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Relationship between the chemical structure of prostaglandins and their vasoactivities in dogs

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Summary

1. The relationship between the chemical structure and the direct vasoactivity of different prostaglandins administered intra-arterially was studied in the dog hindlimb preparation.

2. All of the prostaglandins studied, except $PGF_{1\alpha}$ and $PGF_{2\alpha}$, caused a dose related decrease in the femoral arterial perfusion pressure in dogs in which the femoral arterial blood flow was kept constant, indicating the direct vasodilator action of these prostaglandins.

3. Among the prostaglandins studied, PGE_1 is the most potent vasodilator. Comparing the chemical structure and vasodilator action of PGE_1 with those of different prostaglandins, the following conclusions can be made:

4. The formation of the Δ^5 double bond in PGE_1 causes no change in its vasodilator activity, whereas the saturation of the Δ^{13} double bond of PGE_1 slightly reduces its activity.

5. The alterations in the orientation and length of the carboxyl and alkyl side chains reduce markedly the vasodilator action of PGE- and PGA-compounds.

6. The presence of a carbonyl group at C9 is the most important requirement for the potent vasodilator action of PGE_1. On the other hand, the presence and S-configuration of a hydroxyl group at C15 are essential for the intrinsic action at the receptor sites in the vascular smooth muscle, but may not be responsible for the vasodilator action.

7. The esterification of PGE_1 or PGE_2 and a triple bond formation and the replacement of C7 with oxygen in prostaglandin appear to reduce or abolish their vasodilator action.

Introduction

Several investigators (Bergström & von Euler, 1963 ; Horton & Main, 1963, 1966 ; Pike, Kupiecki & Weeks, 1967 ; DuCharme, Weeks & Montgomery, 1968 ; Nakano & McCurdy, 1968 ; Nakano & Cole, 1969) showed that, when administered intravenously, both PGE- and PGA-compounds are potent depressor agents whereas PGF compounds are moderate pressor agents in rats and dogs. PGE-compounds are effectively inactivated by a single passage through the lungs (Ferreira & Vane, 1967 ; Nakano, 1970a) whereas PGA- compounds are more resistant to metabolism in the lungs (Horton & Jones, 1969 ; McGiff, Terragno, Strand, Lee, Lonigro &

Ng, 1969). Hence, it is rather difficult to assess the relationship between the structure of prostaglandins and their direct vasoactivities when one compares the depressor or pressor effect of different prostaglandins administered intravenously. Recently, as well as naturally occurring prostaglandins, several synthetic prostaglandin analogues have been synthesized by various investigators (Fried, Santhanakishnan, Himizu, Lin, Ford, Rubin & Grigas, 1969 ; Kloeze, 1969 ; Pike *et al.*, 1967 ; Ramwell, Shaw, Corey & Andersen 1969 ; Lippman 1969). Although Kloeze (1969) studied the relationship between the structure and platelet aggregation activity of prostaglandins, no systematic study has been made on the relationships between the structure and direct vasoactivity of different prostaglandins. The present study was undertaken to compare the direct vasodilator effect of the intra-arterial injection of graded doses of twenty-four different prostaglandins in the isolated dog hind limb preparation.

Methods

Fifty-four dogs weighing 15–22 kg were anaesthetized by intravenous administration of sodium pentobarbitone (30 mg/kg). The technique used to study the direct effect of prostaglandins on the femoral vascular beds was described previously (Haddy, Molnar & Campbell, 1961 ; Nakano & McCloy, 1967). A femoral artery was cannulated distally and perfused at a constant and known rate with blood bypassed from a contralateral femoral artery by means of a Sigmamotor pump. Systemic arterial pressure and femoral arterial perfusion pressure were continuously measured with Statham pressure transducers (P23AA) (1 mmHg$\equiv$1·333 mbar). In this preparation, the direct effect of different prostaglandins on the vascular resistance was evaluated readily by changes in the femoral arterial perfusion pressure. Both pressures measured were continuously recorded with a Grass polygraph (Model 7). All prostaglandins and their analogues were dissolved in 20 μl of 95% ethanol and further diluted to 1–10 μl/ml solutions with 0·9% NaCl solution before its intra-arterial injection in dogs. Dilutions were made such that the amount injected never exceeded 0·1 ml. The data in this paper were evaluated statistically using the *t* test (Snedecor, 1956).

Materials

The chemical structure of prostaglandins studied is illustrated in Fig. 1. Prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2), prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), prostaglandin $F_{1\beta}$ ($PGF_{1\beta}$), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin A_1 (PGA_1), prostaglandin A_2 (PGA_2), 8-isoprostaglandin E_1 (8-iso-PGE_1) and 15-R-prostaglandin E_1 (15-R-PGE_1) were generously supplied by Dr. J. E. Pike, the Upjohn Co., Kalamazoo, Mich ; compounds AY-16809, AY-21670 and AY-22093 by Dr. J. F. Bagli, Ayerst Laboratories, Montreal, Canada ; PGE_1-ethylester, PGE_1-isobutylester, ω-homo-PGA_2, PGE_2-m-trifluoro-methylphenylester and PGE_2-p-ethylphenylester by Dr. T. Sano, Ono Pharmaceutical Co., Osaka, Japan ; 7-oxa-13,14-prostanoic acid by Dr. J. Fried, Department of Chemistry, University of Chicago, Chicago, Ill., and 15-R-PGA_2 by Dr. A. Weinheimer, Department of Chemistry, University of Oklahoma, Norman, Oklahoma, respectively. 11α-Hydroxy-9, 15-diketo-13-prostanoic acid (15-keto-PGE_1) was synthesized by MnO_2 oxidation of PGE_1 in this laboratory according to the method described by Attenburrow, Cameron, Chapman, Evans,

Hems, Jansen & Walker (1952). 11α-, 15S-dihydroxy-9-keto-prostanoic acid (dihydro-PGE_1) and 11α-, 15S-dihydroxy-9, 15-diketo-prostanoic acid (15-keto-dihydro-PGE_1) were also synthesized by catalytic hydrogenation (using rhodium as a catalyst) in this laboratory from PGE_1 and 15-keto-PGE_1, respectively, by the method described by Bergström, Ryhage, Samuelsson & Sjövall, 1963). All three metabolites were purified by silicic acid chromatography and thin-layer chromatography using slight modifications (Nakano, 1970a, 1970b, 1971) of the method described by Samuelsson (1963) and Green & Samuelsson (1964).

Results

Almost all naturally occurring prostaglandins such as PGE_1, PGE_2, PGA_1, PGA_2 and 8-iso-PGE_1 decreased the femoral arterial perfusion pressure, essentially in proportion to the dose, indicating their direct vasodilator action in dogs. Since both PGE_1 and PGE_2 are the most potent vasodilators, from the dose-response curves of each prostaglandin the vasodilator potency of the test compound was compared with that of PGE_1 at doses which decreased the perfusion pressure by 20 mmHg. The results are summarized by the order of the magnitude of the vasodilator potency in Fig. 1. No significant qualitative and quantitative difference was observed between the vasodilator action of PGE_1 and that of PGE_2 or between PGA_1 and PGA_2. However, the vasodilator effect of PGE compounds was greater than that of PGA compounds. The vasodilator action of PGA compounds was greater than that of 8-iso-PGE_1. Among the prostaglandins studied, only $PGF_{1\alpha}$ and $PGF_{2\alpha}$ caused a slight increase in the femoral arterial perfusion pressure, indicating a mild vasoconstrictor action in dogs. A PGE_1 metabolite, dihydro-PGE_1, exerts a less potent vasodilator action than PGA_1, but more potent action than 8-iso-PGE_1. However, dihydro-PGE_1 exerts the most potent vasodilator action among the three major PGE_1 metabolites studied. The synthetic prostaglandins such as AY-22093, AY-21670 and AY-16809, ω-homo-PGA_2, PGE_1- and PGE_2-esters, and the stereo-epimers of PGE_1 and PGA_2, 15-R-PGE_1 and 15-R-PGA_2 exerted a very feeble vasodilator action. A compound, 7-oxa-13, 14-prostynoic acid, which inhibits the effect of prostaglandins on non-vascular smooth muscle caused no significant change in the femoral arterial pressure at a dose of up to 100 μg/kg. Furthermore, the intra-arterial injection of high doses (10–100 μg/kg) of this inhibitor had no significant effect on the vasodilator action of PGE_1 in the femoral artery.

Discussion

Horton & Main (1963, 1966), Pike *et al.* (1967), Kloeze (1969) and Ramwell *et al.* (1969) have studied the relationship between the chemical structure and the biological activities of different prostaglandins. Most studies on the structure activity of prostaglandins in the circulatory system have been made on their pressor or depressor activities in rats, guinea-pigs and dogs. Since some prostaglandins are inactivated in the lungs, the precise structure-activity relationship of prostaglandins in the circulation cannot be assessed by studying the effects of intravenous injections.

The structure-activity relationship can be analysed in terms of the chemical modification of the three major components of the structure of a prototype prostaglandin, PGE_1, that is, carboxyl side chain, cyclopentane ring and alkyl side chain.

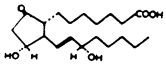
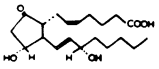
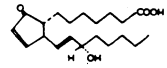
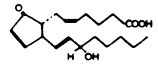
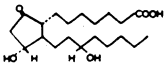
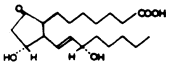
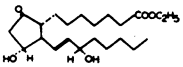
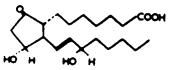
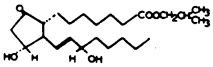
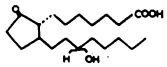
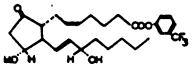
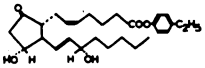
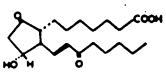
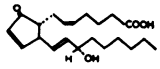
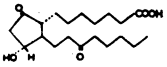
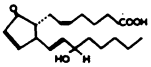
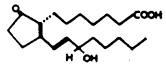
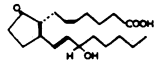
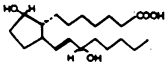
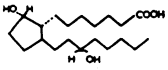
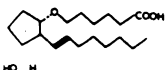
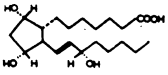
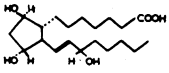
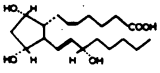
Structure	PG	Relative vasodilator potency
	PGE_1	100·0
	PGE_2	102·0
	PGA_1	32·2
	PGA_2	30·7
	Dihydro-PGE_1	20·7
	8-ISO-PGE_1	8·5
	PGE_1-ethylester	4·1
	15-R-PGE_1	4·0
	PGE_1-isobutylester	3·4
	AY-22093	3·0
	PGE_2-m-CF_3-phenylester	2·1
	PGE_2-p-ethyl-phenylester	2·0
	15-keto-PGE_1	2·0
	ω-Homo-PGA_2	1·2
	Dihydro-15-keto-PGE_1	0·8
	15-R-PGA_2	0·05
	PGB_1	0·02
	PGB_2	0·02
	AY-16809	<0·01
	AY-21670	<0·01
	7-Oxa-13,14-prostynoic acid	0
	$PGF_{1\alpha}$	mild vasoconstrictor
	$PGF_{1\beta}$	0
	$PGF_{2\alpha}$	mild vasoconstrictor

FIG. 1. Chemical structures and the relative vasodilator potency of twenty-four different prostaglandins injected intra-arterially in the dog hindlimb. The vasodilator potency of each prostaglandin was compared with that of PGE_1 (100).

(a) *Carboxyl side chain variations*

The modification of the carboxyl side chain appears to influence greatly the vasodilator action of PGE_1. The magnitude of the vasodilator action of PGE_1 and PGA_1 is almost equivalent to that of PGE_2 and PGA_2, respectively. Hence, as seen with PGE_2 and PGA_2, the unsaturation of PGE_1 at C5 does not appear to modify the vasodilator potency of PGE_1 or PGA_1. PGE_1-ethylester, PGE_1-isobutylester, PGE_2-*m*-trifluoromethylphenylester and PGE_2-*p*-ethylphenylester exert considerably weaker vasodilator action than PGE_1 and PGE_2. This indicates that the esterification of PGE_1 and PGE_2 markedly reduces the vasodilator activity of PGE_1 and PGE_2. As seen in 7-oxa-13, 14-prostynoic acid, the replacement of C7 in PGE_1 by an oxygen atom abolishes the vasodilator action, but it is not certain whether the absence of the vasodilator action in this compound is due to the alteration of the side chain alone, or of the cyclopentane ring. However, similar loss of the biological activities was also observed with 3-oxo-PGE_1 or 7-oxo-PGE_1 (Fried *et al.*, 1969). Furthermore, the nonvascular smooth muscle-stimulating action of PGE_1 is competitively antagonized by 7-oxa-13, 14-prostynoic acid, although no such inhibition was noticed with the vascular smooth muscles in the present study. When the entire trans-carboxyl side chain of PGE_1 is altered to the *cis*-orientation relative to the cyclopentane ring plane, as seen with 8-*iso*-PGE_1, the vasodilator potency of PGE_1 is markedly diminished (Nakano & Kessinger, 1970). In addition, it was found that 8-*iso*-PGE_1 is a mild vasodilator in the systemic circulation but exerts a vasoconstrictor action on the pulmonary vascular beds in dogs. Pike *et al.* (1967) showed that both PGB_1 and PGB_2 cause very little or no effect on the systemic arterial pressure in rats. Likewise, in the present study, both PGB_1 and PGB_2 exert very feeble vasodilator actions. The planar orientation of both alkyl and carboxyl side chains has been markedly altered in PGB_1 and PGB_2, because of the formation of C8, C12-double bond. This appears to reduce the affinity of PGB_1 and PGB_2 to receptor sites and hence results in little or no vasodilator action. Since sufficient amounts of the major urinary metabolites, dinor- or tetranor-prostaglandin compounds, are not available for experimental studies at present, the biological actions of these compounds remain uncertain. However, these metabolites are almost always oxidized at 15 C position and reduced at Δ^{13} double bond as 11-hydroxy-11, 15-diketo-tetranor-prostanoic acid or 11-hydroxy-11, 15-diketo-ω-carboxy-tetranor-prostanoic acid and hence their cardiovascular actions may not be prominent. Furthermore, a previous study from this laboratory (Nakano & Cole, 1969) showed that the injection of PGE_1 (4 μg/kg) in the portal vein does not change systemic arterial pressure significantly, whereas the intravenous injection of the same dose of PGE_1 exerts a marked depressor action. This also suggests that the beta-oxidation of PGE_1 in the liver greatly reduces the circulatory action of PGE_1.

(b) *Cyclopentane ring variations*

PGE and PGA compounds are powerful vasodilators in many species of animals, whereas $PGF_{2\alpha}$ is a moderately potent vasoconstrictor in dogs and rabbits (DuCharme *et al.*, 1968 ; Nakano & Cole, 1969). The presence of a carbonyl group at C9 must be essential for the vasodilator or other biological actions of PGE_1, (Kloeze, 1969). As seen with AY-16809, AY-21670, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, the absence of this radical or the replacement by a hydroxyl group not only reduces the vasodi-

lator activity considerably but also may change $PGF_{1\alpha}$ and $PGF_{2\alpha}$ to vasoconstrictor agents. Lippmann (1969) also found that both AY-16809 and AY-21670 exert very weak antacid action in the rat stomach. As seen with PGA_1 and PGA_2, the removal of a hydroxyl group at C 11 and formation of Δ^{10} double bond in the structure of PGE_1 and PGE_2 slightly reduces the vasodilator action of PGE_1 and PGE_2. Weeks, Sekhar & DuCharme (1969) showed that PGA_1 and PGA_2 have equipotent hypotensive action in dogs. Both PGA_1 and PGA_2 exert 2·5 times more hypotensive action than PGE_1, whereas PGE_1 exerts slightly less depressor action than PGE_2 in dogs. As seen in the present study, the magnitude of the vasodilator action of intra-arterially injected PGA_1 or PGA_2 is slightly smaller than that of intra-arterially injected PGE_1 or PGE_2 in dogs. However, the magnitude of the depressor action of intravenous injection of PGA_1 or PGA_2 is generally greater than that of PGE_1 or PGE_2. Many investigators (Ferreira & Vane, 1967; Horton & Jones, 1969; McGiff *et al.*, 1969; Nakano & Cole, 1969; Pike *et al.*, 1968) have attributed this difference in the depressor response to the greater metabolic degradation of intravenously injected PGE_1 or PGE_2 by 15-hydroxy-prostaglandin dehydrogenase in the lungs (Änggård & Samuelsson, 1964, 1966; Nakano, 1970a). The Δ^{10} double bond appears to exert more potent vasodilator action since the vasodilator action of PGA compounds is much greater than that of AY-22093.

(c) *Alkyl side-chain variations*

The length of this side chain appears to be very critical for the vasodilator action of prostaglandins since ω-homo-PGA_2, which has one excessive ω-carbon, exerts little vasodilator action. The magnitude of the vasodilator action of three metabolites, dihydro-PGE_1, 15-keto-PGE_1 and 15-keto-dihydro-PGE_1, is considerably smaller than that of PGE_1. Among the three metabolites, dihydro-PGE_1 exerts the most potent vasodilator action, indicating that the saturation of Δ^{13} double bond causes little effect on the vasodilator action. In contrast, the oxidation of the secondary alcohol group at C 15 in PGE_1 results in a marked diminution of its vasodilator action. This indicates that the hydroxyl group at C 15, as seen with all prostaglandins, is important in the vasoactive receptor site. Änggård (1966), Pike *et al.* (1967) and Nakano (1971) showed that a prostaglandin metabolite, 15-keto-PGE_1, which lacks a hydroxyl group at 15 C, exerts very little or no biological action in dogs and rats. The stereochemical orientation of this hydroxyl group is critical for the biological actions of prostaglandins. However, this is not a determinant functional group for vasodilator action since $PGF_{2\alpha}$ has a 15-hydroxyl group but is a vasoconstrictor. In the present experiment, no study was made on the vascular action of 19-hydroxylated prostaglandins such as 19-hydroxy-PGA_1 and 19-hydroxy-PGB_1. However, Horton & Jones (1969) showed that the depressor effect of 19-hydroxy-PGA_1 is approximately 1/20 that of PGA_1 whereas 19-hydroxy-PGB_1 has no effect. This indicates that hydroxylation at C 19 in prostaglandins reduces markedly the vasodilator action. With 19-hydroxy-PGB_1, the loss of the vasodilator activity is mostly due to the alteration in the cyclopentane ring rather than to the hydroxylation of C 19. Biologically active prostaglandins have a hydroxyl group at C 15 in the S-orientation (Cahn, Ingold & Prelog, 1955). Nakano (1969) found that PGE_1 (15-S-PGE_1) and PGA_2 (15-S-PGA_2) are very potent vasodilator and hypotensive agents, whereas their epimers, 15-R-PGE_1 and 15-R-PGA_2, have

little or no vasodilator or hypotensive effects in anaesthetized dogs. These observations suggest that the receptor sites in the vascular smooth muscle for prostaglandins are stereospecific for their intrinsic action. It is of interest that, according to recent observations made by Nakano, Änggård & Samuelsson (1969), 15-hydroxyprostaglandin dehydrogenase also has a similar stereospecificity for its substrates. PGE_1 (15-S-PGE_1) is the best substrate for this enzyme whereas its epimer, 15-R-PGE_1, is not a substrate but an inhibitor for this enzyme with PGE_1 as a substrate *in vitro* (Nakano *et al.*, 1969). Recently, 15-methyl-PGA_2 and 15-methyl-PGE_2 have been synthesized but no studies have been reported on their biological actions. No data are yet available on the vasoactivity of omega-hydroxylated prostaglandins.

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