Renal interstitial Ca$^{2+}$

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Mupanomunda, Maria M., Bing Tian, Norio Ishioka, and Richard D. Bukoski. Renal interstitial Ca$^{2+}$. Am J Physiol Renal Physiol 278: F644–F649, 2000.—Renal interstitial fluid Ca$^{2+}$ concentration ([Ca$^{2+}]_{isf}$) was measured in anesthetized Wistar rats by using in situ microdialysis. During perfusion of 20 cm of the proximal small intestine with Ca$^{2+}$-free buffer, renal [Ca$^{2+}]_{isf}$ was 1.63 ± 0.19 mmol/l in the cortex (n = 6) and 1.93 ± 0.12 mmol/l in the medulla (n = 5, P = 0.223). When Ca$^{2+}$ in the intestinal lumen was increased to 3 mmol/l, no change was seen in total or ionized serum Ca$^{2+}$ (SCa), urinary Ca$^{2+}$ excretion (UCa), or Ca$^{2+}$ in a microdialysate of the kidney cortex. Increasing intestinal Ca$^{2+}$ further, to 6 mmol/l, was without effect on SCa but significantly increased UCa by 38% and microdialysate Ca$^{2+}$ by 36% (1.25 ± 0.09 vs. 1.70 ± 0.14 mmol/l, n = 4, P < 0.05). Intravenous infusion of 28 ng·kg$^{-1}$·min$^{-1}$ of parathyroid hormone for 1 h during perfusion of the intestinal lumen with 1 mmol/l Ca$^{2+}$ caused a 7–10% rise in SCa, a 40% fall in UCa, and a 32% increase in microdialysate Ca$^{2+}$ (1.32 ± 0.13 vs. 1.74 ± 0.13 mmol/l, n = 6, P < 0.05). Interlobar arteries with a mean diameter of 120 μm were studied by using a wire myograph to determine whether changes in extracellular Ca$^{2+}$ affect muscle tone. When precontracted with 5 mmol/l serotonin, the arteries relaxed in response to cumulative addition of Ca$^{2+}$ (1–5 mmol/l) with an ED$^{-50}$ value for Ca$^{2+}$ of 3.30 ± 0.08 mmol/l, n = 3. These data demonstrate that [Ca$^{2+}]_{isf}$ changes dynamically during manipulation of whole-animal Ca$^{2+}$ homeostasis and that intrarenal arteries relax in response to extracellular Ca$^{2+}$ varied over the range measured in vivo.

kidney; calcium; microdialysis; relaxation

The concentration of Ca$^{2+}$ in the blood is regulated by coordinated mechanisms involving absorption of the cation from the intestinal lumen by epithelial cells, reabsorption from the urine by renal tubular cells, and deposition and resorption from bone by osteoblasts and osteoclasts (16, 18). At each site where Ca$^{2+}$ undergoes transcellular movement, i.e., from the lumen of the renal tubule into the vascular space, it traverses an interstitial compartment where it is free to interact with adjacent cells. If the cells in these spaces have a mechanism for sensing changes in extracellular Ca$^{2+}$, then the cation might serve as a stimulus to modulate their physiological activity. Recent work from our laboratory (6) has demonstrated that the perivascular network of sensory dilator nerve fibers expresses a Ca$^{2+}$ receptor that is homologous with the Ca$^{2+}$-sensing receptor that was originally described in the bovine and human parathyroid gland (4, 11), kidney (21), and brain (22). This finding has raised the question of whether dynamic changes in the concentration of Ca$^{2+}$ in the interstitial compartment of tissues involved in transcellular Ca$^{2+}$ movement can modulate vascular reactivity and local blood flow by altering the release of vasodilator transmitters from perivascular sensory nerves (5).

In initial efforts to address this question, we found that extracellular Ca$^{2+}$ causes concentration-dependent relaxation of isolated mesenteric branch arteries that is sensory nerve dependent and that the magnitude of this relaxation correlates with the density of Ca$^{2+}$-receptor-positive nerve fibers in the periadventitial surface of the artery (6, 20, 25). To address the question of whether Ca$^{2+}$ in the interstitial fluid of any tissue can achieve levels that are high enough to induce relaxation, we used an in situ microdialysis method to test the hypothesis that ionized Ca$^{2+}$ in the interstitium of the intestinal submucosa changes as a function of the Ca$^{2+}$ content in the intestinal lumen. The results indicated that Ca$^{2+}$ in the duodenal interstitium can vary between 1 and 2 mmol/l (19). Of interest, this range of interstitial Ca$^{2+}$ concentrations is sufficient to induce relaxation of isolated mesenteric branch arteries and supports the hypothesis that duodenal interstitial Ca$^{2+}$ achieves levels sufficient to stimulate Ca$^{2+}$-induced relaxation and modulate local vascular reactivity.

The kidney also plays a key role in the regulation of whole-animal Ca$^{2+}$ homeostasis, serving as a target tissue for the calciotropic hormones, parathyroid hormone (PTH) (3, 13, 24) and 1,25 (OH)$_2$ vitamin D$_3$ (3, 18). In experiments designed to map the distribution of the perivascular sensory nerve Ca$^{2+}$ receptor, we found that, similar to mesenteric arteries, perivascular nerves of intrarenal arteries express Ca$^{2+}$-receptor protein (24). This observation raised the question of whether perturbations of whole-animal Ca$^{2+}$ homeostasis can cause dynamic changes in renal interstitial Ca$^{2+}$ that are sufficient to affect renovascular function, as has been described for other endocrine and paracrine systems (9). To address this question, we have now used in situ microdialysis to measure Ca$^{2+}$ in the renal interstitium during manipulation of whole-animal Ca$^{2+}$ homeostasis and have found that interstitial Ca$^{2+}$ in the kidney varies over a concentration range that can...
modulate contractile activity of isolated intrarenal arteries.

**METHODS**

Animals. All studies were performed by using Wistar rats obtained from Harlan Sprague Dawley and protocols that were approved by the Institutional Animal Care and Use Committee. On delivery to our animal quarters, the rats were housed in microisolator cages maintained at constant temperature, humidity, and fixed dark-light cycles, and given free access to tap water and Purina rodent chow until 8 AM of the morning of the experiment. Microdialysis. Microdialysis was performed by using established methods (19). The animals were anesthetized with a mixture of ketamine and xylazine (100 mg/kg, 5 mg/kg ip) and the right jugular vein was cannulated by using PE-50 tubing for administration of drugs and fluids. The left kidney was then exposed through a midline incision, and a section of the renal cortex or renal medulla was cannulated for perfusion with the buffer described above containing known amounts of Ca$^{2+}$ as described recently (19).

The zero-net-flux method was used to estimate the basal concentration of Ca$^{2+}$ in the interstitial compartment of the renal cortex or renal medulla during perfusion of the intestinal lumen with nominally Ca$^{2+}$-free buffer. After a 90-min equilibration period, the microdialysis probe was perfused at 1 μl/min with buffer containing increasing concentrations of Ca$^{2+}$ (0.5, 1, 2, 3, and 5 mmol/l). After each incremental increase, i.e., from 0.5 to 1.0 mmol/l, a 35-min equilibration period was allowed to elapse before collection of the dialysate over a 15-min period. For each level of Ca$^{2+}$ in the perfusate, the concentration of free ionized Ca$^{2+}$ in both dialysate and perfusate was determined by using a microfluorometric method (20). The difference between the amount of Ca$^{2+}$ in the dialysate (Ca$^{2+}$dialysate) and the perfusate (Ca$^{2+}$perfusate) was then plotted as the dependent variable against the concentration of Ca$^{2+}$ in the perfusate and with the use of linear regression analysis, the point where Ca$^{2+}$dialysate = Ca$^{2+}$perfusate was zero (0-net-flux point) was identified, and used as an estimate of the concentration of Ca$^{2+}$ in the interstitium (17).

Effect of intestinal Ca$^{2+}$ and PTH infusion on renal interstitial Ca$^{2+}$ concentration (Ca$^{2+}_{int}$). Experiments were performed to assess the effect of changing the concentration of Ca$^{2+}$ in the lumen of the gut on Ca$^{2+}$ in the renal cortical interstitium. Animals (n = 4) were prepared as described above, and a microdialysis probe was placed in the renal cortex and perfused with buffer containing 0.5 mmol/l Ca$^{2+}$ whereas the intestinal lumen was cannulated and perfused with Ca$^{2+}$-free buffer. After a 90-min equilibration period, the microdialysate was collected for 15 min and stored until Ca$^{2+}$ was determined. After this collection period, the concentration of Ca$^{2+}$ in the buffer perfusing the intestinal segment was increased to 3 mmol/l and, after a 60-min period, the microdialysate was collected for 15 min and stored. The concentration of Ca$^{2+}$ in the buffer perfusing the intestinal segment was then increased to 6 mmol/l and, after a 60-min period, the microdialysate was collected for a 15-min period. The net effect of increasing intestinal Ca$^{2+}$ on Ca$^{2+}$ in the renal interstitium was estimated by calculating the fractional increase in the concentration of Ca$^{2+}$ in the dialysate that occurred in response to the maneuver. At the end of the experiment, the kidney was removed and fixed in formalin, and sectioned by using a razor blade to verify placement of the microdialysis fiber in the expected site.

Other experiments assessed the effect of PTH-induced increases in serum Ca$^{2+}$ on interstitial Ca$^{2+}$ in the renal cortex. Animals (n = 7) were instrumented, and a segment of the proximal small intestine was perfused with buffer containing 1 mmol/l Ca$^{2+}$. The dialysis membrane was then perfused at a rate of 1 μl/min with buffer containing 0.5 mmol/l Ca$^{2+}$ and, after a 90-min equilibration period, dialysate was collected for 15 min for measurement of the basal concentration of Ca$^{2+}$. PTH was then infused through the jugular cannula at 28 ng·kg$^{-1}$·min$^{-1}$ for a 45-min period, after which the dialysate was collected for 15 min and stored for determination of Ca$^{2+}$ content. The fractional increase in Ca$^{2+}$ was determined as described above. Analysis of serum Ca$^{2+}$. A separate series of animals was used to assess the effect of changing the concentration of Ca$^{2+}$ in the intestinal lumen on or flushing parathyroid hormone on serum total and ionized Ca$^{2+}$ and urinary Ca$^{2+}$ excretion. In these experiments, the rats were anesthetized with ketamine and xylazine, and the jugular vein and carotid artery were cannulated for the administration of compound and withdrawal of blood, respectively; the proximal small intestine was cannulated for lumenal perfusion; and ureters were cannulated for urine collection.

When the effect of increasing concentrations of intestinal Ca$^{2+}$ was assessed, the lumen of the bowel was perfused with nominally Ca$^{2+}$-free buffer for a 1-h equilibration period, during which time urine was collected. After this period a blood sample (1 ml) was taken from the carotid artery for preparation of serum, and volume was replaced by intravenous injection of 1 ml 0.9% saline. The concentration of Ca$^{2+}$ in the buffer perfusing the intestinal segment was then increased to 6 mmol/l, and the perfusion was allowed to continue for a 1-h period, during which time urine was collected and at the end of which a final blood sample was taken. Total Ca$^{2+}$ in the serum and urine was analyzed by using flame photometry, and ionized Ca$^{2+}$ was determined by using a Ca$^{2+}$-selective electrode (Radiometer).

When the effect of PTH was assessed, the cannulated segment of the intestine was perfused with buffer containing 1 mmol/l Ca$^{2+}$ and urine was collected for a 1-h equilibration period. At the end of this period, 1 ml of blood was taken from the carotid artery for preparation of serum and volume was replaced as described above. Infusion of PTH was then initiated as described above in the microdialysis section, and urine was collected for a 1-h period after which a second 1 ml blood sample was taken.

Myography. A separate set of animals (n = 3) was used to study the contractile function of isolated intrarenal arteries. Kidneys were removed from anesthetized rats, immediately hemisected, and placed in ice-cold physiological solution containing (in mmol/l) 140 NaCl; 4.7 KCl; 1.17 MgSO$_4$7H$_2$O; 5 NaHCO$_3$; 1.15 KH$_2$PO$_4$; 1.10 Na$_2$HPO$_4$; 1.0 CaCl$_2$; 20 HEPES; and 5 glucose, pH 7.4. Cortical interlobar arteries were microdissected from the renal matrix and placed on a wire myograph (Kent Scientific, Litchfield, CT) by means of tungsten wires with a 27-µm diameter. After a 15-min equilibration period at 37°C, the arteries were stretched to a circumference that yielded an inner diameter of ~120 µm and allowed to equilibrate for an additional 15 min. The vessels were exposed one time to 5 µmol/l serotonin for a 4–5 min period, washed with fresh physiological salt solution, and then recontracted with 5 µmol/l serotonin. Ca$^{2+}$ was then cumula-
The effect on serum and urine Ca$^{2+}$ of raising the concentration of Ca$^{2+}$ in the gut from 0 to 6 mmol/l was assessed in a separate group of rats (n = 6). Total serum Ca$^{2+}$ during perfusion of the gut with Ca$^{2+}$-free buffer was 9.42 ± 0.26 mg/dl, and this was unaffected by increasing intestinal Ca$^{2+}$ to 6 mmol/l, where serum Ca$^{2+}$ was 9.55 ± 0.17 mmol/l (Table 1). Similarly, serum-ionized Ca$^{2+}$ was unaffected by altering the concentration of Ca$^{2+}$ perfusing the lumen of the bowel; (control = 1.27 ± 0.04 vs 1.26 ± 0.02 mmol/l with 6 mmol/l Ca$^{2+}$). In contrast, increasing Ca$^{2+}$ in the gut from 0 to 6 mmol/l significantly increased urinary Ca$^{2+}$ excretion from 9.91 ± 0.08 to 13.7 ± 0.45 µg/h, P < 0.05, and was without effect on urine volume, 252 ± 7.5 vs. 287.5 ± 88 µl/h (Table 1).

Effect of PTH infusion. The effect of infusion of PTH on renal cortical interstitial Ca$^{2+}$ was determined by measuring the change in the concentration of Ca$^{2+}$ in the dialysate under control conditions and after infusion of PTH. Under basal conditions the concentration of Ca$^{2+}$ in the dialysate was 1.32 ± 0.13 mmol/l and increased to 1.74 ± 0.13 mmol/l after PTH infusion (Fig. 3). This represents a 32 ± 7.5% increase above baseline (significant at P < 0.05). Infusion of the same concentration of PTH into a second set of animals (n = 6) caused an increase in total serum Ca$^{2+}$ from 9.42 ± 0.09 to 10.08 ± 0.12 mg/dl, P < 0.05, and of ionized serum Ca$^{2+}$ from 1.19 ± 0.02 to 1.31 ± 0.03 mmol/l, P < 0.05 (Table 1). PTH also caused a significant fall in urinary Ca$^{2+}$ excretion from 13.0 ± 1.7 to 7.86 ± 0.08 µg/h, P < 0.05, without affecting the volume of urine output (366 ± 25 vs. 335 ± 83 µl/h).
Response of isolated intrarenal arteries to extracellular Ca\textsuperscript{2+}. Renal interlobar arteries were isolated from a separate set of rats (n = 3) and mounted on a wire myograph for measurement of isometric force responses. These arterial segments contracted in response to 5 µmol/l serotonin and relaxed in a dose-dependent manner in response to the cumulative addition of extracellular Ca\textsuperscript{2+} (Fig. 4). The maximal relaxation in response to 5 mmol/l Ca\textsuperscript{2+} was 66 ± 1.7% of the initial tension, and the ED\textsubscript{50} value for Ca\textsuperscript{2+} was 3.30 ± 0.08 mmol/l.

**DISCUSSION**

The present studies were initiated as an extension of our recent work that identified a Ca\textsuperscript{2+}-sensing receptor on the periadventitial nerve network of intrarenal arteries (24), which we have postulated may mediate the relaxation response to physiological concentrations of interstitial Ca\textsuperscript{2+}. In support of this thesis are our demonstration that Ca\textsuperscript{2+}-induced relaxation is sensory nerve dependent (20) and our recent finding that the concentration of free Ca\textsuperscript{2+} in the duodenal interstitium undergoes dynamic changes in response to physiological stimuli over a range that can induce significant relaxation of isolated arteries (19).

Because of the potential importance of this system as a regulatory mechanism and the well-established role of the kidney in transcellular Ca\textsuperscript{2+} transport, the present experiments were designed to determine the basal concentration of Ca\textsuperscript{2+} in interstitial space of the renal cortex and medulla, assess the effect of increasing the concentration of Ca\textsuperscript{2+} present in the lumen of a segment of the small bowel or infusion of PTH on

**Table 1. Effect of PTH and intestinal Ca\textsuperscript{2+} on serum and urinary Ca\textsuperscript{2+}**

<table>
<thead>
<tr>
<th>Maneuver</th>
<th>Pre-PTH</th>
<th>Post-PTH</th>
<th>Lumen Ca\textsuperscript{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mmol/l</td>
<td>6 mmol/l</td>
<td></td>
</tr>
<tr>
<td>Serum total Ca\textsuperscript{2+}, mg/dl</td>
<td>9.42 ± 0.09</td>
<td>10.08 ± 0.12*</td>
<td>9.42 ± 0.26</td>
</tr>
<tr>
<td>Serum ionized Ca\textsuperscript{2+}, mmol/l</td>
<td>1.19 ± 0.02</td>
<td>1.31 ± 0.03*</td>
<td>1.27 ± 0.04</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} excretion, µg/h</td>
<td>13.0 ± 1.7</td>
<td>7.86 ± 0.08*</td>
<td>9.91 ± 0.08</td>
</tr>
<tr>
<td>Urine production, µl/h</td>
<td>366 ± 25</td>
<td>335 ± 83</td>
<td>252 ± 7.5</td>
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</table>

Values are means ± SE. PTH, parathyroid hormone. *P < 0.05.
interstitial Ca\(^{2+}\) in the renal cortex, and to learn whether isolated intrarenal arteries relax in response to graded increases in extracellular Ca\(^{2+}\). The results include what to our knowledge is the first measurement of interstitial Ca\(^{2+}\) in the kidney, the demonstration that the concentration of interstitial Ca\(^{2+}\) changes in response to alterations in whole-animal Ca\(^{2+}\). In these segments, an additional 10% is absorbed (2). In these segments, our subsequent studies were focused on Ca\(^{2+}\) in the small intestine with increasing amounts of the cation. The results indicate that during perfusion of the proximal convoluted tube where ~55% of the filtered Ca\(^{2+}\) load is absorbed, and in the proximal straight tube where an additional 10% is absorbed (2). In these segments, Ca\(^{2+}\) transport is coupled to Na\(^{+}\) and fluid reabsorption (10). Transport of Ca\(^{2+}\) is also passive in the medullary portion of the thick ascending limb of the loop of Henle, where it is driven by the potential gradient created by furosemide sensitive Cl\(^{-}\) reabsorption (7, 23).

Because of the differential handling of tubular Ca\(^{2+}\) by different segments of the nephron, including cortical and medullary aspects, we first tested whether there were differences in the basal concentration of Ca\(^{2+}\) in the interstitium of the cortex vs. the medulla. Our results indicate that during perfusion of the proximal small intestine with Ca\(^{2+}\)-free medium, the basal level of interstitial Ca\(^{2+}\) tends to be higher in the medulla than the cortex, but the difference is not statistically significant. The values in both compartments are somewhat higher than that which we observed in the duodenal submucosa during infusion of the lumen with Ca\(^{2+}\)-free buffer (1.08 mmol/l) (21), and in the absence of data to the contrary, may reflect a true tissue-to-tissue difference. In view of the fact that the renal cortex is a major site of Ca\(^{2+}\) reabsorption and that medullary and cortical Ca\(^{2+}\) levels do not vary significantly, our subsequent studies were focused on Ca\(^{2+}\) in the renal cortex.

As noted above, experiments were performed to learn whether interstitial Ca\(^{2+}\) in the renal cortex could be increased by perfusing the lumen of the proximal small intestine with increasing amounts of the cation. The rationale behind this series was based in part on our observation that increasing Ca\(^{2+}\) in the lumen of the bowel causes a large increase in interstitial Ca\(^{2+}\) in the submucosa and the logical extrapolation that this Ca\(^{2+}\) would be absorbed into the blood and filtered at the glomerulus. The results show that raising Ca\(^{2+}\) in the gut from 0 to 3 mmol/l, which results in a rise in submucosal Ca\(^{2+}\) from 1.0 to 1.45 mmol/l does not alter renal cortical Ca\(^{2+}\), but that increasing Ca\(^{2+}\) further to 6 mmol/l, which increases submucosal Ca\(^{2+}\) to 1.8 mmol/l, causes a significant rise in interstitial Ca\(^{2+}\) in the cortex.

We also studied the effect of infusion of PTH on renal cortical interstitial Ca\(^{2+}\) because the cortex is a major site of the calcitropic action of the peptide on tubular Ca\(^{2+}\) transport (2, 12, 23). As expected, infusion of PTH at 28 ng · kg\(^{-1}\) · min\(^{-1}\) for 1 h resulted in a significant rise in total and ionized serum Ca\(^{2+}\) and a decrease in urinary Ca\(^{2+}\) excretion. Concomitant with these changes in Ca\(^{2+}\) homeostasis, PTH increased the concentration of Ca\(^{2+}\) in the renal cortical dialysate an average of 32%, demonstrating that infusion of PTH can alter renal interstitial Ca\(^{2+}\). Although the amount of PTH that was used is likely to be more pharmacological than physiological, significant increases in serum PTH do occur in conditions such as primary or secondary hyperparathyroidism (14) and can be predicted to accompany calcilytic-based pulse therapy for the treatment of osteoporosis (13).

In view of our studies linking the perivascular sensory nerve CaR with Ca\(^{2+}\)-induced relaxation (6, 19), we have speculated that increasing interstitial Ca\(^{2+}\) in the kidney could serve to cause a local vasodilation (5). The results of our myograph studies show that isolated interlobar arteries relax in response to cumulative addition of extracellular Ca\(^{2+}\). It should be noted that the ED\(_{50}\) for Ca\(^{2+}\) in the interlobar artery is 3.3 vs. ~1.8 mmol/l for the isolated mesenteric branch artery (19).

At the present time it is not known whether the relative decrease in sensitivity of the small renal artery is an intrinsic property of the artery and reflects a true tissue-to-tissue variation, similar to the difference in interstitial Ca\(^{2+}\), or whether it reflects damage to the perivascular nerve network that was caused during isolation of the renal artery segment from the renal parenchyma. Regardless, the data indicate that the intrarenal vasculature is sensitive to elevations of extracellular (or interstitial Ca\(^{2+}\)) as might occur under the conditions of the present study.

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