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Effect of Hydrochlorothiazide on Calcium Excretion by the Isolated Perfused Rat Kidney[1] (40410)

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Diuretic agents have been used, paradoxically, for the treatment of both hypercalcemia (1) and hypercalciuria (2). Most diuretics that inhibit sodium or chloride reabsorption at points distal to the proximal tubule produce a proportionate increase of both calcium and sodium in the urine. This occurs with the powerful loop diuretics (3, 4) and mercurials (5). An important exception is hydrochlorothiazide (6). This drug has a dual action on urinary calcium excretion. Its acute effect involves an increase in urinary calcium excretion, though whether or not this occurs in the same proportion as sodium excretion is disputed (7, 8). After chronic administration thiazides produce hypocalciuria (2).

The mechanism by which thiazides decrease the urinary excretion of calcium is not entirely clear (9). Since the hypocalciuric effect is manifest only after prolonged thiazide administration, it has been thought to be secondary to the contraction of extracellular volume that attends the use of diuretics (10). Others have suggested that hypocalciuria is due to stimulation of parathyroid hormone secretion or sensitization of the kidney to this hormone (10–12). Still others have proposed that thiazides exert a direct action on the kidney increasing the tubular reabsorption of calcium (13). Since experiments *in vivo* are often complicated by reverberating extrarenal influences that may modify renal behavior, their interpretation can be difficult. We have therefore used an isolated perfused rat kidney preparation to examine the acute and direct effects of hydrochlorothiazide and furosemide on calcium excretion by the kidney.

Methods. Male Sprague-Dawley rats weighing 290–440 g and allowed free access to food and water were used for all experiments. Kidney perfusion with 5% bovine albumin in Krebs-Ringer bicarbonate solution was performed according to the technique described by Nishiitsutsuji-Uwo *et al.* (14) as modified by Ross *et al.* (15) and previously described in several publications from our laboratory (16, 17). The concentration of calcium in the perfusate was 2.5 m*M* and the ultrafiltrable calcium concentration was 1 m*M*.

After the right kidney was placed in the perfusion tray inside the perfusion cabinet a period of 15–25 min was allowed to elapse for equilibration. Thereafter, clearance periods of 10 or 15 min duration were collected for the ensuing 75 min. Total time of perfusion was thus 90–100 min. Perfusion pressure was kept constant at 110 Hg and flow remained stable at 26–37 ml/min.

Hydrochlorothiazide (Merck) 1 or 2 mg dissolved in 0.05 *N* NaOH, or furosemide (Hoechst) 10 mg were added to the recirculating perfusate (total vol of 75–80 ml) after two or three initial control periods. An additional 10-min equilibration period was allowed immediately after the addition of diuretics and collections resumed after it for the duration of the perfusion. A separate group of control kidneys was perfused for a similar period of time without addition of drugs. In three of these kidneys 0.05 *N* NaOH was added to the recirculating medium as control for the vehicle in which hydrochlorothiazide was dissolved. Since the function of these kidneys was similar to that of the other controls the results have been pooled.

Glomerular filtration rate was determined using ^{14}C inulin. Sodium and potassium concentrations in perfusate and urine were measured using a flame photometer with an in-

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ternal lithium standard. Total calcium in the perfusate and urine was measured using an atomic absorption spectrophotometer.

Analysis of the results was done by comparison of the clearance periods immediately preceding the addition of the diuretics with two consecutive clearance periods at the height of the diuresis. In addition, comparisons were done with control kidneys perfused for similar periods of time at equivalent times of perfusion. Statistical analysis was done using "paired t" test or standard "t" test wherever applicable. Results are expressed as mean ± SE (n).

Results. Table I summarizes the several parameters of renal function in six control isolated perfused rat kidneys over the time span during which these experiments were conducted. In three of these kidneys 1 ml of 0.05 *N* NaOH was added to the perfusate as control for the vehicle in which hydrochlorothiazide was dissolved at the same time either the latter or furosemide were usually added. Since there was no difference in the time course between these experiments and those in which no vehicle was added, they have been pooled.

Effect of hydrochlorothiazide. Figure 1 summarizes the fractional excretion of calcium vs. fractional excretion of sodium in 48 clearance periods in seven control kidneys and the control periods of the kidneys that received hydrochlorothiazide or furosemide. This figure confirms that the relation between the excretion of calcium and sodium is linear in the isolated perfused rat kidney as in the kidney *in vivo*.

When hydrochlorothiazide 1 or 2 mg was added to the recirculating medium in seven experiments, calcium excretion increased to a lesser extent than did sodium excretion. The ratio of calcium to sodium clearance ($^{C}Ca/^{C}Na$) decreased significantly from 0.65 ± 0.04 to 0.52 ± 0.04, $p < 0.025$. Absolute and fractional excretions of calcium and sodium as well as fractional excretion of potassium increased after the addition of hydrochlorothiazide. Glomerular filtration rate decreased slightly but not significantly. These results are summarized in Table II.

Effect of furosemide. Furosemide 10 mg produced a change in the pattern of excretion of sodium and calcium differing from that caused by hydrochlorothiazide. After the addition of furosemide to the recirculating medium in seven experiments, there was a rapid increase in the urinary excretion of calcium

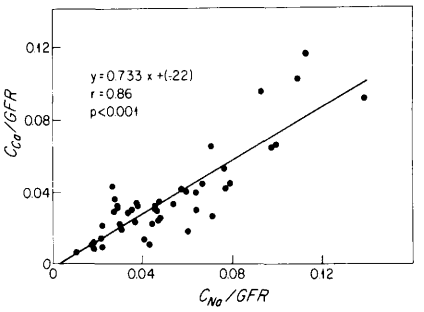

FIG. 1. This figure summarizes the relation between the fractional excretion of calcium and that of sodium in a total of 48 clearance periods observed in seven control isolated perfused rat kidneys and the control periods of those perfused kidneys that received hydrochlorothiazide or furosemide. The relation between calcium and sodium excretion is best described by a line confirming that this relation is linear in the isolated perfused rat kidney as found in the kidney *in vivo*.

TABLE I. PHYSIOLOGICAL PARAMETERS OF ISOLATED PERFUSED RAT KIDNEYS AS A FUNCTON OF TIME.

	20–50 min	50–80 min	*p*
V ml/min	0.03 ± 0.008	0.032 ± 0.01	NS
GFR ml/min	0.47 ± 0.09	0.43 ± 0.11	NS
$U_{Ca}V$ μEq/min	0.035 ± 0.006	0.039 ± 0.013	NS
C_{Ca}/GFR	0.03 ± 0.005	0.04 ± 0.004	NS
$U_{Na}V$ μEq/min	3.09 ± 0.86	2.95 ± 0.73	NS
C_{Na}/GFR	0.05 ± 0.006	0.05 ± 0.005	NS
C_{Ca}/C_{Na}	0.71 ± 0.05	0.76 ± 0.09	NS
C_K/GFR	0.35 ± 0.05	0.38 ± 0.04	NS

TABLE II. EFFECT OF HYDROCHLOROTHIAZIDE ON THE FUNCTION OF THE ISOLATED PERFUSED RAT KIDNEY.

	Control	Hydrochloro-thiazide	*p*
V ml/min	0.033 ± 0.011	0.063 ± 0.013	<0.001
GFR ml/min	0.46 ± 0.06	0.38 ± 0.06	NS
$U_{Ca}V$ μEq/min	0.047 ± 0.015	0.074 ± 0.017	<0.001
C_{Ca}/GFR	0.04 ± 0.01	0.08 ± 0.02	<0.01
$U_{Na}V$ μEq/min	4.05 ± 1.11	7.64 ± 1.25	<0.001
C_{Na}/GFR	0.06 ± 0.01	0.15 ± 0.03	<0.001
C_{Ca}/C_{Na}	0.65 ± 0.04	0.52 ± 0.04	<0.025
C_K/GFR	0.37 ± 0.05	0.84 ± 0.11	<0.001

of the same magnitude as the increase in the urinary excretion of sodium. The ratio of $^{C}Ca/^{C}Na$ remained stable (0.79 ± 0.21 vs. 0.73 ± 0.06, $p < 0.7$). As expected with furosemide, there was a large increase in the absolute and fractional excretion of calcium, sodium and potassium. Net secretion of potassium was apparent during furosemide diuresis. GFR decreased significantly. These results are summarized in Table III.

Discussion. The action of thiazide diuretics on calcium excretion is complex and controversial, in part because it has been difficult to separate direct effects on the kidney from indirect extrarenal effects. In particular, the hypocalciuric effect of chronic thiazide administration has been ascribed to hormonal (10) or direct tubular (13) influences. The use of an isolated perfused kidney offers the possibility of distinguishing these factors. The present experiments indicate that hydrochlorothiazide does indeed have a direct effect on the isolated kidney to produce an increase in the excretion of calcium as well as sodium. Nevertheless, urinary losses of calcium are proportionately smaller than those of sodium, so that the ratio of calcium clearance to sodium clearance falls significantly. Such a fall in $^{C}Ca/^{C}Na$ is not characteristic of the diuresis produced by furosemide in this preparation. Nor does it accompany other forms of diuresis observed in the isolated kidney, such as that accompanying an increase in perfusion pressure or the substitution of chloride for bicarbonate in the perfusion medium (J. Stoff, personal communication, 18). These results, therefore, suggest that thiazides act directly on the kidney to retard renal excretion of calcium relative to that of sodium during sodium diuresis.

A similar conclusion was drawn by Costanzo and Weiner from experiments in which thiazides were infused directly into the renal artery, producing a diuresis of both sodium and calcium, but a marked fall in the ratio of calcium to sodium clearance (19). Micropuncture experiments in intact dogs suggest that hydrochlorothiazide reduces sodium reabsorption in the distal tubule while calcium reabsorption in this segment is depressed little or not at all (6).

While the present expeirments suggest a direct effect of hydrochlorothiazide, they do not of course eliminate additional extrarenal actions, such as those operating on or through the parathyroid glands, or resulting from a reduction in extracellular fluid volume that might produce hypocalciuria *in vivo*. A combination of direct and indirect effects are probably responsible for the tendency of chronic thiazide administration to produce renal retention of calcium.

Summary. The effect of hydrochlorothiazide on calcium excretion was studied in the isolated, perfused rat kidney. Thiazide produced a diuresis of sodium, calcium, potassium and water, but retarded the excretion of calcium relative to that of sodium, so that $^{C}Ca/^{C}Na$ fell by 20%. This was not observed after furosemide, which induced a comparable diuresis of sodium and water but did not change $^{C}Ca/^{C}Na$. A direct action of thiazides on renal tubules may contribute to the relative hypocalciuria produced by these drugs *in vivo*.

TABLE III. EFFECT OF FUROSEMIDE ON THE ISOLATED PERFUSED RAT KIDNEY.

	Control	Furosemide	p
V ml/min	0.037 ± 0.007	0.073 ± 0.006	<.005
GFR ml/min	0.57 ± 0.05	0.34 ± 0.04	<.005
$U_{Ca}V$ μEq/min	0.068 ± 0.025	0.131 ± 0.021	<.001
C_{Ca}/GFR	0.04 ± 0.009	0.14 ± 0.02	<.001
$U_{Na}V$ μEq/min	3.73 ± 0.92	8.46 ± 0.85	<.001
C_{Na}/GFR	0.04 ± 0.01	0.17 ± 0.03	<.001
C_{Ca}/C_{Na}	0.79 ± 0.21	0.73 ± 0.06	NS
C_K/GFR	0.27 ± 0.05	1.22 ± 0.24	<.005

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