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The Effects of Indanyloxyacetic Acid (MK 196) on Electrolyte Excretion in the Rat Kidney (39510)

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A substituted derivative of ethacrynic acid, 6,7-dichloro-2-methyl-1-oxo-phenyl-5-indanyloxyacetic acid (MK 196), has been shown in preliminary studies to be a potent uricosuric and diuretic agent in the rat and monkey (1, 2). Prior studies from this laboratory have localized the nephron site of inhibition of urate reabsorption to the proximal convoluted tubule. The reabsorption of sodium in the proximal tubule, however, is not affected by MK 196, and the sodium diuresis results from impaired reabsorption in the ascending limb of the loop of Henle (1). The current studies were undertaken to examine further the changes in electrolyte excretion induced by this agent and to gain additional inferential information on the nephron site of action.

Methods. Clearance studies. Male Sprague–Dawley rats weighing 200 to 400 g were used in all studies. Animals were anesthetized with Inactin (Promonta, Hamburg, Germany), 100 mg/kg body wt injected intraperitoneally, and prepared for clearance studies as previously described (3). A solution of isotonic saline containing [*methoxy*-^{3}H]inulin (25 μCi/ml) was infused at a rate of 1.2 ml/hr for the duration of the experiment. MK 196 was dissolved in distilled water containing an equal concentration of sodium bicarbonate and infused in a dose of 50 mg/kg body wt/hr in a volume of 1.2 ml/hr. Control animals received the same solution without the drug. In the experimental animals, urinary losses of sodium and water were replaced with a volume of isotonic saline equal to the urine flow rate. Hematocrits were measured before and after the study to ensure the adequacy of volume replacement. After 60 to 90 min of equilibration, blood and urine samples were obtained.

Free water clearance and reabsorption studies. Studies were performed in a separate group of animals that were lightly anesthetized with ether for placement of catheters, placed in restraining cages, and allowed to awaken. A solution of [*methoxy*-^{3}H]inulin (25 μCi/ml) was infused at 1.2 ml/hr for the duration of the study. Free water reabsorption (T^cH_2O) studies were performed in three rats, deprived of food and water for 24 hr. Pitressin tannate in oil (0.5 U) was injected subcutaneously 1 hr prior to study. Two percent saline was infused at a rate of 0.20 ml/min. Free water clearance (C_{H_2O}) studies were performed in three animals deprived of food for 24 hr but allowed free access to 2% dextrose drinking solution. On the day of study a solution containing 1.67% of glucose, 1.3% ethanol, and 0.13% sodium chloride was administered by stomach tube in a volume equal to 3% of body weight. The water diuresis was sustained by the infusion of hypotonic saline (0.225%) at a rate of 0.20 ml/hr. In both groups of animals, blood and urine samples were obtained after a 60-min equilibration period, after which MK 196 was infused as a bolus (50 mg/kg body wt). Additional blood and urine samples were obtained 30 to 120 min after administration of the drug. The rate of saline infusion was not changed.

Radioactivity of blood and urine samples was determined in a modified Bray's solution in a Packard Tri-Carb liquid scintillation counter (Packard Instruments Co., Downers Grove, Ill.). Hematocrits were measured in microhematocrit tubes, Na^+ and K^+ by flame photometry, and uric acid by a specific uricase method utilizing a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometry. PO_4 in serum and urine was determined in an AutoAnalyzer; pH was determined in an Instrumentation Laboratory blood gas analyzer (Instrumentation

Laboratories, Watertown, Mass.). Osmolality was determined by freezing point depression in an Advanced Instruments osmometer. Clearance and fractional excretions were calculated from standard formulae. Clearance periods for each animal were averaged and the results expressed as the mean of means ± SEM. Statistical significance was determined by the *t* test for unpaired data.

Results. Clearance studies (Table I). In the experimental animals, hematocrits measured prior to (43.3 ± 0.42%) and during (42.5 ± 0.23%) the infusion of the drug were not significantly different, indicating the adequacy of volume replacement. The onset of the diuretic response was delayed, averaging 30 min. Compared to controls, the continuous infusion of MK 196 resulted in significantly higher rates of urine flow, of urinary excretion of sodium, and of urinary excretion of potassium. The glomerular filtration rate was not significantly lower in the experimental animals. The clearance and fractional excretion of urate were markedly higher in the MK 196-treated animals as compared to controls. The clearances and fractional excretions of calcium and magnesium were also significantly higher in the drug-treated animals. There was no significant change in phosphate clearance in response to MK 196.

Free water clearance and reabsorption studies. To localize the nephron site of impaired sodium reabsorption, T^cH_2O and C_{H_2O} studies were performed and the results are summarized in Table II. In response to a bolus infusion of MK 196, fractional free water reabsorption as a function of fractional osmolar clearance and fractional free water clearance as a function of fractional volume flow were decreased below values obtained during the control periods.

Discussion. MK 196, a substituted derivative of ethacrynic acid, has previously been reported to be a potent diuretic and uricosuric agent in the rat (1). The current studies confirm our previous findings on the renal effects of MK 196 on sodium and urate excretion and present additional information on its effects on the excretion of calcium, magnesium, phosphate, C_{H_2O}, and T^cH_2O. Following the intravenous infusion

TABLE II. THE EFFECT OF MK 196 ON C_{H_2O} AND T^cH_2O[a].

C_{H_2O} (n=3)			
Fractional volume excretion (%)		Fractional C_{H_2O} (%)	
C	E	C	E
6.63 ± 0.42	9.71 ± 1.58	4.60 ± 0.33	1.36 ± 0.31
P, NS		T^cH_2O (n = 3)	$P < 0.001$
Fractional osmolar clearance (%)		Fractional T^cH_2O (%)	
C	E	C	E
14.52 ± 0.90	15.76 ± 0.78	15.76 ± 0.78	3.4 ± 0.30
P, NS		$P < 0.001$	

[a] Numbers represent mean ± SEM; NS = not significant; C = control; E = experimental; n = number of animals studied.

TABLE I. RENAL EFFECTS OF MK 196.[a]

	Control (6)	Experimental (7)	P
GFR (μl/min/g KW)	1149.0 ± 118.6	906.0 ± 54.6	NS
Volume (μl/min/g KW)	8.9 ± 1.4	119.0 ± 28.8	<0.005
$U_{Na}V$ (μequiv/min)	0.40 ± 0.039	26.1 ± 2.2	<0.001
U_KV (μequiv/min)	3.3 ± 0.6	6.2 ± 0.7	<0.01
C_{osm} (μl/min/g KW)	37.7 ± 5.9	137.0 ± 38.0	<0.025
C_{urate} (μl/min/g KW)	131.8 ± 13.7	464.0 ± 83.5	<0.005
FE_{urate} (%)	11.4 ± 0.3	56.4 ± 10.7	<0.001
$C_{calcium}$ (μl/min/g KW)	4.7 ± 0.6	21.0 ± 0.5	<0.01
$FE_{calcium}$ (%)	0.4 ± 0.05	2.0 ± 0.5	<0.005
C_{Mg} (μl/min/g KW)	35.0 ± 9.0	130.0 ± 19.0	<0.001
FE_{Mg} (%)	3.0 ± 0.8	21.8 ± 5.0	<0.01
$C_{phosphate}$ (μl/min/g KW)	163.0 ± 40.0	179.1 ± 100.0	NS
$FE_{phosphate}$ (%)	17.0 ± 5.9	23.0 ± 1.6	NS
U_{pH}	6.7 ± 0.40	6.5 ± 0.16	NS

[a] Numbers represent mean ± SEM. Numbers in parentheses indicate the number of animals studied.

of MK 196, there is a delayed onset of response which averaged 30 to 45 min. The reason for this delayed response is unknown but may indicate a requirement for further metabolism of the drug. MK 196 administration resulted in significantly higher rates of sodium excretion in the urine and a decrease in both C_{H_2O} and T^cH_2O. It is evident from these results that the major site of action of this agent is in the loop of Henle. These findings are consistent with the results of micropuncture studies which have indicated that fractional reabsorption in the proximal convoluted tubule was not significantly altered by MK 196 (1). C_{H_2O} and T^cH_2O studies, performed during the sustained infusion of the drug, revealed results qualitatively similar to those of the present study utilizing only a single bolus infusion of the drug (1). It would appear, then, that MK 196 has not only a delayed onset of action but also a relatively long duration of action.

In the present studies, the fractional excretion of urate was increased from 11 to 56%. This action on urate is localized to the proximal tubule where MK 196 has been demonstrated to inhibit both the reabsorption and secretion of urate, the net effect being an increase in urate excretion (1). The demonstration of impaired proximal urate reabsorption and normal sodium reabsorption in the proximal convoluted tubule suggests that the reabsorption of these two constituents of the glomerular filtrate is not intimately linked at this nephron site and that the effects of MK 196 on urate transport are specific.

The renal handling of other electrolytes may then be used to gain further inferential information on the nephron sites of action of MK 196. In the nonparathyroidectomized animal, the bulk of phosphate reabsorption occurs in the proximal tubule (4, 5). In the current studies, neither the clearance nor fractional excretion of phosphate was altered by MK 196. Although some degree of inhibition of phosphate reabsorption cannot definitely be excluded, it seems likely that MK 196 has little effect on phosphate transport, and these results further support the conclusion that the effects of MK 196 on urate transport are specific and not the result of a generalized inhibition of proximal tubular function. Recent evidence has suggested that, in the rat, calcium is reabsorbed both in the proximal tubule and at distal nephron sites (6). Magnesium reabsorption, on the other hand, appears to occur mostly at sites beyond the proximal convoluted tubule (6, 7). The increase in calcium and magnesium excretion, in the absence of changes in phosphate excretion, are consistent with the conclusion that the increases in calcium and magnesium excretion are the result of inhibition of the reabsorption of these ions at a distal site.

Taken together, the results of these studies indicate that MK 196 is a potent loop-acting diuretic resulting in significant increases in the urinary excretion of sodium, calcium, magnesium, and water. In addition, MK 196 induces a marked increase in the urinary excretion of uric acid and thus might be a useful agent in some clinical settings.

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