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THE EFFECTS OF PROSTAGLANDINS E_1, E_2 AND $F_{2\alpha}$ ON THE CUTANEOUS VASCULATURE OF THE RAT

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1 The responses of the rat cutaneous vasculature to prostaglandins E_1, E_2 and $F_{2\alpha}$ have been investigated by a photomicrographic technique.

2 Prostaglandin E_1 produced transient arterial constriction which was blocked by local pretreatment of preparations with compound 48/80 or methysergide. It was concluded that prostaglandin E_1 produced vasoconstriction by release of 5-hydroxytryptamine (5-HT) from mast cells. The magnitude of the vasoconstrictor response appeared to be subject to seasonal variation.

3 Prostaglandin $F_{2\alpha}$ produced arterial constriction of longer duration which was not blocked by compound 48/80, methysergide or phenoxybenzamine.

4 Preparations pretreated with prostaglandin $F_{2\alpha}$ were found to be more sensitive to the venous constrictor effect, and less sensitive to the arterial constrictor effect, of noradrenaline.

5 Prostaglandin E_2 produced arterial constriction which was usually partially blocked by compound 48/80 and methysergide and it was concluded that a major component of the vasoconstrictor response to prostaglandin E_2 was of the E_1 type but some component was of the $F_{2\alpha}$ type.

Introduction

Prostaglandins E_1 and E_2 have been widely reported to produce vasodilatation in a number of vascular beds whereas prostaglandin $F_{2\alpha}$ has been found to produce variable responses (see Higgins & Braunwald, 1972; Muirhead, 1973; Nakano, 1973). Few studies have been made on the cutaneous vasculature of the rat. Since this species is so often used for tests of inflammatory responses a study of the effects of these three prostaglandins on rat cutaneous vasculature was undertaken.

Methods

Male rats derived from the Wistar strain weighing 100 to 180 g were anaesthetized with urethane (1250–1500 mg/kg, i.p.). A longitudinal incision was made in the skin of either the abdomen or the outer thigh. The skin was carefully separated from the underlying tissues and a flap with the inner surface exposed, was placed over a translucent, perspex table (1.5 cm diameter) on a microscope stage. The flap was gently anchored in place with threads and transilluminated with a tungsten-halogen light source. Magnification was 19×. Photomicrographs of the silhouettes were taken at timed intervals with an Olympus PM-6 camera. Although the field was too deep to be all in focus, semiquantitative estimates of changes in diameter of the blood vessels were possible.

The skin flap was kept moist with regular applications of Tyrode solution (composition g/l: NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, $NaHCO_3$ 1.0, glucose 1.0) at 37°C. Drugs were diluted in Tyrode at 37°C immediately before use and were dropped directly on to the tissue from a Pasteur pipette. Photomicrographs were usually taken at 15 s intervals for the first minute after application of a drug and thereafter at 1 min intervals until the response was maximal (2 to 4 minutes). The drug was then washed from the tissue with several changes of warm Tyrode solution. Two consistent responses to active drugs were always obtained before the addition of antagonists.

Drugs

The following drugs were used: compound 48/80 (Wellcome), histamine diphosphate (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), methysergide hydrogen maleate (Sandoz), (–)-noradrenaline bitartrate (1-arterenol bitartrate, Sigma), phenoxybenzamine hydrochloride (Smith, Kline & French), prostaglandins E_1 (free acid), E_2 (free acid) and $F_{2\alpha}$ tromethamine salt (Upjohn). Concentrations given refer to weight of salt per ml of solution, except for prostaglandins E_1 and E_2 where concentrations refer to weight of free acid.

Results

Effects of prostaglandins on abdominal and outer thigh skin vasculature

Prostaglandin E_1 (10–200 ng/ml) produced transient constriction of the arteries of both abdominal skin and outer thigh skin, which usually was more marked on the vessels of the outer thigh skin. There was little effect on the veins. The constrictor effect appeared at about 45 s after application of prostaglandin E_1 and usually had disappeared by 2 minutes. It was often preceded by a brief, marked dilatation which was apparent within 15 seconds. Higher concentrations of prostaglandin E_1 (500 ng/ml to 10 μg/ml) did not produce constrictor effects (Figure 1 e–h); dilatation often occurred at these concentrations. There was some tachyphylaxis to the constrictor effect of prostaglandin E_1 but the responses could be repeated several times at 10 min intervals.

A seasonal variation in the constrictor response to prostaglandin E_1 was found, the response being much less during the summer months (October to April) than during the winter months (May to September). On many preparations tested during the summer months it produced only a vasodilator effect but vaso-

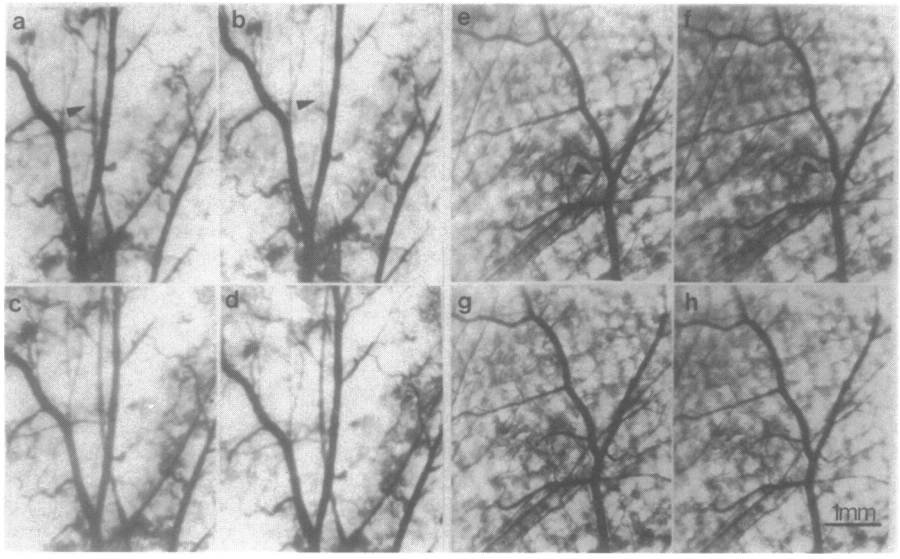

Figure 1 Effect of compound 48/80 on the response of rat cutaneous vasculature to prostaglandin E_1 (100 ng/ml) (outer thigh skin): (a) Control; (b) 2 min after application of prostaglandin E_1 (note arterial constriction at arrow); (c) control after two applications of 48/80 10 μg/ml; (d) 2 min after application of prostaglandin E_1 (note absence of arterial constriction). Effect of low and high concentrations of prostaglandin E_1 on rat cutaneous vasculature (outer thigh skin): (e) Control; (f) 2 min after application of prostaglandin E_1, 10 ng/ml (note arterial constriction at arrow); (g) control; (h) 2 min after application of prostaglandin E_1, 10 μg/ml (note absence of arterial constriction).

Table 1 Vasoconstrictor responses to prostaglandins in three seasons

	First winter		*Summer*		*Second winter*	
	Tested	*Constricted*	*Tested*	*Constricted*	*Tested*	*Constricted*
Prostaglandin E_1						
Abdomen	6	4	11	2 (slight)	3	3
Thigh	6	6	27	7 (slight)	6	5
Prostaglandin E_2						
Abdomen	–	–	–	–	5	5
Thigh	–	–	4	0	7	7
Prostaglandin $F_{2\alpha}$						
Abdomen	–	–	3	3	3	3
Thigh	–	–	4	4	5	5

Abdomen—abdominal skin, Thigh—outer thigh skin. A dash indicates that no tests were made.

constrictor effects were observed during two winter periods (Table 1).

Prostaglandin E_2 (100–200 ng/ml) also produced a constrictor effect on arteries. However on 4 preparations where both prostaglandin E_1 and E_2 were tested, it was found that the constrictor effect of prostaglandin E_2 was longer lasting and more generalized over the whole field than was that of E_1. The constrictor effect of prostaglandin E_2 was also preceded by a phase of dilatation.

Prostaglandin $F_{2\alpha}$ (50 ng/ml to 1 μg/ml) produced marked constriction of arteries, sometimes preceded by dilatation. The constrictor effect developed more slowly than that of prostaglandin E_1 and appeared between 1 and 2 min after addition of the drug. The constrictor response to prostaglandin $F_{2\alpha}$ differed from that to E_1 in the more generalized nature of the response (Figure 3b). In some preparations slight venous constriction also occurred. The preparations exhibited marked tachyphylaxis to the constrictor effect and to obtain reproducible responses additions of prostaglandin $F_{2\alpha}$ were made at 30 min intervals. There was no evidence of seasonal variation in the constrictor response to prostaglandin $F_{2\alpha}$ (Table 1).

Effect of compound 48/80 on responses to prostaglandins

Addition of compound 48/80 (10 or 100 μg/ml) to the preparations produced marked constriction of the arteries with little effect on the veins (Figure 2a,b). Very low concentrations of 5-HT (1 ng/ml) produced vasodilatation. Higher concentrations of 5-HT (10 to 100 ng/ml), but not histamine (100 ng/ml to 10 μg/ml), produced marked constriction of the arteries (Figure 2e–h). It therefore seemed likely that the effects observed with compound 48/80 were due to 5-HT released from mast cells. In order to deplete the mast cells of amines, two or three additions of compound 48/80 were made until there was no constrictor response (Figure 2c,d). The response of the preparations to 5-HT was not affected by pretreatment with compound 48/80.

The constrictor response to prostaglandin E_1 was completely abolished by pretreatment with compound 48/80 on all preparations tested (4 thigh skin and 2 abdominal skin preparations) (Figure 1a–d). The effect of prostaglandin E_2 was partially blocked on 2 thigh skin and 1 abdominal skin preparation and completely blocked on 1 thigh skin and 1 abdominal skin preparation. The action of prostaglandin $F_{2\alpha}$ was not blocked on any of the preparations tested (3 thigh skin and 3 abdominal skin preparations). The dilator responses to prostaglandins E_1 and E_2 did not appear to be blocked by pretreatment with compound 48/80.

Effect of methysergide on responses to prostaglandins

Methysergide (1 μg/ml) dripped on to the preparations for 5 min produced complete block of the constrictor

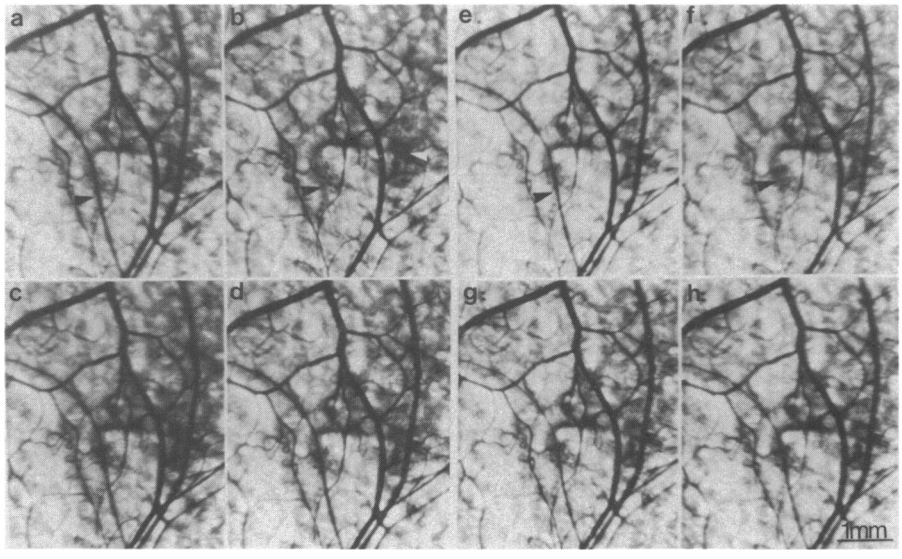

Figure 2 Effect of compound 48/80, 5-hydroxytryptamine (5-HT) and histamine on rat cutaneous vasculature (outer thigh skin). Effect of first and second application of compound 48/80 (10 μg/ml): (a) control; (b) 2 min after first application of compound 48/80 (note complete closure of both arteries at arrows); (c) control; (d) 2 min after second application of same amount of compound 48/80 (note absence of arterial constriction). Effect of 5-HT (10 ng/ml): (e) control; (f) 1 min after application of 5-HT (note almost complete closure of one artery at arrow). Effect of histamine (10 μg/ml): (g) control; (h) 2 min after application of histamine (note absence of arterial constriction).

response to 5-HT (100 ng/ml). The responses to the prostaglandins were tested before and after treatment of the preparations with methysergide (1 μg/ml) for 5 minutes. The constrictor response to prostaglandin E_1 was completely blocked by methysergide (5 thigh skin preparations), that to prostaglandin E_2 was partially blocked (4 thigh skin and 3 abdominal skin preparations), and that to prostaglandin $F_{2\alpha}$ was unaffected (2 thigh skin and 1 abdominal skin preparation).

Effect of phenoxybenzamine on the response to prostaglandin $F_{2\alpha}$

Phenoxybenzamine (1 μg/ml) dripped on to the preparations for 5–15 min produced complete block of the constrictor response to noradrenaline (100–200 ng/ml) (Figure 3c–d, g–h). On the 2 abdominal skin preparations tested the constrictor response to prostaglandin $F_{2\alpha}$ was not blocked by phenoxybenzamine and may even have been enhanced (Figure 3a–b, e–f). On these preparations it was also noted that noradrenaline produced more marked constriction of veins than arteries whereas prostaglandin $F_{2\alpha}$ produced marked arterial constriction (Figure 3). In both experiments noradrenaline was tested after application of prostaglandin $F_{2\alpha}$ to the preparations.

Discussion

The rat cutaneous vascular preparation used in these experiments was very reactive to topically applied drugs and, in contrast to the rat mesenteric vascular preparation studied by Furness & Marshall (1974) was capable of responding to drugs with virtually complete closure of the arteries (Figure 2).

The results presented in this paper have shown that prostaglandins E_1, E_2 and $F_{2\alpha}$ produce constrictor responses of the rat cutaneous vasculature. However the nature of the responses and the mechanisms by which they were produced appeared to differ. Prostaglandin E_1 produced arterial constriction of relatively short duration which was blocked by compound 48/80 and by methysergide. Since it is known that rat mast cells contain 5-HT as well as histamine (Benditt, Wong, Arase & Roeper, 1955) and 5-HT and compound 48/80 both produced arterial constriction of this preparation, while histamine did not, it would appear that the constrictor effect of prostaglandin E_1 was due to 5-HT released from mast cells. In other species where histamine is the only amine present in mast cells, and where histamine produces vasodilatation, a vasodilatator response only to prostaglandin E_1 would be expected. Vasodilatation to prostaglandin E_1 has in fact been observed on most vascular beds where it has been tested (see Higgins &

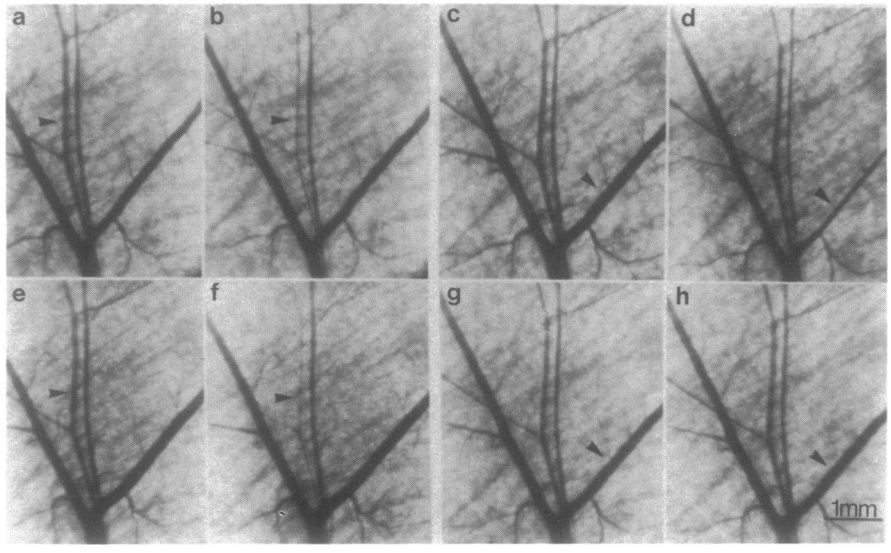

Figure 3 Effect of phenoxybenzamine (1 μg/ml) on the responses of rat cutaneous vasculature to prostaglandin $F_{2\alpha}$ (200 ng/ml) and noradrenaline (100 ng/ml): (a) control; (b) 2 min after application of prostaglandin $F_{2\alpha}$ (note arterial constriction at arrow) (c) control; (d) 2 min after application of noradrenaline (note venous constriction at arrow); (e) control after application of phenoxybenzamine for 15 min; (f) 2 min after application of prostaglandin $F_{2\alpha}$ (note arterial constriction still present); (g) control after application of phenoxybenzamine; (h) 2 min after application of noradrenaline (note absence of venous constriction at arrow).

Braunwald, 1972; Muirhead, 1973; Nakano, 1973), although constriction of dog nasal blood vessels (Stovall & Jackson, 1967) and rat mesocaecum metarterioles (Viguera & Sunahara, 1969) has been reported. Our results indicate that dose might be an important factor in determining the response observed with prostaglandin E_1 since higher concentrations of the drug (500 ng/ml to 10 μg/ml) did not produce vasoconstriction, presumably because some other action masked the constrictor response. The mechanisms of the dilator responses to prostaglandin E_1 observed at very low doses and very high doses were not investigated. However low doses of 5-HT produced vasodilatation and it is possible that the vasodilatation observed with low doses of prostaglandin E_1 might also be due to released 5-HT.

The seasonal variation observed in the response to prostaglandin E_1 might reflect some variation in the storage or release of 5-HT from rat mast cells. 5-HT levels in the central nervous system have been found to fall in rats in a hot environment and to rise in rats in a cold environment (Corrodi, Fuxe & Hökfelt, 1967), but it is not known whether there is any temperature or seasonal variation in the levels of 5-HT in rat mast cells. Prostaglandin E_1 has been shown to produce oedema by causing release of amines from mast cells (Crunkhorn & Willis, 1971) but Freeman & West (1972) found marked differences in the skin response to prostaglandin E_1 between rats from different breeding colonies. It is possible that seasonal variation might have been responsible for some of the differences observed by these workers. The greater sensitivity of the outer thigh skin vessels to the constrictor action of prostaglandin E_1 which was often observed, might reflect a larger number of mast cells in the skin of this area than in abdominal skin, or greater storage of 5-HT in mast cells of the outer thigh skin. Parratt & West (1957) found that the 5-HT levels in the skin of the groin of the rat were higher than those in the abdominal skin.

A major component of the constrictor response to prostaglandin E_2 also appeared to be due to released 5-HT but there was some component of the response which was not blocked by compound 48/80 or methysergide, and which, in time course and appearance, resembled the constrictor response to prostaglandin $F_{2\alpha}$. The constrictor response to prostaglandin $F_{2\alpha}$ was of longer duration, was more generalized over the whole lengths of the arteries, sometimes involved the small veins, and was more markedly tachyphylactic than that to prostaglandin E_1.

The site and mechanism of action of prostaglandin $F_{2\alpha}$ on the peripheral vasculature is controversial (see Muirhead, 1973; Nakano, 1973). Results from our experiments suggest that prostaglandin $F_{2\alpha}$ has two actions. Pretreatment with this prostaglandin appeared to enhance the venous constrictor effect of noradrenaline but antagonize the arterial constrictor effect. At the concentrations used in our experiments prostaglandin $F_{2\alpha}$ also produced a vasoconstrictor effect, predominantly on arteries, which did not involve 5-HT or adrenergic mechanisms since it was not blocked by methysergide or phenoxybenzamine. It would seem therefore that the observed response to prostaglandin $F_{2\alpha}$ might vary with both the dose and the level of the natural adrenergic tone. This might explain the apparently conflicting observations in the literature. In the presence of high adrenergic tone, prostaglandin $F_{2\alpha}$ might produce venoconstriction. A venopressor action, which was dependent on sympathetic innervation, has indeed been observed in the perfused dog limb (Ducharme, Weeks & Montgomery, 1968). However, an arterial constrictor effect independent of sympathetic innervation has also been described in the dog (Nakano, Perry & Denton, 1968). The decrease in sensitivity to the arterial constrictor action of noradrenaline might explain the lack of potentiation of noradrenaline on the perfused dog hind paw preparation (Kadowitz, Sweet & Brody, 1971; 1972), and the vasodilator action of prostaglandin $F_{2\alpha}$ in the cat (Änggård & Bergström, 1963). Further experiments are required to clarify the interaction of prostaglandins with adrenergic mechanisms on the cutaneous vasculature.

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