Br. J. Pharmac. (1980), **71**, 529–531

EFFECT OF INDOMETHACIN ON HYDRALAZINE-INDUCED RENIN AND CATECHOLAMINE RELEASE IN THE CONSCIOUS RABBIT

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1 The effects of hydralazine on mean arterial pressure (MAP) heart rate (HR), plasma renin activity (PRA) and plasma catecholamines were examined in conscious rabbits before and after prostaglandin synthesis inhibition with indomethacin.

2 Hydralazine (3 mg/kg, i.v.) produced a 12% decrease in MAP and significant increases in HR, PRA and plasma noradrenaline and adrenaline.

3 Indomethacin (5 mg/kg, s.c.) failed to alter significantly the control MAP, HR, PRA or plasma catecholamines but inhibited renal venous prostaglandin E_2 by 56% ($P < 0.02$).

4 Indomethacin inhibited the hydralazine-induced tachycardia by 24% and augmented its hypotensive effects by 6%.

5 The hydralazine-stimulated increase in PRA was also inhibited 75% ($P < 0.001$) by indomethacin whereas noradrenaline and adrenaline concentrations were not significantly reduced.

6 Indomethacin inhibits hydralazine-induced renin release in the presence of elevated concentrations of plasma catecholamines; these findings suggest that renal prostaglandins function as important mediators of sympathetically-induced renin release.

Introduction

Hydralazine is a potent dilator of vascular smooth muscle used in the treatment of hypertension (Gottlieb, Katz & Chidsey, 1972). When administered to man or experimental animals, the fall in blood pressure is accompanied by an increase in heart rate and renin release (Meyer, Peskar, Tauchmann & Hertting, 1971; Gottlieb *et al.*, 1972; Pettinger, Campbell & Keeton, 1973; Pettinger & Keeton, 1975). These responses are thought to be mediated by the reflex activation of the sympathetic nervous system resulting from the reduction in blood pressure since the β-adrenoceptor antagonist, propranolol, will inhibit these effects and enhance the fall in blood pressure produced by the drug (Meyer *et al.*, 1971; Gottlieb *et al.*, 1972; Pettinger *et al.*, 1972; Pettinger & Keeton, 1975). In recent studies in the rat, we observed that inhibitors of prostaglandin synthesis also block the release of renin and the tachycardia which followed hydralazine administration (Campbell, Graham & Jackson, 1979). The present studies were designed to examine further in conscious rabbits the effects of inhibition of prostaglandin synthesis on the hypotension, tachycardia, renin release, and catecholamine release associated with hydralazine administration.

Methods

New Zealnd white rabbits (3 to 4 kg) of either sex were used and were maintained on a standard laboratory diet and given tap water *ad libitum*. Under local anaesthesia, the central ear artery and vein were cannulated with polyethylene tubing as previously described (Weber, Graham, Gain & Stokes, 1977). The venous catheter was used for intravenous injection. The arterial catheter was connected to a Narco-RP-1500 pressure transducer for the measurement of heart rate and blood pressure which were recorded on a Grass Model 7 Polygraph. The arterial catheter was also used to obtain blood samples.

Following surgical placement of the catheters, 1 to 2 h were allowed for the blood pressure and heart rate to stabilize. At time zero, the rabbits were injected with oil (1 ml/kg) or indomethacin (5 mg/kg) (Sigma Chemical) in oil subcutaneously. After 90 min, 0.9% w/v NaCl solution (saline, 1 ml/kg) or hydralazine (3 mg/kg) (Ciba) was given intravenously and blood samples were collected at 110 min for estimation of plasma renin activity and plasma catecholamine concentrations. Blood pressure and heart rate were

0007-1188/80/140529-03 $01.00

measured continuously throughout the experimental period.

In another group of rabbits, a polyethylene catheter was placed in the left lumboadrenal vein and advanced into the renal vein (Weber *et al.*, 1977) while the animals were under pentobarbitone (30 mg/kg, i.v.) anaesthesia. The catheter was exteriorized and secured on the animal's back. After a 24 h recovery period, two renal venous blood samples were obtained, one before and one 110 min after indomethacin, as described above. These samples were used for determination of plasma prostaglandin E_2 concentrations.

Plasma renin activity was measured by the antibody trapping method of Poulsen & Jorgenson (1974) and plasma catecholamines by a radioenzymatic assay (Peuler & Johnson, 1977). The measurement of renal venous prostaglandin E_2 (PGE_2) was performed by the method of Dray, Charbonner & Maclouf (1975) involving acid–lipid extraction, silicic acid chromatography, and a radioimmunoassay. The anti-PGE_2 antibody was found to cross react 14% with PGE_1 but less than 0.5% with PGA_2, PGB_2, PGD_2, 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$ and the 15-keto metabolites of PGE.

Statistical analysis was by an unpaired Student's *t* test when different groups of animals were compared and a paired test when responses in the same animals were compared. For multiple analyses, an analysis of variance was used (Snedecor & Cochran, 1967).

Results

Hydralazine caused a 12% decrease in blood pressure at 20 min following its injection, ($P < 0.001$) (Table 1). This fall in blood pressure was associated with a marked increase in heart rate at the same time intervals (75%, $P < 0.001$). In the rabbits pretreated with indomethacin, hydralazine cause a slightly greater decrease in blood pressure (18%, $P < 0.001$) and a smaller, (51%) increase in heart rate. Oil and saline or indomethacin and saline failed to change blood pressure or heart rate.

Hydralazine caused a significant increase in plasma renin activity 20 min after its administration ($P < 0.001$) which was inhibited by 75% ($P < 0.001$) by indomethacin pretreatment (Table 1). Indomethacin when given with saline failed to alter the plasma renin levels but decreased the renal venous levels of PGE_2 by 56% ($P < 0.02$).

Hydralazine produced a significant increase in both the plasma noradrenaline ($P < 0.001$) and adrenaline ($P < 0.001$) concentrations (Table 1). Indomethacin failed to alter significantly the basal or hydralazine-stimulated catecholamine levels. Interestingly, plasma adrenaline levels tended to increase further in the indomethacin-hydralazine group when compared with hydralazine alone; however, this effect was highly variable and so failed to attain statistical significance.

Discussion

These data indicate that the fall in blood pressure produced by hydralazine is accompanied by significant increases in heart rate, plasma renin activity, and plasma catecholamines. Since plasma catecholamines increase the release of renin and heart rate (Johnson, Davis & Witty, 1971; Pettinger, Augusto & Leon, 1972; Leenen, Redmond & McDonald, 1975), the release of catecholamines from the adrenal medulla and/or the increase in sympathetic nerve activity would appear to be the primary compensatory event which accompanies hydralazine-induced hypotension. Thus, the release of renin and the tachycardia would be secondary mechanisms brought into play by activation of the sympathetic nervous system. This con-

Table 1 Effect of indomethacin on hydralazine-induced renin and catecholamine release in the conscious rabbit

	Olive oil		*Indomethacin*	
	Control	*Hydralazine*	*Control*	*Hydralazine*
MAP (mmHg)	68.9 ± 2.0	60.8 ± 1.7***	69.2 ± 2.8	56.7 ± 3.3
HR (beats/min)	182 ± 11	318 ± 12***	194 ± 12	293 ± 11
PRA (ng $ml^{-1}h^{-1}$)	2.2 ± 0.7	22.5 ± 3.2***	1.2 ± 0.5	7.2 ± 1.2†
NA (pg/ml)	148.7 ± 23.3	638.5 ± 88.6***	188.4 ± 20.1	589.0 ± 61.0
Ad (pg/ml)	15.3 ± 2.8	249.6 ± 53.4***	37.5 ± 9.5	688.9 ± 243
PGE_2 (pg/ml)	40.1 ± 4.4	—	17.5 ± 4.1	—

Each value represents the mean ± s.e. mean for 8 rabbits.
Mean arterial pressure (MAP); heart rate (HR); plasma renin activity (PRA); noradrenaline (NA); adrenaline (Ad); prostaglandin (PG).
***$\beta < 0.001$ versus oil-saline control; †$P < 0.001$ versus oil-hydralazine treatment.

tention is suggested by our previous findings that propranolol, a β-adrenoceptor blocking drug, blocks hydralazine-induced renin release and tachycardia (Pettinger *et al.*, 1973; Pettinger & Keeton, 1975).

Frame & Hedqvist (1975) found that PGE_2 and its fatty acid precursor, arachidonic acid, would inhibit the neuronal release of noradrenaline produced by sympathetic nerve stimulation *in vitro*. Indomethacin, by blocking the synthesis of prostaglandins, removed this inhibitory influence and caused an enhancement of the release of noradrenaline. In the present studies, failure of indomethacin to inhibit hydralazine-induced catecholamine release would be consistent with these known effects of indomethacin and prostaglandins on the sympathetic neurone.

In recent studies in the rat, indomethacin and meclofenamate were found to inhibit hydralazine, isoprenaline, H133/22, and dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP)-induced renin release (Campbell *et al.*, 1979). Since isoprenaline, H133/22 (a selective β_1 agonist), and db cyclic AMP release renin by directly stimulating renal juxtaglomerular cells, it was concluded that inhibitors of prostaglandin synthesis inhibit sympathetically mediated renin release at a site distal to the juxtaglomerular β-adrenoceptor. In the present studies, the suppression of hydralazine-induced renin release by indomethacin in the presence of elevated circulating levels of plasma cateholamines would also support our previous finding. We conclude that renal prostaglandins mediate sympathetically stimulated renin release at the level of the juxtaglomerular cell.

In summary, the data indicate that indomethacin blocks renin release and reduces the tachycardia associated with hydralazine-induced hypotension and thereby enhances the antihypertensive action of the drug. Furthermore, indomethacin produces these effects without reducing the plasma catecholamine concentrations. These studies, therefore, provide indirect evidence that prostaglandins function as important mediators of sympathetically-induced renin release.

The authors are grateful for the technical assistance of Ms Beverley Adams and Mr William Stephenson and the secretarial assistance of Ms Cathy Johns. We appreciate the gift of the angiotensin I antiserum provided by Dr Knud Poulsen. Support was provided by grants from the Texas Affiliate of the American Heart Association and the National Heart, Lung and Blood Institute (HL 21066). The studies were performed while E.K.J. was a predoctoral fellow of the National Institutes of Health (NIGMS GMO7062) and R.M.G. was the recipient of the Faculty Development Award of the Pharmaceutical Manufacturer's Association.

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(Received February 25, 1980. Revised April 22, 1980.)