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Potentiation of angiotensin II-induced natriuresis by indomethacin in the rat

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High doses of angiotensin II (Ang-II) markedly decrease rat renal tubular electrolyte reabsorption thereby inducing natriuresis. (Malvin & Vander, 1967). In view of the recent demonstration by Malik & McGiff (1975) that Ang-II is a potent stimulant of prostaglandin formation in rat kidney we have investigated the involvement of prostaglandin biosynthesis in Ang-II-induced natriuresis in the rat, using indomethacin, a known inhibitor of prostaglandin synthesis in rat kidney (Armstrong, Blackwell, Flower, McGiff & Mullane, 1975).

In untreated rats Ang-II, (1 $\mu g\ kg^{-1}\ min^{-1}$) increased urine flow from 19.8 ± 2.7 µl/min to 34.1 ± 3.4 µl/min ($n=8$) within 20 minutes. Pretreatment of animals with indomethacin (10 mg/kg, orally), 3 h before anaesthesia potentiated the Ang-II response increasing urine flow almost 5-fold from 16.6 ± 3.3 to $81.5 \pm 6.8\ \mu l\ min^{-1}\ 100^{-1}\ g$. Similar changes were observed with sodium excretion which increased from 3.37 ± 0.37 to $5.4 \pm 0.35\ \mu Eq\ min^{-1}\ 100^{-1}\ g$ after angiotensin in untreated rats and from 2.42 ± 0.49 to $12.52 \pm 1.58\ \mu Eq\ min^{-1}\ 100^{-1}\ g$ in indomethacin pretreated animals.

Calcium excretion increased from 0.049 ± 0.005 to $0.101 \pm 0.005\ \mu Eq\ min^{-1}\ 100^{-1}\ g$ in the untreated group and from 0.040 ± 0.007 to $0.197 \pm 0.005\ \mu Eq\ min^{-1}\ 100^{-1}\ g$ following indomethacin pretreatment. Ang-II increased calcium excretion more markedly than sodium excretion resulting in a decreased Na/Ca-ratio. Pretreatment with indomethacin, however, resulted in an increase in the Na/Ca-ratio after Ang-II. Only small changes were observed in potassium excretion.

Malik and McGiff (1975) and Armstrong *et al.* (1975) demonstrated an increase in renal vascular resistance after intra-arterial injection of PGE_2 in the rat; consequently an attenuation of the Ang-II induced constriction of renal blood vessels might be expected after indomethacin pretreatment.

In our experiments, however, there was no significant difference in glomerular filtration rate or renal blood flow between untreated and indomethacin treated rats. Therefore, bearing in mind a possible redistribution of local intrarenal bloodflow, a decreased tubular reabsorption of sodium is likely to contribute to the potentiated natriuresis following Ang-II after indomethacin pretreatment, as suggested by the elevated Na/Ca-ratio.

The above results suggest that the natriuretic effect of Ang-II is enhanced following inhibition of intrarenal prostaglandin biosynthesis in the rat.

References

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

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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.