References

ARMSTRONG, J.M., BLACKWELL, G.J., FLOWER, R.J., McGIFF, J.C. & MULLANE, K. (1975). Possible contribution of prostaglandins to genetic hypertension in rats: identification of a biochemical lesion. *Br. J. Pharmac.*, **55**, 244P.

MALIK, K.U. & McGIFF, J.C. (1975). Modulation by prostaglandins of adrenergic transmission in the isolated perfused rabbit and rat kidney, *Circ. Res.*, **36**, 599–609.

MALVIN, R.L. & VANDER, A.J. (1967). Effects of angiotensin infusion on renal function in the unanaesthetized rat. *Am. J. Physiol.*, **213**, 1205–1208.

Production of 6-oxo-prostaglandin $F_{1\alpha}$ by rat, guinea-pig and sheep uteri, *in vitro*

R.L. JONES, N.L. POYSER & N.H. WILSON

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, Scotland

We have recently shown that 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) is the major prostaglandin produced following the incubation of pseudopregnant rat uterine homogenates (Fenwick, Jones, Naylor, Poyser & Wilson, 1977). We have now extended these studies to guinea-pig and sheep uteri. Whole uterine homogenates were incubated at 37°C for 90 min and the prostaglandins produced extracted by organic solvent (Poyser, 1972). Guinea-pig and rat extracts were further purified by silicic acid column chromatography. PGE and PGF fractions were obtained with 6-oxo-$PGF_{1\alpha}$ (and PGD_2) being present in the PGE fraction. Amounts of PGE_2 and $PGF_{2\alpha}$ produced were estimated by both radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS).

Sheep uterine extracts were purified by straight-phase gel partition chromatography (Brash & Jones, 1974). The order of elution from the column is PGD_2 and 6-oxo-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$. These substances were submitted to methyl ester (Me), n-butyloxime (BuO) and trimethylsilyl ether (TMS) derivitization for identification and estimation by GC-MS (see Table 1).

In the rat and sheep samples, 6-oxo-$PGF_{1\alpha}$ was the major product identified, whereas in the guinea-pig more $PGF_{2\alpha}$ than 6-oxo-$PGF_{1\alpha}$ was produced. Sheep uteri were able to use exogenous arachidonic acid as evidenced by the incorporation of radioactivity in the 6-oxo-$PGF_{1\alpha}$ molecule when [1–^{14}C] arachidonic acid was added to the homogenate. Also, deuterated 6-oxo-$PGF_{1\alpha}$ was formed when 5, 6, 8, 9, 11, 12, 14, 15-octadeutero-arachidonic acid was used as substrate. The deuterated 6-oxo-$PGF_{1\alpha}$ showed complete loss of one deuterium atom (from carbon 6) and partial loss of a second since the deuterium atom on carbon 5 is now adjacent to the 6-oxo group and is thus exchangeable with the medium. Recovered deuterated $PGF_{2\alpha}$ showed negligible loss of deuterium.

6-oxo-$PGF_{1\alpha}$ runs as a single substance on GC provided that the ketone group is protected by oxime of 6-oxo-$PGF_{1\alpha}$. This suggests that the cyclic ether can be produced from the hemiacetal form of 6-oxo-

Table 1 Prostaglandin production by rat, guinea-pig and sheep uteri, *in vitro*

Species	*Added Arachidonic Acid (μg/ml)*	*Amount of PGs recovered (ng/g)*			
		6-oxo-$PGF_{1\alpha}$	*$PGF_{2\alpha}$*	*PGE_2*	*PGD_2*
Rat (pooled pseudopregnant)	nil	3000	800+	200+	30
Guinea-pig (pooled, dioestrus)	nil	250	600+	100+	20
Sheep (pooled, nonpregnant)	10	3200	<50	<100	<100
Sheep (pooled, nonpregnant)	10	1800	<10	<20	<20
Sheep (pooled, nonpregnant)	10	530	20	55	<10

+ Values obtained by radioimmunoassay; other values from gas chromatography-mass spectrometry.

formation (though 2 oxime isomers are formed). If oximation is omitted and the Me-TMS derivative is formed, two peaks are seen on GC analysis. The major peak has a retention time (C value = 24.3) and mass spectrum identical to 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (cyclic ether), as described by Pace-Asciak and Wolfe (1971) and derived from rat stomach homogenates. The second peak (C value = 25.7) is probably the open chain form $PGF_{1\alpha}$ during derivitization and gas chromatography and that it is not present in the original extracts. The biosynthetic route from arachidonic acid to 6-oxo-$F_{1\alpha}$ in the uterus is under investigation.

This study was supported by the Medical Research Council.

References

BRASH, A.R. & JONES, R.L. (1974). Straight phase separation of prostaglandin methyl esters on lipophilic gels. *Prostaglandins*, **5**, 441–454.

FENWICK, L., JONES, R.L., NAYLOR, B., POYSER, N.L. & WILSON, N.H. (1977). Production of prostaglandins by the pseudopregnant rat uterus, in vitro, and the effect of tamoxifen with the identification of 6-keto-prostaglandin $F_{1\alpha}$ as a major product. *Br. J. Pharmac.*, **59**, (in press).

PACE-ASCIAK, C. & WOLFE, L.S. (1971). A novel prostaglandin derivative formed from arachidonic acid by rat stomach homogenates. *Biochemistry*, N.Y., **10**, 3657–3664.

POYSER, N.L. (1972). Production of prostaglandins by the guinea-pig uterus. *J. Endocr.*, **54**, 147–159.

The potentiation of exogenous noradrenaline by prostaglandins $F_{2\alpha}$ C_2 and D_2 on the canine saphenous vein

R.R. JEFFREY & G.W. SMITH (introduced by A. UNGAR)

Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ, Scotland

Kadowitz, Sweet & Brody (1971) have shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) potentiates the response of venous smooth muscle to exogenous noradrenaline (NA). PGD_2 acts similarly to $PGF_{2\alpha}$ on several preparations (Horton & Jones, 1974) whilst PGC_2 has similar actions to PGE_2 (Jones, Kane & Ungar, 1974). Experiments are reported here which compare the potencies of $PGF_{2\alpha}$, PGD_2 and PGC_2 on a single preparation.

The method used was a modification of that described by Webb-Peploe & Shepherd (1968) and was used to determine the venous response to exogenous NA before and during infusion of the various PGs. Both lateral saphenous veins were cannulated at the ankle and perfused separately, one serving as a control for the other. NA was injected proximal to the pump and once a dose dependent relationship was obtained, the PG infusion was commenced.

$PGF_{2\alpha}$ (1 μg/min) caused a significant potentiation of the response and the calculated potency ratio (R) from the pooled results of three preparations was 6. PGD_2 (1 μg/min) was not as potent (R = 3, from six preparations) and PGC_2 (1 μg/min) was the least potent (R = 2, from three preparations). Using linear regression analysis, the results during PG infusion (1 μg/min) were significantly different from control ($P < 0.05$). In the two preparations, $PGF_{2\alpha}$ (0.1 μg/min) was found to potentiate the response (R = 2). However, at 5 μg/min there were considerable baseline changes and no satisfactory tests were performed. At 5 μg/min PGC_2 caused similar baseline changes. Up to 10 μg/min, PGD_2 was apparently no more potent than at 1 μg/min and no baseline shift occurred.

The results with $PGF_{2\alpha}$ are largely in agreement with those of Kadowitz *et al.* (1971) and a similar potency ratio for $PGF_{2\alpha}$ (1 μg/min) has been obtained. We have found PGD_2 to be considerably less potent than $PGF_{2\alpha}$, whereas it is up to 60 times more potent than $PGF_{2\alpha}$ as a direct vasoconstrictor in the sheep (Jones, 1975). PGC_2 which from our results is only weakly active in potentiating the effects of NA, is in contrast a potent dilator of both resistance and capacitance vessels in the dog (Jones, Kane & Ungar, 1974).

References

HORTON, E.W. & JONES, R.L. (1974). Biological activity of prostaglandin D_2 on smooth muscle. *Br. J. Pharmac.*, **52**, 110–111P.

JONES, R.L. (1975). Actions of prostaglandins on the arterial system of the sheep: some structure-activity relationships. *Br. J. Pharmac.*, **53**, 464P.

JONES, R.L., KANE, A.K. & UNGAR, A. (1974). Cardiovascular actions of PGC_2 in the cat and dog. *Br. J. Pharmac.*, **51**, 157–160.

KADOWITZ, P.J., SWEET, C.S. & BRODY, M.J. (1971). Potentiation of adrenergic venomotor responses by Angiotensin, $PGF_{2\alpha}$ and cocaine. *J. Pharmac. exp. Ther.*, **176**, 167.

WEBB-PEPLOE, M.M. & SHEPHERD, J.T. (1968). Response of large hindlimb veins of the dog to sympathetic nerve stimulation. *Am. J. Physiol.*, **215**, 299–307.