978) Eur. J. Pharmacol. 49: 65–72

Fink, G. D., Chapnick, B. M., Goldberg, M. R., Paustian, P. W., Kadowitz, P. J. (1977) Circ. Res. 41: 172–178

Fitzpatrick, T. M., Johnson, M., Kot, P. A., Ramwell, P. W., Rose, J. C. (1977) Br. J. Pharmacol. 59: 269–273

Goldberg, M. R., Chapnick, B. M., Joiner, P. D., Hyman, A. L., Kadowitz, P. J. (1976) J. Pharmacol. Exp. Ther. 198: 357–365

Juan, H. (1977) Naunyn-Schmiedeberg's Arch. Pharmacol. 300: 77–85

McGiff, J. C., Terragno, N. A., Malik, K. U., Lonigro, A. J. (1972) Circ. Res. 31: 36–43

Morcillo, E., Reid, P., Dubin, N., Ghodgaonkar, R., Pitt, B. (1977) Circulation 56: III–122 (Abstract)

Morcillo, E., Pitt, B., Reid, P. (1978) Am. J. Cardiol. 41: 367 (Abstract)

Needleman, P., Key, S. L., Denny, S. E., Isakson, P. C., Marshall, G. R. (1975) Proc. Natl Acad. Sci. USA 72: 2060–2063

Pitlick, F., Dreyer, B., Mulrow, P., Tan, S. Y., Sweet, P. (1977) Circulation 56: III–121 (Abstract)

Ryan, M. J., Kraft, E., Sugawara, K., Zimmerman, B. G. (1977) J. Pharmacol. Exp. Ther. 200: 606–613

Vane, J. R., Ferreira, S. H. (1975) Life Sci. 16: 804–805

Vargaftig, B. (1966) Experientia 22: 182–183