J. Pharm. Pharmac., 1974, **26**, 270

COMMUNICATIONS

Prostaglandins in rat tissues

During an investigation into the effects of increasing the blood volume in anaesthetized rats using the blood-bathed organ technique we detected prostaglandin-like substances (PLS) which were released following the rise of the blood pressure produced by infusion of either saline or blood. In experiments in which blood was cross-circulated in rats the blood pressure of the recipient dropped while that of the donor rose following the increase of circulating blood volume produced by infusion of either saline or blood. The phenomenon was almost imperceptible when the animals were binephrectomized (Papanicolaou, 1972).

We now have evidence that the prostaglandins PGA, PGE and PGF were released following the rise of blood pressure produced either by infusion of 10 ml of saline, or 8 ml of blood, at 2 ml min^{-1}, in anaesthetized rats. PLS were also detected using the blood-bathed organ technique when the rats were binephrectomized, but the amounts were less than those in normal rats (work submitted for publication). It was suggested that a large amount of PLS was released from the kidneys. The rat blood pressure preparation is less sensitive in the detection of some PGs than is the blood-bathed organ technique (Horton & Jones, 1969), which could explain why it was possible to detect PLS by using the latter but not the former method, when rats were binephrectomized.

This report concerns the isolation of biologically active substances in rat renal medulla, brain, spleen, liver and lung, having the chromatographic behaviour and the bioassay properties of PGs. No PGs were detected in rat renal cortex.

Fig. 1 shows the procedure used for the extraction, identification and the quantitative bioassay estimation of PGs in 10 g of each tissue. The same procedure was used for the recovery.

Usually 20 g of each tissue was taken from several rats and homogenized for 2 min in 400 ml of saline (20 ml g^{-1} of tissue), 400 ml of ethanol (20 ml g^{-1} of tissue) and 200 mg of (−)-ascorbic acid g^{-1} of tissue. Half (400 ml, 10 g of tissue) was extracted for PGs content and half to estimate recovery using 1000 ng each of PGA_2, PGE_2 and $PGF_{2\alpha}$. In initial experiments (rat renal medulla and brain), 30 g of each tissue was used, 10 g for extraction of PGs, 10 g for recovery procedures (where ascorbic

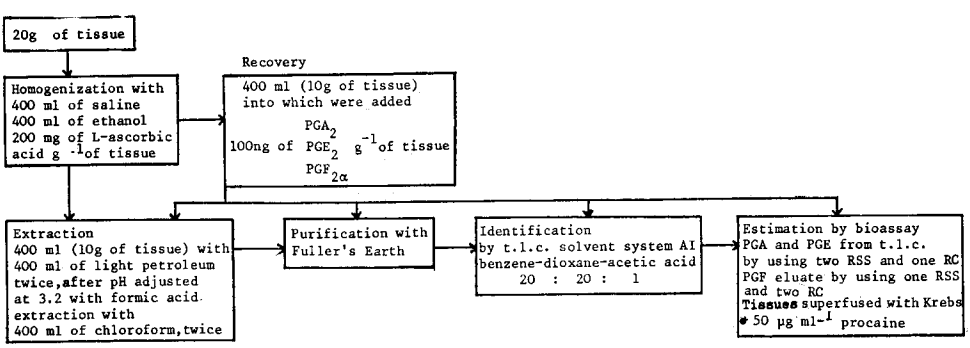

FIG. 1. A schematic portrayal of the procedure used for the extraction, identification and quantative bioassay estimation of prostaglandins.

acid was used) and 10 g for recovery procedures, without ascorbic acid. Three experiments were made on each tissue.

Prostaglandins were extracted and purified by using the method of Unger, Stamford & Bennett (1971). To remove neutral fats and carotine, the mixture was extracted twice with equal volumes of light petroleum. After adjustment of the pH to 3–3·5 with formic acid (1–3% v/v), the solution was extracted twice with chloroform. The extract was evaporated to dryness and formic acid was removed by oxygen-free nitrogen. Fuller's earth was used for further purification.

The residue was dissolved into 1·0 ml of chloroform and 100 μl was applied to thin-layer chromatographic plates (Ready plastic sheet, F1500, silica gel 20 × 20 cm, acid resistant: Carl Scheicher & Schüll D-3354 Dassel, W. Germany). After development of standard PGA_2, PGE_2 and $PGF_{2\alpha}$, and the extracted material in the AI solvent system of Green & Samuelsson (1964), the standards were visualized with phosphomolybdic acid and heat. Corresponding areas of the extract chromatogram opposite the PGA_2, PGE_2 and $PGF_{2\alpha}$ standard spots were scraped off and the biologically active substances were eluted from silica gel with 2 × 10 ml of chloroform–methanol (5 : 5 v/v).

The eluates were evaporated to dryness and the residues were stored at −20 . They were dissolved in 2 ml of Krebs solution just before bioassay. The eluates of the corresponding areas in the space between the standard spots were also assayed for biological activity and when biological activity was found we rejected the experiments. Frequently this biological activity was due to prostaglandins which did not migrate well, because the layer was overloaded which was the reason for using 100 μl of the eluates at each spot.

Three organs, two rat stomach strips and one rat colon were used for the assay of the PGE and PGA compounds, and one stomach strip and two colons for PGF compounds. The tissues were superfused with Krebs solution at the rate of 10 ml min^{-1} by a roller pump. The composition of the Krebs in g $litre^{-1}$ of distilled water was as follows: NaCl 6·56, KCl 0·35, $CaCl_2$ 0·40, KH_2PO_4 0·16, $MgSO_4\,7H_2O$ 0·29, $NaHCO_2$ 0·86, Na_2HPO_4 0·41, NaH_2PO_4 0·13, glucose 1·00. The solution was gassed with a mixture of 5% carbon dioxide in oxygen. Procaine (50 μg ml^{-1} of Krebs) was also added. After equilibration of the whole system for 2 h to acquire more sensitivity and base-line level stability, 1·0 ml of various concentrations of solutions of PGE_2 was perfused and the contractions recorded. Then 1·0 ml of eluate from the thin-layer chromatogram area corresponding to the PGE_2 standard spot, dissolved into 2·0 ml of Krebs, was similarly perfused and the contraction recorded. A second measurement was then made with the other half of the eluate after calibration with PGE_2. A similar procedure was adopted for the eluates of the areas corresponding to the PGA_2 and $PGF_{2\alpha}$ standard spots. When the response to the first half of the eluate was more than that to 100 ng of standard PGs, the second half was further diluted with Krebs solution.

The AI solvent system used separate groups of PGs (PGA from PGE and PGF) but does not separate PGs according to their degree of unsaturation (PGE_1 from PGE_2 and PGE_3). Each group of PGs was assayed against PGE_2, A_2 and $F_{2\alpha}$, respectively. To increase the sensitivity of the assay organs particularly to the PGA series, which is 5–10 times less active than PGE (Horton & Jones, 1969), we used procaine (50 μg ml^{-1} of Krebs) (Coceani & Wolfe, 1966; Dunham & Zimmerman, 1970) at five times the concentration used by Dunham & Zimmerman (1970). This further increased the sensitivity to 2·5 to 5·0 ng ml^{-1} of PGA_2.

We did not use antagonists to increase the specificity of the assay organs. Like Unger & others (1971), we found that substances like acetylcholine, histamine, noradrenaline, 5-hydroxytryptamine, angiotensin II, vasopressin and bradykinin, which

Table 1. *The detected prostaglandins and the percentage of the recovery* (means ± s.e. n = 3).

Tissue	PGA assayed as ng of PGA_2 equivalent	PGE assayed as ng of PGE_2 equivalent	PGF assayed as ng of $PGF_{2\alpha}$ equivalent	Recovery (%) PGA_2	Recovery (%) PGE_2	Recovery (%) $PGF_{2\alpha}$	Recovery (%) without ascorbic acid PGA_2	Recovery (%) without ascorbic acid PGE_2	Recovery (%) without ascorbic acid $PGF_{2\alpha}$
Renal Medulla	86 ± 18	108 ± 16	65 ± 8	41·6 ± 6·0	51·6 ± 4·4	40·0 ± 8·6	18·0 ± 1·5	19·6 ± 3·1	19·0 ± 1·5
Brain	33 ± 6	36 ± 4	51 ± 6	40·0 ± 2·8	45·0 ± 5·0	46·6 ± 6·6	20·0 ± 2·5	21·6 ± 4·4	16·0 ± 3·0
Spleen	76 ± 12	81 ± 10	33 ± 11	41·6 ± 4·4	70·0 ± 5·7	45·0 ± 2·8			
Liver	ND	24 ± 3	28 ± 10	40·0 ± 8·6	68·3 ± 10·1	58·3 ± 7·2			
Lung	56 ± 9	88 ± 7	98 ± 10	38·3 ± 1·6	63·3 ± 6·0	71·6 ± 7·2			
Mean recovery for PGA_2 PGE_2 $PGF_{2\alpha}$ with and without ascorbic acid				40·3 ± 2·0	59·6 ± 3·6	52·3 ± 3·9	19·0 ± 1·3	20·6 ± 2·4	17·5 ± 1·6
n				15	15	15	6	6	6
t				6·446	6·546	5·495			
P<				0·0005	0·0005	0·0025			

affect the assay organs, were not extracted by the method.

We used two stomach strips and one colon to assay the PGA and PGE series, because these PGs contract the strip better than the colon. The PGF series was assayed on one stomach strip and two colons because the colon is the more sensitive (Vane, 1971).

Table 1 shows the mean PG values (±s.e.) in three experiments on each tissue together with the percentage recovery with and without ascorbic acid (first two cases). Recovery was varied, and was markedly lower when ascorbic acid was not used as others have also found (Edwards, Strong & Hunt, 1969; Dunham & Zimmerman, 1970). The values were not corrected according to recovery. The concentration of PGs in the rat renal medulla was high and this might explain why PGs were not detected when the less sensitive method of the rat blood pressure preparation (cross-circulating rats) was used with binephrectomized rats, but they were detected by the more sensitive blood-bathed organ techique (Horton & Jones, 1969).

The authors wish to thank Dr. J. R. Pike of the Upjohn Company, Kalamazoo Michigan, who kindly provided PGs. This work was supported by a grant from I.N.S.E.R.M. (ATP 17 N° de contrat 73 5 479 17).

Centre De Recherches Sur L'Hypertension Arterielle,
I.N.S.E.R.M. (U-28),
Hopital Broussais,
96, Rue Didot, 75014-Paris,
France

N. Papanicolaou
S. Makrakis
J. Bariety
P. Milliez

September 15, 1973

REFERENCES

Coceani, F. & Wolfe, L. (1966). *Can. J. Physiol. Pharmac.*, **44**, 933–950.
Dunham, J. & Zimmerman, B. (1970). *Am. J. Physiol.*, **219**, 1279–1285.
Edwards, W., Strong, C. & Hunt, J. (1969). *J. Lab. clin. Med.*, **74**, 389–399.
Green, K. & Samuelsson, B. (1964). *J. Lipid Res.*, **5**, 117–120.
Horton, E & Jones, R. (1969). *Br. J. Pharmac.*, **37**, 705–722.
Papanicolaou, N. (1972). *Experientia*, **28**, 275–276.
Unger, W., Stamford, Y. & Bennett, A. (1971). *Nature*, **233**, 336–337.
Vane, J. (1971). *Nature New Biology*, **231**, 232–235.