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Effect of prostaglandins E_1, E_2 and A_2 on vascular resistance and responses to noradrenaline, nerve stimulation and angiotensin in the dog hindlimb

PHILIP J. KADOWITZ

Department of Pharmacology, School of Medicine, Tulane University, New Orleans, Louisiana, USA

Summary

1. The relationship between the vasodilator and inhibitory effects of prostaglandin E_1 (PGE_1), E_2 and A_2 on responses to nerve stimulation, noradrenaline and angiotensin was evaluated in the dog hindlimb preparation.

2. PGE_1 and PGE_2 were equipotent as vasodilators in the hindlimb; however, PGE_1 was much more potent as an inhibitor of vasoconstrictor responses to nerve stimulation, noradrenaline and angiotensin.

3. PGA_2 and PGE_2 were approximately equal as inhibitors of vasoconstrictor responses to noradrenaline, nerve stimulation and angiotensin; however, PGE_2 was far more potent as a vasodilator.

4. Since there is no relationship between the vasodilator and inhibitory effects of PGE_1, E_2 and A_2, and since the inhibitory effect of PGA_2 was present at a time when hindlimb perfusion pressure had returned to control value, it is concluded that the inhibitory action is probably not the result of a physiological antagonism.

5. Since each prostaglandin inhibited responses to nerve stimulation and noradrenaline to approximately the same extent and responses to angiotensin were also inhibited, it is suggested that these agents antagonize vasoconstrictor responses by a nonspecific depressant effect on smooth muscle cells.

Introduction

Prostaglandins (PGs) are natural acidic lipids which are released from several organs when vasoconstrictor substances are infused or the sympathetic nerves are stimulated (Davies, Horton & Withrington, 1968; Gilmore, Vane & Wyllie, 1968; McGiff, Crowshaw, Terragno & Lonigro, 1970; Dunham & Zimmerman, 1970). They modify vasoconstrictor responses to noradrenaline and sympathetic nerve sitmulation in a variety of vascular beds in several species (Hedqvist & Brundin, 1969; Holmes, Horton & Main, 1963; Hedqvist, 1970a; Weiner & Kaley, 1969; Viguera & Sunahara, 1969; Hedwall, Abdel-Sayed, Schmidt & Abboud, 1970; Hedwall, Abdel-Sayed, Schmidt, Mark & Abboud, 1971; Kadowitz, Sweet & Brody, 1971a and b; Sweet, Kadowitz & Brody, 1971). It has therefore been suggested that prostaglandins may regulate sympathetic neurotransmission to vascular smooth muscle (Hedqvist, 1970b). However, prostaglandins fail to modify responses to noradrenaline and angiotensin in some tissues (Davies & Withrington, 1967; Nakano & McCurdy, 1967; Carlson & Orö, 1966). This study compares the

effects of equivalent doses of prostaglandins, E_1, E_2 and A_2 on vascular resistance and vasoconstrictor responses to noradrenaline, nerve stimulation and angiotensin in the perfused dog hindlimb preparation.

Methods

Mongrel dogs (12–18 kg) were anaesthetized with pentobarbitone sodium (30 mg/kg i.v.) and, after cannulation of the trachea were respired with room air using a 'Harvard' respirator. Arterial pressure (1 mmHg≡1·33 mbar) was measured through a catheter inserted into the aorta through a carotid artery. Drugs were injected through a catheter in the jugular vein. After administration of heparin sodium (5 mg/kg i.v.), the left hindlimb was perfused (Beck, DuCharme, Gebber, Levin & Pollard, 1966) through the iliac artery by a Sigmamotor pump, Model T6, with blood supplied from the abdominal aorta. Flow, initially set to provide a perfusion pressure that approximated the systemic pressure and not thereafter changed, averaged 160±5 ml/min in 24 experiments. Since the flow was constant, changes in perfusion pressure reflected changes in vascular resistance. Hindlimb perfusion pressure was monitored from a T-piece in the tubing between pump and limb. A 'Harvard' shielded electrode was placed on the left lumbar chain between L5 and L6 and the limb was decentralized by crushing the chain at L3 to prevent reflex changes in perfusion pressure; the nerve was stimulated with rectangular pulses, 2-ms duration, 10–18 V, 0·3 to 30 Hz for 15 s periods with a Tektronix assembly. All pressures were measured by Statham transducers and recorded on a Glass polygraph.

Intra-arterial injections of (−)-noradrenaline bitartrate (Levophed bitartrate, Winthrop, dose in terms of base) and angiotensin II amide (Hypertensin Ciba) were made in small volumes (0·03–0·3) ml into the perfusion circuit. Prostaglandins, E_1, E_2 and A_2 were dissolved in 95% ethyl alcohol (1 mg/ml) and stored in the freezer. On the day of use the stock solution was diluted to a volume of 10 ml with saline and infused close to the limb with a 'Harvard' infusion pump.

Data were analysed with Student's *t* test for paired and group analysis (Snedecor, 1956). The 5% probability level was the criterion for significance.

Results

Close intra-arterial infusion of PGE_1, PGE_2 and PGA_2 at 4 μg/min, in 3 groups of animals reduced hindlimb perfusion pressure (Table 1). The onset of the dilator response was rapid and the peak effect was attained in 3 to 5 minutes. The decrease

TABLE 1. *Effect of infusion of PGE_1, PGE_2 and PGA_2 on hindlimb perfusion pressure and mean arterial pressure*

		Perfusion pressure (mmHg)						Mean arterial pressure (mmHg)		
			Minutes after onset of infusion				Minutes after infusion		Minutes after onset	Minutes after infusion
	n	Control	1	3	5	10	30	Control	10	30
PGE_1	8	150± 7	104±6*	94±6*	91±5*	91±5*	110± 4*	121±4	119±5	129±3
PGE_2	7	123±10	87±8*	75±3*	73±3*	76±2*	113±11	111±5	112±7	116±7
PGA_2	9	139± 6	121±5*	119±5*	126±6*	136±6	144± 6	127±5	95±9*	121±4

* Significantly different from control ($P<0·05$), paired comparison.

in perfusion pressure was well maintained during infusions of PGE_1 and PGE_2 but not during infusion of PGA_2 (Table 1). The maximum dilator actions of PGE_1 and PGE_2 in the hindlimb were similar, both agents possessing significantly greater dilator activity than PGA_2 (Fig. 1). There was little tendency for perfusion pressure to return towards control values when infusion of PGE_1 was terminated and perfusion pressure was still significantly lower than control 30 min after infusion (Table 1). Perfusion pressure returned towards the control value when the PGE_2 infusion was terminated and was not significantly different from control 30 min after infusion (Table 1). In experiments in which the vasodilator effects of PGA_2 were evaluated, perfusion pressure returned to control value during constant infusion (Table 1). PGA_2 decreased systemic arterial pressure by 36 ± 6 mmHg whereas PGE_1 and PGE_2 were without significant effect on the arterial blood pressure (Table 1).

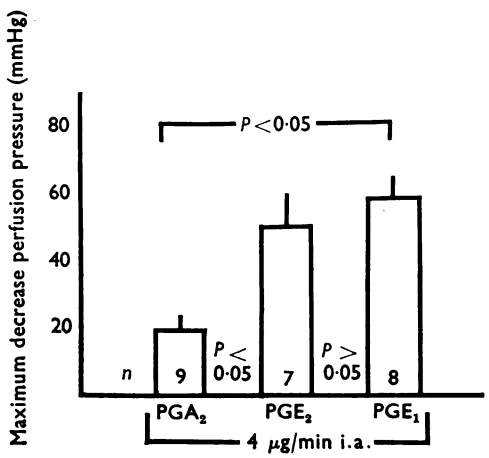

FIG. 1. Effect of intra-arterial infusion of PGA_2, PGE_2 and PGE_1 (4 μg/min) on perfusion pressure in the dog hindlimb preparation. The maximum vasodilator responses to PGE_1 and PGE_2 were significantly greater than the maximum response to PGA_2; however, the responses to PGE_1 and PGE_2 were not significantly different from each other.

The effects of PGE_1, PGE_2 and PGA_2 on vasoconstriction induced by sympathetic nerve stimulation, noradrenaline and angiotensin were measured by constructing dose and frequency response relationships prior to, during, and 30 min after prostaglandin infusion in the 3 groups of animals. Vasoconstrictor responses to a wide range of doses of noradrenaline and angiotensin and to nerve stimulation were decreased at each dose and stimulus frequency during PGE_1 infusion. There was little tendency for responsiveness to return after PGE_1 infusion and all responses were significantly decreased 30 min after infusion (Fig. 2A).

Constrictor responses to all doses of noradrenaline and angiotensin and to all but the highest stimulus frequency were decreased significantly during PGA_2 infusion and all responses were not significantly different from control 30 min after termination of the infusion (Fig. 2B).

Effects of all doses of noradrenaline and of 0·3 and 1·0 μg of angiotensin were decreased significantly during PGE_2 infusion. Effects of nerve stimulation at 0·3, 1 and 3 Hz were also decreased significantly during PGE_2 infusion and all responses returned to control value 30 min after the infusion (Fig. 2C).

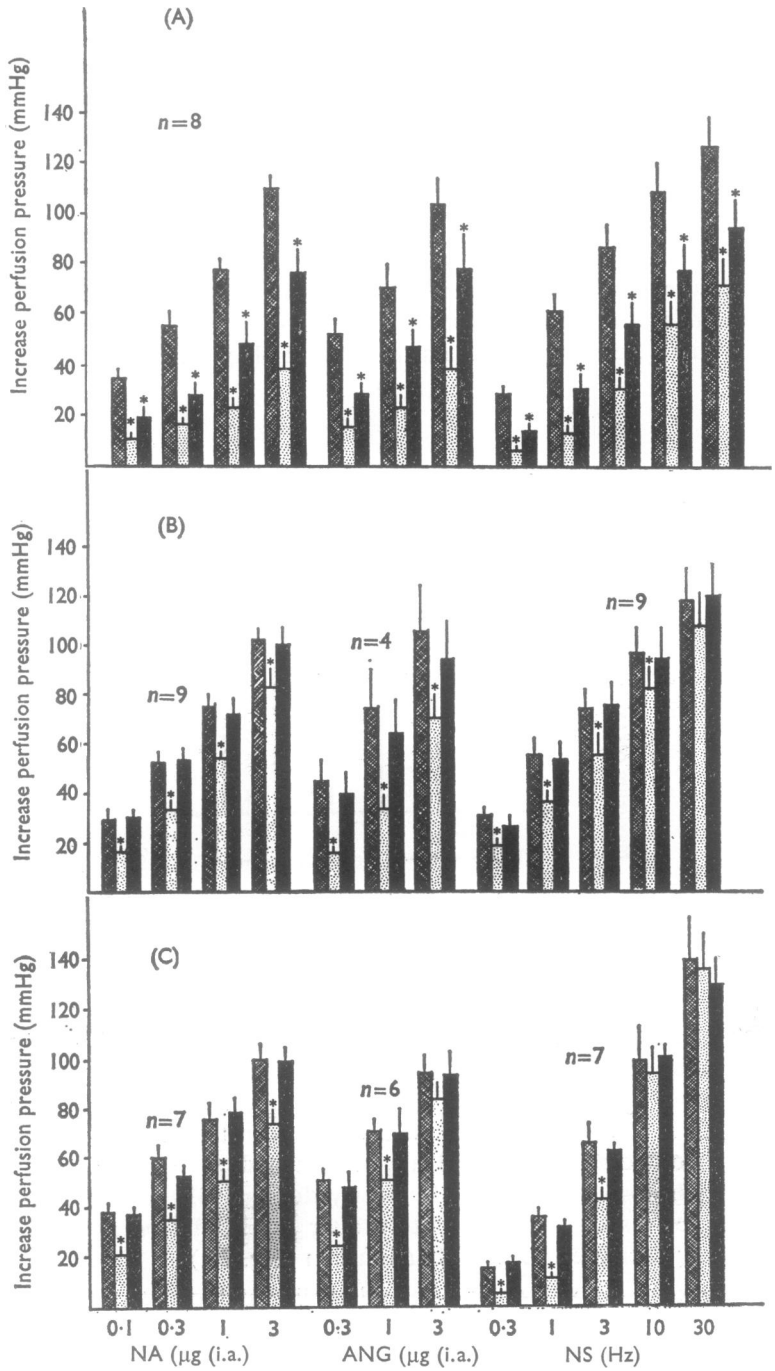

FIG. 2. Effect of (A) PGE_1, (B) PGA_2 and (C) PGE_2 (4 μg/min) on vasoconstriction induced by noradrenaline (NA), angiotensin (ANG) and nerve stimulation (NS) in the dog hindlimb preparation. Responses to NA, ANG, and NS were obtained in the control period (hatched bars), during infusion of the prostaglandin (stippled bars), and 30 min after termination of the infusion (solid bars) in each of 3 series of experiments. *$P<0.05$.

Discussion

Prostaglandins E_1, E_2 and A_2 antagonize vasoconstriction induced by sympathetic nerve stimulation, noradrenaline and angiotensin in dog hindlimb but there is little or no relationship between the vasodilator and blocking effects. If the inhibitory activity of prostaglandins E_1, E_2 and A_2 were due to a physiological antagonism, the vasodilator and blocking effects should be related. However, PGE_1 is far more potent than PGE_2 as an antagonist of hindlimb vasoconstriction but these two agents are equipotent as vasodilators. Similarly, PGE_2 and PGA_2 inhibit equally hindlimb vasoconstrictor responses but PGE_2 is more potent as a vasodilator. In addition, the effect of PGA_2 on vascular resistance and vasoconstrictor responses can be dissociated in that responses to vasoconstrictor stimuli are inhibited at a time when hindlimb perfusion pressure has returned to control value during the infusion. These findings in the hindlimb along with those in the hindpaw and gracilis muscle (Kadowitz *et al.*, 1971a & b ; Hedwall *et al.*, 1971) indicate that the inhibitory effect of prostaglandins on adrenergic responses is not merely the consequence of vasodilation, as suggested by Carlson & Orö (1966). Although the mechanism by which prostaglandins inhibit vasoconstriction is unknown, the observation that responses to exogenous and nerve released noradrenaline are depressed to approximately the same extent indicates that the site of action is primarily postjunctional. Since responses to angiotensin are also inhibited, this effect is probably a nonspecific one on hindlimb vascular smooth muscle.

The effect of PGE_2 on noradrenaline and nerve stimulation is different in hindlimb and hindpaw (Kadowitz *et al.*, 1971a). In the hindpaw, PGE_2 enhances effects of nerve stimulation ; however, the hindpaw is primarily cutaneous tissue whereas the hindlimb is mainly skeletal muscle. Thus the effect of PGE_2 on responses to noradrenaline and nerve stimulation may be dependent on the tissue studied. Although PGE_2 does not alter vasoconstrictor responses to nerve stimulation and noradrenaline in the dog spleen perfused with blood (Davies & Withrington, 1967), it had a variable dose-related effect on vasoconstriction induced by nerve stimulation and noradrenaline in the isolated cat spleen perfused with Krebs solution in that it inhibited responses to nerve stimulation at low concentrations but enhanced responses to noradrenaline at high concentrations (Hedqvist, 1970a). Thus the effect of PGE_2 on responses to nerve stimulation and noradrenaline may depend on organ system, species, and concentration used.

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REFERENCES

BECK, L., DUCHARME, D. W., GEBBER, G. L., LEVIN, J. A. & POLLARD (1966). Inhibition of adrenergic activity at a locus peripheral to the brain and spinal cord. *Circulation Res.*, **18**, Suppl. 1, 55–69.

CARLSON, L. A. & ORÖ, L. (1966). Effect of prostaglandin E_1 on blood pressure and heart rate in the dog. *Acta physiol. scand.*, **67**, 89–99, 1966.

DAVIES, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1968). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *Br. J. Pharmac. Chemother.* **32**, 127–135.

DAVIES, B. N. & WITHRINGTON, P. G. (1967). The effects of prostaglandins E_1 and E_2 on the smooth muscle of the dog spleen and on its responses to catecholamines, angiotensin and nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 136–144.

DUNHAM, E. H. & ZIMMERMAN, B. G. (1970). Release of prostaglandin-like material from dog kidney during nerve stimulation. *Am. J. Physiol.*, **219**, 1279–1285.

GILMORE, N., VANE, J. R. & WYLLIE, J. H. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135–1140.

HEDQVIST, P. (1970a). Control by prostaglandin E_2 of sympathetic neurotransmission in the spleen. *Life Sci.*, **9**, 269–278.

HEDQVIST, P. (1970b). Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. *Acta physiol. scand.*, Suppl. **345**, 1–40.

HEDQVIST, P. & BRUNDIN, J. (1969). Inhibition by prostaglandin E_1 or noradrenalin release and of effector response to nerve stimulation in the cat spleen. *Life Sci.*, **8**, 389–395.

HEDWALL, P. R., ABDEL-SAYED, W. A., SCHMIDT, P. G. & ABBOUD, F. M. (1970). Inhibition of venoconstrictor responses by prostaglandin E_1. *Proc. Soc. exp. Biol. Med.*, **135**, 757–759.

HEDWALL, P. R., ABDEL-SAYED, W. A., SCHMIDT, P. G., MARK, A. L. & ABBOUD, F. M. (1971). Vascular responses to prostaglandin E_1 in gracilis muscle and hindpaw of the dog. *Am. J. Physiol.*, **221**, 42–47.

HOLMES, S. W., HORTON, E. W. & MAIN, I. H. M. (1963). The effect of prostaglandin E_1 on responses of smooth muscle to catecholamines, angiotensin and vasopressin. *Br. J. Pharmac. Chemother.*, **21**, 538–543.

KADOWITZ, P. J., SWEET, C. S. & BRODY, M. J. (1971a). Differential effects of prostaglandins E_1, E_2, $F_1\alpha$ and $F_2\alpha$ on adrenergic vasoconstriction in the dog hindpaw. *J. Pharmac. exp. Ther.*, **176**, 167–173.

KADOWITZ, P. J., SWEET, C. S. & BRODY, M. J. (1971b). Blockade of adrenergic vasoconstrictor responses in the dog by prostaglandins E_1 and A_1. *J. Pharmac. exp. Ther.*, **179**, 563–572.

MCGIFF, J. C., CROWSHAW, K., TERRAGNO, H. A. & LONIGRO, A. J. (1970). Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circulation Res.*, **27**, Suppl. 1, 121–130.

NAKANO, J. & MCCURDY, J. R. (1967). Cardiovascular effects of prostaglandin E_1. *J. Pharmac. exp. Ther.*, **156**, 538–547.

SNEDECOR, C. W. (1956). *Statistical Methods*, Iowa State College Press, Ames, Iowa.

SWEET, C. S., KADOWITZ, P. J. & BRODY, M. J. (1971). Another humoral substance that enhances adrenergic responsiveness during acute renal ischemia. *Nature, Lond.*, **231**, 263–265.

VIGUERA, M. G. & SUNAHARA, F. A. (1969). Microcirculatory effects of prostaglandins. *Can. J. Physiol. Pharmac.*, **47**, 627–634.

WEINER, R. & KALEY, G. (1969). Influence of prostaglandin E_1 on the terminal vascular bed. *Am. J. Physiol.*, **217**, 563–566.

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