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PROSTAGLANDINS, KININ AND INFLAMMATION IN THE RAT

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1 Prostaglandin E_1 and bradykinin exert synergistic effects on vascular permeability in rat skin and in rat paws. Prostaglandin E_2 is inactive in this respect.

2 Prostaglandin $F_{2\alpha}$ reduces the bradykinin response in rat skin but not in rat paws. It also markedly reduces the response of the skin to dextran, 5-hydroxytryptamine and histamine.

3 Prostaglandin E_1 and dextran exert synergistic effects in rat skin when threshold doses of each agent are used.

4 Rats genetically resistant to dextran fail to respond to intradermal dextran in the presence of prostaglandin E_1.

Introduction

It has recently been reported (Crunkhorn & Willis, 1971; Freeman & West, 1972) that prostaglandins of the E series (e.g. prostaglandins E_1 and E_2) increase vascular permeability in the skin of rats whereas those of the F series (e.g. prostaglandin $F_{2\alpha}$) antagonize the effects of those of the E series. As the amounts of prostaglandins recoverable from inflammatory lesions are generally insufficient to account for the observed degrees of inflammatory reaction, they may interact with other putative mediators of inflammation.

Recent studies by Ferreira, Moncada & Vane (1973) showed that prostaglandins E_1 and E_2 potentiated, on intra-arterial injection into the dog spleen, the reflex increase in arterial blood pressure induced by bradykinin although they themselves were vasodepressor. Thomas & West (1973) found that threshold doses of prostaglandin E_1 injected into the skin of rats exerted synergistic effects on the increase in vascular permeability induced by threshold doses of bradykinin but not by those of histamine or 5-hydroxytryptamine (5-HT). These latter authors also showed that the responses in the skin to prostaglandin E_2 and to bradykinin were only additive although this prostaglandin was the one detected by Willis (1969) in exudates from carrageenan oedema. Furthermore, prostaglandin $F_{2\alpha}$ inhibited the vascular permeability-increasing potential of bradykinin, histamine and 5-HT, as well as that of dextran.

Using a different method of evaluation, Harrison (1973) showed that in the skin of guinea-pig and rabbit prostaglandin E_2 (in microgram doses) enhanced the effect of bradykinin but not that of histamine and prostaglandin $F_{2\alpha}$ failed to inhibit the effect of bradykinin on vascular permeability. These two results are in direct contrast to those previously mentioned for rats, although, as in rats, prostaglandin $F_{2\alpha}$ always antagonized the effect of prostaglandin E_2 alone.

We have studied in detail the interaction of prostaglandins and kinin in rat skin and rat hindpaws as well as the effect of some anti-inflammatory agents on these responses. The relative sensitivity of rats genetically resistant to dextran (Harris & West, 1963) has also been tested.

Methods

Female Wistar rats (150-210 g) were used. They were obtained from the ASH colony at Margate (hereinafter referred to as R rats as they reacted to dextran with the anaphylactoid reaction) and from the colony maintained at the North East London Polytechnic (referred to as NR rats as they always failed to react to dextran). A few animals from the Tuck colony at Rayleigh were also used. All animals had free access to food (diet 41B) and drinking water.

Skin tests

Groups of 10 rats had their backs shaved 24 h before being anaesthetized with pentobarbitone (40 mg kg^{-1} i.p.). After the intravenous injection of azovan blue (20 mg kg^{-1}), intradermal injections of prostaglandins E_1, E_2, or $F_{2\alpha}$, and

bradykinin, histamine, 5-HT or dextran, alone or as mixtures, were made into the shaved areas in dose volumes of 0.05 ml Tyrode solution. Control injections of solvent were always made in each animal. When anti-inflammatory agents were tested, they were administered either orally 3 h or 1 h, or intravenously 10 min, before the intradermal injections. Forty-five minutes later, the rats were killed and the effects of the agents on vascular permeability were measured spectrophotometrically by estimating the amount of dye (μg) in each weal, by the method of Harada, Takeuchi, Fukao & Katagiri (1971). To determine when potentiation or inhibition had occurred, the results obtained with the agents given alone were added and the total was subtracted from the results obtained when the two agents were given together. The differences were then expressed as percentages of the totals of the responses when the agents were given separately. In each experiment, the amount of dye extracted from the skin after control Tyrode injections was subtracted from all other values before calculations were made.

Paw volume tests

Groups of five rats were used. Prostaglandins E_1, E_2, $F_{2\alpha}$, bradykinin, or dextran, alone or as mixtures were injected subcutaneously into one hindpaw in a volume of 0.05 ml Tyrode solution, the other hindpaw receiving the same volume of Tyrode solution. The volume of both hindpaws was measured on a volume differential meter before and at 20 min after the injections. When dextran was used, further observations at 45 min were also made. The percentage increases in paw volume in each rat were then calculated, those in the control paw being subtracted from the values in the treated paw to give the calculated increase in paw volume resulting from the treatment. To determine when potentiation or inhibition had occurred, the results obtained with the agents given alone were added and the total was subtracted from the results obtained when the two agents were given together. The differences were expressed as percentages of the totals of the responses when the agents were given separately.

Results

Sensitivity to agents injected intradermally

Doses producing threshold increases in vascular permeability in R rats were 100 ng for bradykinin, 25 ng for prostaglandins E_1 and E_2, 1,000 ng for prostaglandin $F_{2\alpha}$, 10 μg for dextran, 20 ng for 5-HT, and 500 ng for histamine. Similar values were obtained in NR rats except for dextran which failed at all dose levels to produce an increased vascular permeability in the skin. Prostaglandins E_1 and E_2 were thus as active as 5-HT by this route in both colonies of rats.

Sensitivity to agents injected subcutaneously

Doses producing threshold increases in paw volume in both R and NR rats were 1,000-2,000 ng for bradykinin, 25 ng for prostaglandins E_1 and E_2, and 1,000 ng for prostaglandin $F_{2\alpha}$. Hence, the doses of the prostaglandins were similar to those used in the intradermal tests but that of bradykinin had to be increased 10 to 20 times. In R rats, the dose of dextran producing a threshold increase in paw volume was 25 μg.

Interaction of prostaglandins and bradykinin intradermally

When bradykinin was injected with prostaglandin E_1 in both R and NR rats, a synergistic effect

Table 1 Effect of intradermal prostaglandins (PG) and bradykinin (100 ng), together and separately, on vascular permeability in the skin of R rats.

	Interaction of bradykinin with:		
Agent injected	*PGE_1 (25 ng)*	*PGE_2 (25 ng)*	*$PGF_{2\alpha}$ (1,000 ng)*
Bradykinin with PG (a)	17.3 ± 2.8	9.8 ± 2.2	2.7 ± 0.5
PG alone (b)	5.2 ± 1.1	6.5 ± 1.3	0.2 ± 0.1
Bradykinin alone (c)	4.0 ± 0.9	3.9 ± 0.9	5.6 ± 1.0
(b) + (c)	9.2	10.4	5.8
% change in response $\frac{(a) - (b + c)}{(b + c)} \times 100$	+88	−6	−53
Significance on *t*-test	$P < 0.005$	N.S.	$P < 0.05$

Mean values ± s.e. mean of dye (μg) extracted from skin of 10 rats are shown. N.S. = not significant.

was observed when the dose range of bradykinin was 50-200 ng and that of prostaglandin E_1 was 5-100 ng. A typical result in R rats is shown in Table 1. Synergistic effects were not obtained with doses as low as 1 ng prostaglandin E_1 or as high as 400 ng bradykinin. The synergistic effect of bradykinin and prostaglandin E_1 also occurred in Wistar rats obtained from the Tuck colony (which respond to dextran).

Also shown in Table 1 are typical results of injecting into R rats mixtures of bradykinin and prostaglandin E_2 (where only an additive effect was found) and of bradykinin and prostaglandin $F_{2\alpha}$ (where a reduced effect was observed). Similar results with these two prostaglandins were obtained with NR rats and with Tuck rats.

When histamine (500 ng) or 5-HT (20 ng) was mixed with bradykinin (100 ng), a simple additive response was obtained. Similarly, 5-HT with histamine had only an additive effect.

Interaction of prostaglandins and other agents intradermally

When dextran was injected with prostaglandin E_1 into R rats, enhancement of the response occurred only when threshold doses were used (Table 2). This result contrasts with that previously reported by Thomas & West (1973) using higher doses of both agents. Prostaglandin E_1 did not significantly potentiate 5-HT or histamine (Table 2). Prostaglandin E_2 at the dose levels tested was additive with these agents, as well as with dextran, whilst prostaglandin $F_{2\alpha}$ inhibited the response of all three agents (Table 2). In NR rats, dextran and prostaglandin E_1 failed to give a response greater than that to prostaglandin E_1 alone.

Effect of anti-inflammatory agents

The interaction of prostaglandin E_1 and bradykinin intradermally in both R and NR rats was not modified by indomethacin (9 mg kg^{-1}, orally or 5 mg kg^{-1}, intravenously), aspirin (200 mg kg^{-1}, orally), or phenylbutazone (90 mg kg^{-1}, orally).

Interaction of prostaglandins and bradykinin subcutaneously

Bradykinin, injected subcutaneously into the hindpaw of both R and NR rats, exerted a synergistic effect with prostaglandin E_1. A typical result is shown in Table 3. Prostaglandin E_2 and bradykinin were additive, but prostaglandin $F_{2\alpha}$, in contrast to the effect found intradermally, failed to reduce the kinin effect.

When prostaglandin E_1 (25 ng) was injected with dextran (25 μg) into R rats, a synergistic effect was not obtained. Dextran at higher doses (up to 1 mg) did not produce a response in NR rats even in the presence of prostaglandin E_1 (25 ng).

Discussion

The present results suggest that the effects on vascular permeability of prostaglandin E_1 and bradykinin are synergistic when injected intradermally or subcutaneously into the hindpaw of rats. The amounts of both agents used to obtain synergism are similar to those previously extracted from inflammatory sites (Starr & West, 1967; Willis, 1969). Since the two agents presumably act independently, their effects on vascular permeability when applied together would be expected to be a simple addition of their individual responses; however, the effects were found to be more than additive.

Ferreira *et al.* (1973) found that both prostaglandin E_1 and E_2 potentiated the bradykinin response in the dog spleen and Harrison (1973) reported that prostaglandin E_2 enhanced the bradykinin response in the skin of rabbits and guinea-pigs. In the experiments on rats reported

Table 2 Interaction of intradermal prostaglandins (PG) with dextran, 5-hydroxytryptamine (5-HT) and histamine on vascular permeability in the skin of R rats.

	Interaction of agent with:				
	PGE_1		*PGE_2*		*$PGF_{2\alpha}$*
Agent injected	*(25 ng)*	*(100 ng)*	*(25 ng)*	*(100 ng)*	*(1,000 ng)*
Dextran (10 μg)	+ 77	N.D.	+ 14	N.D.	N.D.
Dextran (25 μg)	N.D.	− 4	N.D.	− 6	− 70*
5-HT (20 ng)	+ 20	+ 24	+ 6	− 6	− 70*
Histamine (500 ng)	+ 23	+ 25	+ 5	+ 8	− 60*

Mean values for percentage change in response in 10 rats are shown.
N.D. = not done. * = significant at $P < 0.05$.

Table 3 Effect of subcutaneous prostaglandins (PG) and bradykinin (2,000 ng), together and separately, on paw volume of R rats.

	Interaction of bradykinin with:		
Agent injected	*PGE_1 (25 ng)*	*PGE_2 (25 ng)*	*$PGF_{2\alpha}$ (1,000 ng)*
Bradykinin with PG (a)	30.1 ± 3.3	20.3 ± 2.1	11.6 ± 3.0
PG alone (b)	4.6 ± 2.1	4.4 ± 2.0	4.1 ± 1.7
Bradykinin alone (c)	9.9 ± 2.2	14.4 ± 1.7	9.3 ± 3.3
(b) + (c)	14.5	18.8	13.4
% change in response $\frac{(a)-(b+c)}{(b+c)} \times 100$	+ 108	+ 7	− 14
Significance on *t*-test	$P < 0.01$	N.S.	N.S.

Mean values ± s.e. mean of percentage increase in paw volume of 5 rats are shown. N.S. = not significant.

here prostaglandin E_2 and bradykinin were found to be only additive in the skin; so there are species differences in response. It is possible, however, that the optimal conditions for obtaining synergism of prostaglandin E_2 and bradykinin in the rat have so far not been found.

The interaction of prostaglandin E_1 with bradykinin was relatively specific as no such enhancement occurred when prostaglandin E_1 was mixed with 5-HT or histamine and injected intradermally into rat skin. We have not tested the interaction of prostaglandin E_1 and histamine or 5-HT in the rat paw as Glenn, Bowman & Rohloff (1972) have already reported a simple additive effect. With dextran in R rats, it was possible to obtain potentiation by prostaglandin E_1 by using doses producing only threshold responses but the effects of larger doses were additive. Dextran releases 5-HT and histamine when injected into R rats and slight but not significant potentiation of these responses by prostaglandin E_1 were found (Table 2) and may therefore account for the potentiation of dextran by prostaglandin E_1. However, a high dose of dextran (1 mg subcutaneously) in NR rats was ineffective even in the presence of amounts of prostaglandin E_1 which produced

With anti-inflammatory agents, Ferreira *et al.* (1973) found in dog spleen that intravenous indomethacin reduced the response to intra-splenic bradykinin which was then increased again by intra-splenic prostaglandin E_1 or E_2. The prostaglandin released within the spleen (by bradykinin) was considered to sensitize the sensory nerve endings to bradykinin and indomethacin abolished the prostaglandin-induced facilitation, possibly by inhibiting prostaglandin synthesis and release. However, in the present experiments with rats, indomethacin, aspirin and phenylbutazone all failed to modify the synergism between intradermal bradykinin and prostaglandin E_1.

potentiation in R rats. Therefore it is concluded that prostaglandin E_1 was incapable of lowering the threshold to dextran in NR rats and dextran remained ineffective in these animals. It is also unlikely that NR rats possess excess prostaglandin $F_{2\alpha}$, an effective inhibitor of the dextran response in R rats, as such amounts would also markedly raise the threshold of NR rats to 5-HT and histamine; such was not the case as the sensitivity of all R and NR rats tested, to prostaglandins E_1, E_2, and $F_{2\alpha}$, histamine, 5-HT and bradykinin, was similar.

References

CRUNKHORN, P. & WILLIS, A.L. (1971). Cutaneous reactions to intradermal prostaglandins. *Br. J. Pharmac.*, **41**, 49-56.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1973). Further experiments to establish that the analgesic action of aspirin-like drugs depends on the inhibition of prostaglandin biosynthesis. *Br. J. Pharmac.*, 47, 629-630P.

FREEMAN, P.C. & WEST, G.B. (1972). Skin reactions to prostaglandins. *J. Pharm. Pharmac.*, **24**,, 407-408.

GLENN, E.M., BOWMAN, B.J. & ROHLOFF, N.A. (1972). Proinflammatory effects of certain prostaglandins. In: *Prostaglandins in Cellular Biology*, vol. 1, ed. Ramwell, P.W. & Pharris, B.B. pp. 329-342. New York & London: Plenum Press.

HARADA, M., TAKEUCHI, M., FUKAO, T. & KATAGIRI, K. (1971). A simple method for the quantitative extraction of dye extravasated into skin. *J. Pharm. Pharmac.*, **23**, 218-219.

HARRIS, J.M. & WEST, G.B. (1963). Rats resistant to

the dextran anaphylactoid reaction. *Br. J. Pharmac. Chemother.*, **20**, 550-562.

HARRISON, R.G. (1973). Prostaglandins, kinins and amines–synergistic effects on skin vascular permeability. *I.R.C.S.* (In press.)

STARR, M.S. & WEST, G.B. (1967). Bradykinin and oedema formation in heated paws of rats. *Br. J. Pharmac. Chemother.*, **31**, 178-187.

THOMAS, G. & WEST, G.B. (1973). Prostaglandins as modulators of bradykinin responses. *J. Pharm. Pharmac.*, **25**, 747-748.

WILLIS, A.L. (1969). Parallel assay of prostaglandin-like activity in rat inflammatory exudate by means of cascade superfusion. *J. Pharm. Pharmac.*, **21**, 126-128.

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