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Effects of Indomethacin on Furosemide-Induced Changes in Renal Blood Flow[1] (38711)

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The significance of the role of the prostaglandins in the regulation of renal hemodynamics remains unclear (1–3). Indomethacin administration to dogs has been shown to result in redistribution of intrarenal blood flow presumably as a result of inhibition of prostaglandin synthesis (4). The observation that furosemide and ethacrynic acid inhibit prostaglandin 15-hydroxy dehydrogenase activity *in vitro* (5) suggested that the prostaglandin system may be involved in the increase in renal blood flow produced by these diuretics (6, 7). Therefore, it was decided to determine the effect of indomethacin on the changes in renal blood flow caused by furosemide. The results suggest that the effects of furosemide on renal hemodynamics may be related to the prostaglandin system.

Materials and Methods. Male mongrel dogs were anesthetized with intravenous pentobarbital sodium (30 mg/kg). All animals were artificially ventilated with a Harvard respirator. The femoral artery was cannulated to monitor arterial blood pressure using a strain-gauge transducer (Statham P23AC) and a femoral vein cannulated for administration of additional anesthetic agent, furosemide[3] and indomethacin[4]. The left kidney was exposed via a retroperitoneal flank incision and the left ureter cannulated. Renal venous blood was obtained from a curved 18-gauge needle placed directly into the renal vein and attached to polyethylene tubing. An electromagnetic flowmeter probe (Carolina Medical Electronics, Inc.) was placed on the renal artery. A 16-gauge catheter (Bard A-cath Intravenous Placement Unit) was positioned percutaneously through the chest wall into the left ventricle for injection of radioactive microspheres. A minimum of 1 hr was allowed for recovery of the animal after completion of surgery.

Intrarenal cortical blood flow distribution was estimated using radioactive microspheres (Minnesota Mining and Manufacturing Co.) (5, 8). Approximately 200,000 microspheres, 15 $\pm$ μm in diameter, were diluted in 20% dextran and injected into the left ventricular catheter. At the completion of the experiment, the kidneys were removed and tissue obtained from four cortical zones and counted (5, 8).

Three series of experiments were performed with nine animals in each group. In the first series, intrarenal blood flow distribution was determined before and after intravenous administration of indomethacin (3.5 mg/kg). In the second series, intrarenal blood flow distribution was determined before and after the intravenous administration of furosemide (5 mg/kg) in animals pretreated with indomethacin (3.5 mg/kg).

In all experiments microspheres labeled with ^{141}Ce or ^{85}Sr were alternated to determine intrarenal flow distribution before and after drug administration. Calculations were made as described by Stein *et al.* (8) The four cortical zones were numbered I through IV, with I being the outermost and IV the innermost cortical zone. Total renal blood flow and mean arterial blood pressure were obtained from a direct-writing oscillograph recording (Grass Instruments Polygraph). Renal blood flow was expressed as milliliters per minute per gram kidney weight (ml/min/g). Plasma renin activity was determined using a radioimmunoassay for angiotensin I (9). Renin secretion was calculated

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as the product of the renal venous–arterial renin activity difference and the renal plasma flow. Data were statistically analyzed using Student's *t* test for paired comparisons. Data on intrarenal blood flow are presented as fractional distribution (% of total flow to each zone) and zonal perfusion rate (ml/min/g of each zone).

Results. Intravenous administration of furosemide alone significantly increased total renal blood flow (Table I) whereas after indomethacin total renal blood flow fell in all animals studied. Pretreatment of animals with indomethacin blocked the increase in total renal blood flow produced by furosemide (Table I).

Both furosemide and indomethacin when administered alone to dogs induced redistribution of intrarenal blood flow. Furosemide produced redistribution away from the outer cortex and toward the midcortical zone (Table II). Indomethacin resulted in redistribution away from deep cortical zones III and IV toward the superficial cortex (Table III). Since total renal blood fell after indomethacin, perfusion rate to all four zones was significantly decreased (Table III).

In the nine animals pretreated with indomethacin, there was no significant change in total renal blood flow after furosemide when all of the experiments were pooled (Table I). However, this group did appear to constitute two populations. In four animals, total renal blood flow fell from 3.0 to 2.4 ml/min/g ($P \geq 0.05$) while in the other five animals, total renal blood flow was unchanged (2.0–2.2 ml/min/g, $P > 0.1$). The fractional changes in intrarenal blood flow in these two groups of animals are shown in Table IV. In the group in which total flow decreased, there was a shift of flow from the juxtamedullary toward the superficial cortex. On the other hand, in the four animals in which

TABLE I. EFFECTS OF FUROSEMIDE AND INDOMETHACIN ON TOTAL RENAL BLOOD FLOW IN DOGS.[a]

Condition	F	I	I + F
Control[b]	4.6	3.9	2.7
Experimental[b]	5.0	3.1	2.5
Mean Difference[b]	0.4[c]	−0.7[c]	−0.3
± SEM	0.1	0.1	0.2

[a] F = Furosemide ($N = 8$); I = Indomethacin ($N = 7$); I + F = Furosemide administered to animals pretreated with Indomethacin ($N = 9$).
[b] Renal blood flow in ml/min/g.
[c] Significant difference ($P < 0.05$).

TABLE II. EFFECT OF FUROSEMIDE ON FRACTIONAL DISTRIBUTION OF BLOOD FLOW AND ZONAL PERFUSION RATES TO CORTICAL ZONES.

	Fractional distribution (%)			
	I	II	III	IV
Control	55	26	13	6
Experimental	53	29	14	5
Mean difference	−2.5[a]	3.0[a]	0.3	−0.8
± SEM	0.1	0.01	0.01	0.1
	Zonal perfusion rate (ml/min/g)			
	I	II	III	IV
Control	9.5	5.3	3.5	2.2
Experimental	9.8	6.5	3.9	2.0
Mean difference	0.4	1.2[a]	0.3	−0.2
± SEM	0.4	0.2	0.2	0.2

[a] Significant difference ($P < 0.05$) $N = 8$.

TABLE III. EFFECT OF INDOMETHACIN ON FRACTIONAL DISTRIBUTION OF BLOOD FLOW AND ZONAL PERFUSION RATES TO FOUR CORTICAL ZONES.

	Fractional distribution (%)			
	I	II	III	IV
Control	49	30	14	8
Experimental	51	31	13	5
Mean difference	3[a]	1	−1[a]	−3[a]
± SEM	1	1	0.5	0.7
	Zonal perfusion rate (ml/min/g)			
	I	II	III	IV
Control	6.8	5.3	3.1	2.8
Experimental	5.8	4.5	2.3	1.4
Mean difference	−1.0	−0.8[a]	−0.8[a]	−1.2[a]
± SEM	0.2	0.2	0.2	0.3

[a] Significant difference ($P < 0.05$).

flow was unchanged, flow to the midcortical zone increased, a pattern similar to that seen with furosemide alone (Table II).

Renin secretion decreased after indomethacin in three of four animals (Table V). After furosemide, the rate of renin secretion increased in all four animals.

Discussion. The mechanism by which furosemide causes an increase in renal blood flow is unclear. Recently, Paulsrud and Miller demonstrated *in vitro* inhibition by furosemide and other diuretics of prostaglandin 15-hydroxy dehydrogenase isolated from human placenta (5). Inhibition of the renal enzyme responsible for degradation of prostaglandins could result in increased levels of these vasoactive lipids within the kidney and resultant decrease in renal resistance. The findings in this study are compatible with such a mechanism. Pretreatment of dogs with indomethacin, a drug which inhibits prostaglandin synthesis, prevented the increase in total renal blood flow usually seen after furosemide (Table I). Williamson and coworkers have recently made a similar observation using both furosemide and ethacrynic acid (10). Furthermore, these investigators reported prostaglandin E concentration increased in renal venous blood after furosemide or ethacrynic acid in untreated but not in indomethacin-pretreated animals (10).

Furosemide modifies intrarenal blood flow distribution (11). The decrease in fractional flow to the outer zone I and an increase in the midcortical zone II (Table II) are similar to those previously reported (11). Indomethacin also redistributes intrarenal blood flow away from the deep cortical zones toward the superficial cortex (4) (Table III). Since prostaglandins are primarily formed in the renal medulla, the greater decreases in blood flow to zones III and IV after indomethacin may be due to diminished concentration of these vasoactive lipids. On the other hand, it is also possible that indomethacin itself is a vasoconstrictor, and the decrease in renal blood flow may be due to a direct effect of the drug on the juxtamedullary circulation. The effect of indomethacin on changes in blood flow distribution produced by furosemide were not definitive

TABLE IV. EFFECT OF FUROSEMIDE ON FRACTIONAL DISTRIBUTION OF RENAL BLOOD FLOW TO FOUR CORTICAL ZONES IN ANIMALS PRETREATED WITH INDOMETHACIN.

	Total decreased flow (N = 5)			
	I	II	III	IV
Control	51	30	14	5
Experimental	54	31	13	3
Mean difference	3[a]	1	1	−2
± SEM	1	1	2	0.4
	Total unchanged flow (N = 4)			
	I	II	III	IV
Control	52	32	13	4
Experimental	50	34	13	3
Mean difference	2	2[a]	0	1
± SEM	1	0.5	0.7	0.5

[a] Significant difference ($P < 0.05$).

TABLE V. EFFECT OF FUROSEMIDE ON RENIN SECRETION IN INDOMETHACIN-TREATED ANIMALS.

	Renin secretion		
Expt.	Control (ng/min)	Indomethacin (ng/min)	Furosemide (ng/min)
1	357	368	1,314
2	4,390	1,218	10,460
3	1,081	97	337
4	5,493	274	664

(Table IV). In reviewing the original data we found that the animals appeared to fall into two groups. In five animals renal blood flow decreased after furosemide and in four it remained unchanged. The pattern of intrarenal blood flow distribution also appeared to be different in the two groups. The significance of this inconsistency is not clear. Possibly blockade of prostaglandin synthesis was incomplete in those animals in which total renal blood flow did not fall and in which the pattern of redistribution was similar to that of furosemide alone.

Furosemide stimulates renal renin secretion possibly by a direct effect on the macula densa even when urinary losses are replaced or after volume expansion (12, 13). Never-

theless, furosemide causes a decrease in renal vascular resistance, reflecting dilatation (13). When prostaglandin synthesis was blocked by indomethacin, furosemide caused an increase in renin secretion (Table V) and renal blood flow either fell or remained unchanged. Thus, the changes in renal hemodynamics produced by furosemide appear to reflect a balance between the renin-angiotensin system and the prostaglandins. Administration of furosemide would normally increase the intrarenal concentration of both angiotensin II and prostaglandins, with the action of the latter being dominant. The direct stimulus to an increase in intrarenal prostaglandin activity could be inhibition of the catabolic enzymes (5). On the other hand, renal arterial infusion of angiotensin II stimulates excretion of prostaglandins (2) suggesting the intrarenal generation of angiotensin II could lead to prostaglandin release.

Summary. The effects of indomethacin on furosemide induced changes in renal blood flow were determined in dogs. Furosemide alone caused an increase in total renal blood flow while indomethacin alone decreased renal blood flow. When furosemide was administered to animals pretreated with indomethacin the increase in renal blood flow was blocked. Changes in intrarenal blood flow distribution were also measured using radioactive microspheres. The pattern of blood flow distribution after furosemide was modified in some of the animals pretreated with indomethacin. Stimulation of renin secretion occurred after furosemide in indomethacin-treated animals. The data suggest that the changes in renal blood flow produced by furosemide may be modulated by the prostaglandin system.

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