Stimulation of Renin Release by Intrarenal Calcium Infusion

Vicente Lahera, Mary J. Fiksen-Olsen, and J. Carlos Romero

The effects of intrarenal infusions of calcium gluconate (10 and 100 μg Ca/kg/min) on renin secretion were studied in anesthetized mongrel dogs. In one group, the two doses of calcium were infused for 30 minutes each (1 ml/min). In a second group, the same doses were administered 30 minutes after the start of infusion of prostaglandin synthesis inhibitors (indomethacin 10 μg/kg/min intrarenal or injection of meclofenamate 5 mg/kg i.v. bolus). Mean arterial pressure, renal blood flow, and glomerular filtration rate remained unchanged during the infusion of calcium in both groups. The infusion of 10 μg Ca/kg/min increased renin secretion 77% and sodium excretion 123%. During the infusion of 100 μg Ca/kg/min, renin secretion was not different from precalcium values, whereas urinary 6-keto-PGF₁α, urine flow, sodium, potassium, and calcium excretion rates were increased (p<0.05). During the administration of prostaglandin synthesis inhibitors, the urinary 6-keto-PGF₁α levels were reduced, and the infusion of 10 μg Ca/kg/min failed to increase renin secretion, sodium excretion, or 6-keto-PGF₁α excretion rates. The infusion of 100 μg Ca/kg/min during prostaglandin synthesis inhibition did not modify urine flow or sodium excretion; however, potassium and calcium excretions increased. It is concluded that 1) the intrarenal infusion of small doses of calcium gluconate is capable of stimulating renin secretion through a prostaglandin-mediated mechanism, and 2) the stimulation of renin secretion as well as the increase in sodium excretion induced by calcium are independent of hemodynamic alterations. (Hypertension 1990;15(suppl I):I-149–I-152)

The manner in which calcium influences renin secretion rate has been investigated using a variety of experimental conditions. Although in vitro studies have yielded some inconclusive results, there is a general consensus that the increase of calcium entry into the juxtaglomerular cells decreases renin secretion. It has been proposed that calcium plays a fundamental role in coupling the renin secretion responses to the myogenic vasoconstriction that is seen during renal blood flow (RBF) autoregulation. The juxtaglomerular cells located in the afferent arterial wall are morphologically differentiated from the smooth muscle. Consequently, the juxtaglomerular and smooth muscle cells can be subjected to similar changes in calcium kinetics induced by alterations in renal arterial pressure.

Calcium entry has also been found to stimulate the synthesis of prostaglandin I₂ (PGI₂) in the endothelium. This prostaglandin is known to produce vasodilation and to increase renin release in vitro. These findings suggest that the calcium-induced suppression of renin secretion might be modulated by the calcium-stimulated endothelial response of PGI₂. The hypothesis tested by this study was that a small increase in the concentration of calcium into the renal artery will stimulate synthesis of PGI₂, thus enhancing renin secretion. This effect, which was first suggested by Nadler et al., should be prevented by prostaglandin synthesis inhibition. At higher concentrations, calcium can act directly on the juxtaglomerular cells, reducing renin secretion, as suggested by the study of Watkins et al. Therefore, this study was undertaken to determine the effect on renin secretion of two doses of calcium gluconate infused into the renal artery of anesthetized dogs in the presence or in the absence of prostaglandin synthesis inhibition. The two doses of calcium gluconate (10 and 100 μg/kg/min) were chosen from preliminary pilot studies in which a range of smaller doses than those reported by Watkins et al. and Kotchen and Guthrie were tested.

**Methods**

The experiment was performed in 16 mongrel dogs of either sex (16–23 kg) anesthetized with...
sodium pentobarbital (25 mg/kg i.v.). Before the experiment, the dogs were maintained on a normal diet and tap water ad libitum. A femoral artery was cannulated and mean arterial pressure (MAP) was monitored with a pressure transducer (Statham P23ID, Gould Inc., Hato Rey, Puerto Rico) and recorded on a polygraph (Gould, Inc., Cleveland, Ohio). A femoral vein was cannulated for the administration of 2% inulin solution (1 ml/min) and additional anesthetic. The dogs were ventilated mechanically with a respirator. The left kidney was exposed through a flank incision. RBF was measured with a noncannulating electromagnetic flow probe (Carolina Medical Electronics, Inc., King, North Carolina) placed around the renal artery. Distal to the flow probe, a curved needle (23 gauge) attached to polyethylene tubing was inserted into the renal artery for infusion of saline or calcium solutions (1 ml/min) or indomethacin (0.3 ml/min). Another curved needle (21 gauge) attached to polyethylene tubing was inserted into the renal vein for blood sampling. The left ureter was cannulated for urine flow evaluation and sampling.

After completion of the surgical preparation, the dogs were allowed to equilibrate for 90 minutes before two 15-minute renal clearance periods. Saline was infused intravenously during both the equilibration and precalciium infusion periods. After the basal clearances, intrarenal infusion of indomethacin (10 \( \mu \)g/kg/min) in a saline vehicle was begun and continued throughout the experiment in four dogs, whereas an intravenous bolus injection of meclofenamate (5 mg/kg) in a saline vehicle was administered to the other four dogs. Thirty minutes later, the eight dogs underwent an identical protocol in which two doses (10 and 100 \( \mu \)g/kg/min) of calcium were sequentially given intrarenally for 30 minutes each. Calcium doses were isotonic solutions made from a calcium gluconate solution containing 0.465 meq Ca/ml. The remaining eight dogs received saline without prostaglandin synthesis inhibitors during the 30 minutes after the basal clearances, followed by the infusion of the two doses of calcium gluconate.

In all the dogs, urine samples were collected during the last 15 minutes of each 30-minute infusion. Blood samples from femoral artery and from renal vein were drawn at the middle of the 15-minute clearance period.

As previously mentioned, two different cyclooxygenase inhibitors were used to rule out any specific side effects inherent to the drug that are not related to prostaglandin inhibition. The results obtained with indomethacin and meclofenamate were pooled because no quantitative or qualitative differences were detected.

Renin activity in renal vein plasma and in artery plasma were determined by radioimmunoassay. Renin secretion was calculated as the product of the difference between venous and arterial plasma renin activities and renal plasma flow. Urinary 6-keto-prostaglandin F1a (6kPGF1a), the stable metabolite of PGI2, was measured by radioimmunoassay.

Sodium and potassium concentrations in plasma and in urine were measured with an electrolyte analyzer (Beckman E2A, Beckman Instrs., Inc., Brea, California). Calcium concentrations in plasma and in urine were measured by an atomic absorption spectrophotometer (model 357, Instrumentation Laboratory Inc., Lexington, Massachusetts). Plasma and urine inulin concentration was measured with the Anthrone method. All values are expressed as means±SEM. Data were analyzed with randomized block analysis of variance, Newman-Keuls multiple range test and Student's unpaired t test; \( p \) values less than 0.05 were considered significant.

Results

The intrarenal infusion of 10 \( \mu \)g Ca/kg/min induced a 77% increase of renin secretion from 112.1±26.1 to 198.7±56 ng angiotensin I (Ang I)/min (Figure 1). During the infusion of 100 \( \mu \)g Ca/kg/min, however, the values returned to precalcium levels (113.7±75.4 ng Ang I/min). The administration of prostaglandin inhibitors reduced the basal levels of renin secretion from 139.5±42 to 63.3±32.5 ng Ang I/min. After the administration of prostaglandin inhibitors, the infusion of 10 and 100 \( \mu \)g Ca/kg/min failed to produce any...
significant alteration in renin secretion (24.1±11.2 and 61.4±2.8 ng Ang I/min, respectively) (Figure 1).

The precalcium control levels of MAP and RBF in both groups were 115±7 mm Hg and 113±11 ml/min without prostaglandin inhibitors and 118±6 mm Hg and 114±31 ml/min in the presence of indomethacin or meclofenamate. The intrarenal infusion of calcium did not induce significant alterations in MAP or RBF in the absence or in the presence of prostaglandin inhibitors. The observed increase in renin secretion during the intrarenal infusion of 100 μg Ca/kg/min suggests a stimulus for renin secretion through a prostaglandin-mediated mechanism. This stimulation of renin secretion was not accompanied by any significant hemodynamic alterations but occurred in the presence of a significant increase in UNaV. Furthermore, the results also show that the increase in renin secretion induced by the infusion of 10 μg Ca/kg/min was reversed to control levels by the infusion of 100 μg Ca/kg/min. This latter effect was accompanied by a further increase in UNaV, whereas the hemodynamic parameters remained unchanged.

Three possible mechanisms could be involved in the regulation of renin secretion under our experimental conditions. 1) An increase of calcium influx into the juxtaglomerular cells would tend to decrease renin secretion. 2) Calcium might decrease renin secretion indirectly by increasing the prevalence of the stimulatory effect of PGI2 over the two inhibitory mechanisms mentioned above. 3) Calcium can stimulate the endothelial production of PGI2, which could enhance renin secretion. The observed increase in renin secretion during the infusion of 10 μg Ca/kg/min suggests a prevalence of the stimulatory effect of PGI2 over the two inhibitory mechanisms mentioned above.

Peripheral plasma concentration of sodium, potassium, and calcium were not altered by the infusion of either dose of calcium given in the presence or in the absence of prostaglandin inhibitors.

**Discussion**

The present study demonstrates that the intrarenal infusion of 10 μg Ca/kg/min increases renin secretion through a prostaglandin-mediated mechanism. This stimulation of renin secretion was not accompanied by any significant hemodynamic alterations but occurred in the presence of a significant increase in UNaV. Furthermore, the results also show that the increase in renin secretion induced by the infusion of 10 μg Ca/kg/min was reversed to control levels by the infusion of 100 μg Ca/kg/min. This latter effect was accompanied by a further increase in UNaV, whereas the hemodynamic parameters remained unchanged.

Three possible mechanisms could be involved in the regulation of renin secretion under our experimental conditions. 1) An increase of calcium influx into the juxtaglomerular cells would tend to decrease renin secretion. 2) Calcium might decrease renin secretion indirectly by increasing the delivery of sodium to the macula densa during natriuresis. 3) Calcium can stimulate the endothelial production of PGI2, which could enhance renin secretion. The observed increase in renin secretion during the infusion of 10 μg Ca/kg/min suggests a prevalence of the stimulatory effect of PGI2 over the two inhibitory mechanisms mentioned above.

During the infusion of 10 μg Ca/kg/min, there were no changes in the U6kPGF1α, however, it has been reported that endothelial release of PGI2 is likely to influence the smooth muscle in amounts that are not high enough to diffuse into circulation. During the renal PGI2 stimulation, the release of PGI2 can be significantly increased and detected in the renal venous effluent and in urine, in amounts that are not proportional to the quantities that are being synthesized. This assumption is supported by these results, in which the inhibition of prostaglandin synthesis prevents the stimulation of renin secretion. Iwao et al also reported an increase in renin secretion during the intrarenal
infusion of 0.68 M of calcium chloride; however, they did not measure prostaglandin or UNaV.

The return of renin secretion to control levels during the infusion of 100 μg Ca/kg/min could indicate that the stimulatory effect of PGI₂ was countered by an inhibitory mechanism. A greater influx of calcium into the juxtaglomerular cells might initiate an inhibitory effect on renin secretion that offsets the stimulatory effects of PGI₂. This could explain the normalization of renin secretion despite elevated levels of 6kPGF₁₀ in urine.

The effect of the larger dose of calcium on renin secretion is in agreement with the results of Kotchen and Guthrie.⁹ These authors found that the intrarenal infusion of 300 μg/kg/min of calcium chloride or calcium gluconate reduced renin secretion. They postulated that calcium could stimulate sodium delivery to distal tubules, inducing a decrease in renin secretion through the macula densa. In our experiment, this mechanism might have been important during the administration of 100 μg Ca/kg/min because renin secretion was reversed to control values in the presence of increased UNaV. The relative importance of the macula densa mechanism in the inhibition of renin secretion was suggested by Watkins et al.,⁹ in that the infusion of 250 μg Ca/kg/min in sodium-depleted dogs inhibited renin secretion without altering UNaV. Furthermore, they found that the intrarenal infusion of calcium can reduce renin secretion in nonfiltering kidneys in which the tubular function has been abolished.⁹ They concluded that the inhibitory effect of calcium on renin secretion was produced through a direct action on juxtaglomerular cells.

An important experimental condition for detecting the stimulatory effect of calcium on renin secretion can be the basal levels before its administration. In the experiments by Watkins and Kotchen and their colleagues,⁹,¹⁰ in which calcium induced a decrease in renin secretion, the dogs had high base line renin levels. In contrast, our results and those of Iwao¹⁵ were obtained in dogs with low basal levels of renin secretion.

These results suggest that 1) the intrarenal infusion of small doses of calcium gluconate can be of stimulating renin secretion through a prostaglandin-mediated mechanism, and 2) the stimulation of renin secretion as well as the increase in UNaV induced by calcium are independent of hemodynamic alterations.

Acknowledgments

We thank Jay A. Kachelski for his technical assistance and Paulette J. Peterson for her secretarial assistance.

References

5. Brotherton AF, Hoak JC: Role of Ca and cAMP in the regulation of the production of PGI₂ by the vascular endothelium. Proc Natl Acad Sci USA 1982;79:495-499

Key Words: prostaglandin • indomethacin • natriuresis • renal function
Stimulation of renin release by intrarenal calcium infusion.
V Lahera, M J Fiksen-Olsen and J C Romero

Hypertension. 1990;15:I149
doi: 10.1161/01.HYP.15.2_Suppl.I149

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/2_Suppl/I149

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/