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Effects of Indomethacin and Tolmetin on Furosemide-Induced Changes in Renin Release[1] (40309)

BYRON NOORDEWIER,[2] MICHAEL D. BAILIE, AND JERRY B. HOOK

Departments of Pharmacology, Physiology, and Human Development, Michigan State University, East Lansing, Michigan 48824

The diuretic furosemide increases renin release by the kidney, an effect independent of volume depletion which accompanies diuresis (1). The stimulus for renin release by furosemide appears to be related to both changes in renal arteriolar resistance (2, 3) and a direct tubular effect subsequent to blockade of sodium and chloride reabsorption prior to or at the macula densa (1, 2).

Furosemide-induced renin release is blocked by the prostaglandin synthetase inhibitor, indomethacin, by an undefined mechanism (4). After indomethacin, the ability of furosemide to increase renal blood flow is blunted, while the natriuretic effect is unaffected (4–6).

Calcium has recently been suggested to play a role in renin secretion (7, 8). Although furosemide is acutely calciuretic, the significance of this effect with respect to renin release has not been evaluated. The purpose of these experiments was to determine if the blockade of furosemide induced renin release by prostaglandin synthetase inhibitors, indomethacin and tolmetin, was correlated with changes in the calciuretic response to furosemide.

Materials and methods. Surgical. Male mongrel dogs, 15–25 kg, were used in all experiments. The animals were anesthetized with sodium pentobarbital (30 mg/kg), intravenously, and a cuffed endotracheal tube was inserted. The dogs were artificially ventilated with a Harvard respirator. Catheters were placed in the left femoral artery and in both femoral veins. Normal saline (0.9% NaCl) was infused into one femoral vein to replace fluid losses from surgery and to hydrate the animal until total urine flow was 0.5–2.0 ml/min. The saline infusion was then reduced to equal urine flow. Inulin was infused into the other femoral vein at a rate calculated to maintain plasma inulin concentration between 30–50 mg/dl. Arterial blood pressure was monitored with a Statham P23AC transducer.

Experimental protocols. I. Effect of indomethacin on renal responses to intravenous furosemide. Glomerular filtration rate estimated by the clearance of inulin (C_{IN}), urinary excretion of Na, K, and Ca and plasma renin concentration (PRC) were measured during two control 10-min clearance periods and during the intravenous infusion of furosemide (2 mg/kg/hr). Following the furosemide clearance periods, each dog received increasing doses of indomethacin (0.01, 0.05, 0.1, 0.5 mg/kg, iv). Furosemide infusion continued during the administration of indomethacin. Twenty minutes after each dose of indomethacin, two clearance periods were obtained. In addition to the dogs treated with indomethacin, three dogs were injected with saline instead of indomethacin in an experimental protocol identical to that described above (4 injections at 40 min intervals). These dogs are referred to as "time" control dogs.

II. Effect of indomethacin or tolmetin pretreatment on renal response to intrarenal furosemide. After two control clearance periods, furosemide was infused (15 μg/kg/min) into the renal artery of the experimental kidney, and two clearance periods obtained. The infusion of furosemide was stopped and 30 minutes were allowed for urine flow to return toward control. Two additional control periods were then run and tolmetin (5 mg/kg) or indomethacin (2 mg/kg) was administered intravenously. After 20 min, two more clearances were taken and furosemide again infused intrarenally. Two additional clearance periods were obtained during furosemide infusion. Excretion of Na, K, and Ca, as well

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as C_{IN}, and PRC were measured during each clearance period. The dose of furosemide in two of the tolmetin treated dogs was 5 μg/kg/min but since the results did not differ from the results from the two dogs given 15 μg/kg/min the data were pooled.

Analytical. Urinary and plasma Na and K were determined by flame photometry and Ca by atomic absorption spectroscopy. Inulin concentrations were determined by the method of Walser *et al.* (9). Plasma renin concentration was estimated by incubating plasma with excess homologous renin substrate. The amount of angiotensin I generated was then determined by radioimmunoassay (10). The data were analyzed utilizing analysis of variance with a randomized block design. The 0.05 level of probability was used as the criterion of significance.

Results. Furosemide infused intravenously (2 mg/kg/hr) increased the urinary excretion of sodium and calcium (Table I). Plasma renin concentration (PRC) was also increased. All values remained elevated throughout drug administration (Table I). Glomerular filtration rate was not affected by furosemide. Increasing doses of indomethacin produced dose related decreases in PRC and calcium excretion (Table II). Sodium excretion and GFR were not changed when indomethacin was given during furosemide infusion (Table II). Potassium excretion (not shown) also increased after furosemide and was not affected by indomethacin.

Furosemide, infused into the renal artery, also increased urinary excretion of sodium, potassium and calcium (Figs. 1 and 2). PRC also increased when furosemide was given. Electrolyte excretions and PRC returned toward control when the infusion of furosemide was stopped. Indomethacin (Fig. 1) and tolmetin (Fig. 2) had little effect on electrolyte excretion although each parameter tended to be lower than the previous control. Similarly, PRC tended to decrease after indomethacin or tolmetin. A second infusion of furosemide increased sodium, potassium, and calcium excretion but PRC was not affected by furosemide after administration of indomethacin (Fig. 1) or tolmetin (Fig. 2).

Discussion. Although the role of calcium in renin release is still obscure, there is increasing evidence that movement of this ion within the juxtaglomerular cell may be an important regulatory mechanism. Addition of calcium to kidney slices incubated in calcium free media produces an immediate, large increase in renin release (7). Similarly, the isolated

TABLE I. EFFECT OF TIME ON FUROSEMIDE-INDUCED CHANGES IN RENAL FUNCTION

			Saline dose			
Parameter	Control	Furosemide[a]	1	2	3	4
GFR (ml/min) SE	39.9 ± 12.0	29.3 ± 9.7	31.6 ± 10.0	32.7 ± 10.8	31.6 ± 9.4	35.1 ± 9.5
PRC (ng AI/ml/hr) SE	14.9 ± 3.2	38.9[b] ± 13.1	32.2[b] ± 13.6	30.0[b] ± 6.6	30.4[b] ± 7.1	29.7[b] ± 3.0
$U_{Na}V$ (μEq/min) SE	152 ± 32	538[b] ± 61	649[b] ± 47	848[b] ± 92	924[b] ± 150	717[b] ± 36
$U_{Ca}V$ (μEq/min) SE	0.61 ± 0.07	11.2[b] ± 1.9	15.1[b] ± 3.6	12.6[b] ± 1.6	10.9[b] ± 0.9	11.9[b] ± 3.2

[a] Furosemide was infused at a rate of 2 mg/kg/hr, iv.
[b] Significantly different than control ($P < .05$).

TABLE II. EFFECT OF INCREASING DOSES OF INDOMETHACIN ON FUROSEMIDE-INDUCED CHANGES IN RENAL FUNCTION

			Indomethacin dose (mg/kg)			
Parameter	Control	Furosemide[a]	0.01	0.05	0.1	0.5
GFR (ml/min) SE	43 ± 4	39 ± 4	37 ± 5	36 ± 6	38 ± 7	34 ± 5
PRC (ng AI/ml/hr) SE	16.3[c] ± 7.1	40.1[b] ± 9.6	27.6[b,c] ± 6.8	17.4[c] ± 4.7	14.5[c] ± 4.5	13.6[c] ± 5.5
$U_{Na}V$ (μEq/min) SE	130[c] ± 33	844[b] ± 96	779[b] ± 56	876[b] ± 20	854[b] ± 22.1	656[b] ± 59
$U_{Ca}V$ (μEq/min) SE	0.80[c] ± 0.13	17.6[b] ± 3.2	13.4[b] ± 1.9	14.1[b] ± 2.8	10.8[b,c] ± 2.0	9.8[b,c] ± 2.2

[a] Furosemide was infused at a rate of 2 mg/kg/hr, iv.
[b] Significantly different than control ($P < .05$).
[c] Significantly different than furosemide ($P < .05$).

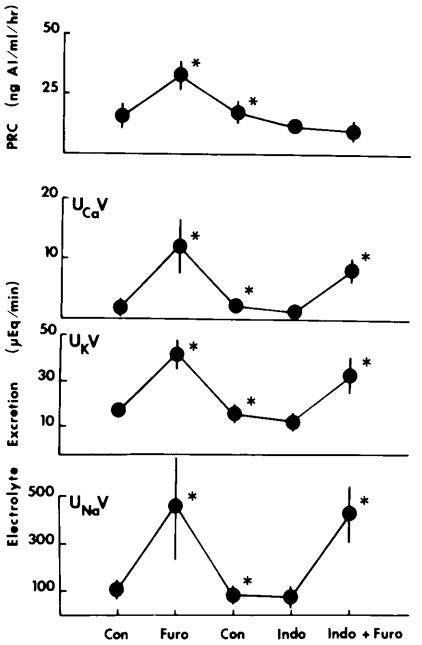

FIG. 1. Effect of indomethacin on renal response to furosemide. Excretion rates of sodium ($U_{Na}V$), potassium (U_KV), and calcium ($U_{Ca}V$) and plasma renin concentration were measured. After a control period (CON), furosemide was infused into the renal artery (FURO). After the furosemide infusion was stopped, control values were measured (CON), and indomethacin was given iv (INDO). Forty minutes after indomethacin, furosemide was infused again (INDO + FURO). The mean and 1 SE are given ($N = 4$). * different from previous clearance period ($P < .05$).

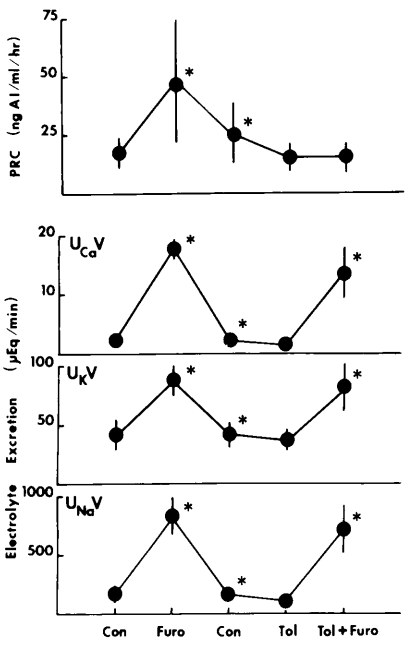

FIG. 2. Effect of tolmetin on renal response to furosemide. Excretion rates of sodium ($U_{Na}V$), potassium (U_KV) and calcium ($U_{Ca}V$) and plasma renin concentration (PRC) were measured. After a control period (CON), furosemide was infused into the renal artery (FURO). After the furosemide infusion was stopped, control values were measured (CON), and tolmetin was given iv (TOL). Forty minutes after tolmetin, furosemide was infused again (TOL + FURO). The mean and 1 SE are given ($N = 4$). * different from previous clearance periods ($P < .05$).

perfused kidney of the cat releases renin in response to calcium only after prior exposure to calcium free perfusate (8). These data indicate that an increase in intracellular free calcium may be involved in renin release.

The present experiments demonstrate that blockade of furosemide-induced renin release by indomethacin or tolmetin does not depend on alterations in net tubular transport of sodium, potassium or calcium. The major stimulus for renin release during furosemide administration appears to be inhibition of sodium (or chloride) flux at the macula densa similar to that observed in the cells of the thick ascending limb of the loop of Henle (1). Since the prostaglandin synthetase inhibitors failed to alter the urinary excretion of sodium in this study or in previous work (4), it is unlikely that the effect of indomethacin or tolmetin on renin release could involve changes in sodium transport at the macula densa.

Similarly, alterations in calcium load to the macula densa do not appear to be important in the action of tolmetin or indomethacin. Although there was a small dose related decrease in calcium excretion after indomethacin, calcium excretion rate was well above control even after the highest dose of indomethacin tested (Table I). In contrast, PRC had decreased dramatically. In addition, pretreatment with neither indomethacin nor tolmetin altered the increase in calcium excretion to intrarenal furosemide, while both drugs blocked any increase in PRC (Figs. 1 and 2). Thus, blockade of furosemide-induced renin release by prostaglandin synthetase inhibitors does not require an alteration in the calciuretic effect of furosemide. Lester and Rubin also found extracellular calcium

was not a determinant in the release of renin following furosemide (8). Since prostaglandin synthetase inhibitors, such as indomethacin or tolmetin, do not appear to affect sodium or calcium load at the macula densa, their site of action is probably subsequent to the signal perceived by the macula densa. Whether their action involves alterations in the state of intracellular calcium remains to be investigated.

Summary. Prostaglandin synthetase inhibitors, indomethacin and tolmetin, blocked furosemide-induced increase in renin secretion whether the furosemide was given intravenously or into the renal artery. Tolmetin and indomethacin did not affect the natriuretic, kaliuretic or calciuretic response to furosemide. Therefore, blockade of furosemide-induced renin release does not appear to require an alteration in sodium or calcium load at the macula densa. Thus, the site of action of prostaglandin synthetase inhibitors on renin release is probably subsequent to the signal perceived by the macula densa.

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