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Relationship between Extra- and Intracellular Calcium in Distal Segments of the Renal Tubule. Role of the Ca^{2+} Receptor RaKCaR

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Abstract. The effect of extracellular calcium ($[Ca^{2+}]_e$) on cytosolic calcium ($[Ca^{2+}]_i$) was investigated in thick ascending limbs and collecting ducts from the rat kidney, using the fluorescent dye fura-2. In cortical collecting ducts, basolateral but not apical changes in $[Ca^{2+}]_e$ were associated with parallel changes in $[Ca^{2+}]_i$. Basal $[Ca^{2+}]_i$ was hardly modified by nifedipine and verapamil but was decreased by 60% by basolateral La^{3+}. Increasing peritubular $[Ca^{2+}]_e$ triggered Ca^{2+} release from intracellular stores. This effect was not reproduced by agonists of the renal Ca^{2+}-receptor RaKCaR, e.g., Ba^{2+}, Mg^{2+}, Gd^{3+}, and neomycin, but was reproduced by Ni^{2+}. Ni^{2+}-induced mobilization of intracellular Ca^{2+} was larger in the inner medullary collecting duct, a segment which poorly responds to increasing $[Ca^{2+}]_e$.

In the cortical thick ascending limb, removing basolateral Ca^{2+} hardly altered $[Ca^{2+}]_i$ but increasing $[Ca^{2+}]_e$ or adding Ba^{2+}, Mg^{2+}, Gd^{3+} and neomycin released intracellular calcium.

These data demonstrate that (1) basolateral influx of calcium occurs in cortical collecting ducts, under basal conditions; (2) this influx occurs through nonvoltage gated channels, permeable to Ba^{2+}, insensitive to verapamil and nifedipine, and blocked by La^{3+}; (3) increasing $[Ca^{2+}]_e$ stimulates the influx and triggers intracellular calcium release, independently of the phospholipase C-coupled receptor RaKCaR; (4) RaKCaR is functionally expressed in thick ascending limbs; (5) another membrane receptor, sensitive to Ni^{2+} but not to Ca^{2+} is present in the collecting duct.

Key words: Rat kidney — Fura-2 fluorescence — Cytosolic calcium — Single tubule — Thick ascending limb — Collecting duct — Ca^{2+} receptor

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Introduction

In most cell types, cytosolic calcium concentration ($[Ca^{2+}]_i$) is low—about four orders of magnitude below that of extracellular fluids—and remains constant when the extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) varies. This may be explained, firstly by the fact that cell membranes are poorly permeable to Ca^{2+} under resting conditions, and secondly by the great efficiency of Ca^{2+} extrusion mechanisms (calcium pumps and/or Na/Ca exchanger) which ensure the maintenance of this high transmembrane Ca^{2+} gradient and regulate $[Ca^{2+}]_i$ variations. In some cell types, however, variations of $[Ca^{2+}]_e$ are associated with parallel changes in $[Ca^{2+}]_i$. This unusual property concerns some endocrine tissues (glomerulosa cells of adrenal glands [8], parathyroid [34], and thyroid C cells [15]), but it has also been found in the cornea [29], and in discrete terminal portions of the mammalian nephron, in particular the cortical collecting duct (CCD) [37]. According to the cell type studied, three different mechanisms have been proposed to account for the dependency of $[Ca^{2+}]_i$ upon $[Ca^{2+}]_e$, namely (1) Ca^{2+} influx through membrane channels which are open under basal conditions [12, 37]; (2) Ca^{2+} influx through a Na/Ca exchanger functioning in the reverse mode [4]; and (3) intracellular Ca^{2+} release and Ca^{2+} influx mediated by a phospholipase C-coupled receptor, sensing extracellular Ca^{2+}. This latter process, which has been described for the first time in bovine parathyroid cells [6, 23, 33], implies that the events associated with an increase in $[Ca^{2+}]_e$ are not limited to an increase in $[Ca^{2+}]_i$, but also involve an enhanced production of diacylglycerol and an activation of protein kinase C.

An isoform of the bovine Ca^{2+}-receptor (BoPCaR, [7]) has been found recently in the rat kidney (RaKCaR, [28]). *In situ* hybridization experiments carried out on

renal tissue slices to localize the mRNA have shown an intense labeling in discrete regions of the cortex and the outer medulla—the so called ''medullary rays''—but no labeling in the inner medulla. According to preliminary results of reverse transcription-polymerase chain reaction (RT-PCR), the mRNA would be present in the thick ascending limb of Henle's loop (cortical and medullary portion) and in the cortical collecting duct [27], a result suggesting that the Ca^{2+}-receptor may participate to $[Ca^{2+}]_i$ variations subsequent to changes in extracellular Ca^{2+} concentration.

The mechanisms responsible for $[Ca^{2+}]_e$-induced $[Ca^{2+}]_i$ variations in rat CCD have been studied some years ago, before the discovery of Ca^{2+}-receptors [37, 38]. The question whether a rise in $[Ca^{2+}]_e$ triggers an intracellular Ca^{2+} release, therefore, has not been addressed. On the basis of their data, Taniguchi et al. concluded that the Na/Ca exchanger is weakly active in CCD and does not significantly contribute to Ca^{2+} entry or Ca^{2+} extrusion under normal conditions [38]. According to these authors, $[Ca^{2+}]_i$ variations secondary to changes in $[Ca^{2+}]_e$ would rather result from changes in a passive influx of Ca^{2+} through voltage-independent membrane channels [37]. However, neither the pharmacological properties nor the localization of these putative channels to apical and/or basolateral membranes have been established in this study.

Because of these uncertainties, and because Ca^{2+} plays a primary role in the regulation of most cell functions, we thought it necessary to clarify which mechanism(s) underlie(s) the sensitivity of rat CCD to changes in $[Ca^{2+}]_e$. The goal of our experiments was thus (1) to determine whether $[Ca^{2+}]_e$-induced $[Ca^{2+}]_i$ variations originate from the apical and/or basolateral side of tubular cells; (2) to establish whether they only concern CCD or also take place in the outer and inner medullary portions of the collecting duct (OMCD, IMCD); (3) to investigate whether Ca^{2+} release from intracellular pools participates—together with Ca^{2+} influx—in Ca^{2+} signals evoked by increasing $[Ca^{2+}]_e$ and, if so, (4) whether the Ca^{2+}-receptor RaKCaR is involved in this process. Since high levels of the RaKCaR mRNA have been found in the thick ascending limb [27], cortical portions of this segment (CTAL) were studied in parallel, as controls.

Materials and Methods

Preparation of Tubules and Fluorescence Measurements

The technique used for measuring $[Ca^{2+}]_i$ in single microdissected tubules has already been described [9, 35 37]. Briefly, experiments were carried out on male Sprague-Dawley rats (90–120g body weight) fed a usual laboratory diet with free access to water. On the day of the experiment, the animals were stunned and bled by section of the carotid arteries. The kidneys were then quickly removed and cut into coronal slices for free-hand microdissection at 4°C in standard medium (*see below*) containing $CaCl_2$ 2 mM and 0.1% BSA (wt/v). Collecting duct fragments were isolated from medullary rays in the middle cortex (CCD), from the inner stripe of outer medulla (OMCD) and from the tip of papilla (IMCD). Cortical and outer medullary fragments of the thick ascending limb of Henle's loop (CTAL, MTAL) were also prepared in some experiments. All microdissected tubules were kept on ice until use. Sequential loading of these samples with acetomethoxyester of fura-2 (fura-2 AM 10 μM for 1 hr at room temperature) was initiated every 20–25 min (e.g., the usual duration of fluorescence measurements on a single tubule). Each fura-2-loaded tubule was then transferred to a perfusion chamber, sucked at both ends within the tip of glass holding pipettes and, according to the requirements of our experiments, either microperfused with a usual device of concentric pipettes, or simply superfused as already described [35]. Under both conditions, peritubular fluid was continuously exchanged at a rate of about 10 ml/min. All media were stored until use in individual reservoirs. Their temperature was increased to 37°C with a heating jacket just before they entered the perfusion chamber. Luminal fluid was perfused by gravity at a slow rate (2–5 nl/min). Exchange of luminal solution, when necessary, was completed within 20–30 secs.

Double wavelength measurements of fura-2 fluorescence were carried out after a 5–10 min equilibration period with a standard photometric set up (model MSP 21, Zeiss, Germany) assisted by a microcomputer [9]. The tubular portion selected for fluorescence measurements included about 20–30 cells. As a rule, all tubules were studied within less than 3 hr following their microdissection.

Calculations

Net fluorescence intensities of fura-2 at 340 nm (S) and 380 nm (L) were obtained by substracting corresponding autofluorescence values from all measurements [9, 35]. $[Ca^{2+}]_i$ was calculated from the equation of Grynkiewicz et al. [17], using a dissociation constant of fura-2 for calcium of 224 nM and calibration parameters determined as previously described [35, 37].

Unless otherwise indicated, results from several tubules were expressed as mean values ± SEM. Each experimental condition was tested on one or several tubules per rat in at least three different rats. Statistical significance of the data was evaluated either by Student's *t* test (paired or unpaired, as appropriate), or by variance analysis (ANOVA) when several groups were compared. If significant differences between groups were detected, they were evaluated using the Scheffé *F* ratio test. $P < 0.05$ was considered statistically significant.

Solutions

The standard medium contained (in mM): NaCl 150; KCl 1; $KHCO_3$ 4; Na_2HPO_4 0.33; NaH_2PO_4 0.44; $MgCl_2$ 1; $MgSO_4$ 0.8; $CaCl_2$ 2; D-glucose 5; N-2-hydroxy-ethyl-piperazine-N-2-ethanesulfonic acid (HEPES) 10; pH 7.4. Osmotic pressure was 310 mOsm/kg.

The so-called Ca^{2+}-free medium was identical to the standard medium except that it had no added $CaCl_2$ and contained 0.1 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA).

When a Na^+-free solution was used, NaCl and Na-phosphates were replaced by tetramethylammonium-chloride and K-phosphates, respectively. It should be noted that TMA^+ ions may induce hormone-like $[Ca^{2+}]_i$ variations by interacting with cholinergic receptors [21]. Since the rat collecting duct is a target site for cholinergic agonists [19,

35], 10^{-4} M atropine was always added to the medium when TMA^+ was present.

Finally, phosphate and bicarbonate-free media were used to test the effects of lanthanum (La^{3+}), barium (Ba^{2+}), and gadolinium (Gd^{3+}).

Materials

Salts used in the standard solution were products from Merck (Darmstadt, Germany); fura-2 (free acid) and fura-2-AM (acetomethoxyester) were purchased from Molecular Probes (Eugene, OR); ouabain, atropine, neomycin, nifedipine and verapamil were from Sigma Chemical (St Louis, MO).

Results and Discussion

Effect of Extracellular Calcium Concentration on $[Ca^{2+}]_i$

Changes in Peritubular Calcium

Experiments were carried out on superfused segments to investigate the effect of removing peritubular calcium on $[Ca^{2+}]_i$ in the different portions of the collecting duct. As mentioned in Materials and Methods, the tubules were tightly mounted on glass holding pipettes, preventing any access of superfusate to the lumen. Therefore, changes applied to superfusate composition only concerned basolateral membranes. Results in Fig. 1 show that CCD, OMCD, and IMCD segments bathed in standard medium exhibited the usual $[Ca^{2+}]_i$ level found in most cell types (80–250 nM). In CCD and OMCD, removing calcium induced a steep 50% decrease in $[Ca^{2+}]_i$ (CCD: from 213 ± 8 nM to 101 ± 4, $n = 14$; OMCD: from 137 ± 4 to 74 ± 4, $n = 13$; $P < 0.001$ for both segments). In contrast, $[Ca^{2+}]_i$ dropped by only 22% in IMCD (from 102 ± 5 to 79 ± 4 nM, $n = 13$, with and without Ca^{2+}, respectively; $P < 0.005$).

In CCD and OMCD the pattern of $[Ca^{2+}]_i$ decrease was monophasic and of exponential type but the time constant of the exponential process (k min^{-1}) was significantly higher in CCD ($P < 0.001$). Following the initial decrease, no further fall of $[Ca^{2+}]_i$ could be observed in any of these segments, but after 15 min in Ca^{2+}-free medium the intracellular Ca^{2+} release elicited by the peptidic hormone vasopressin (Fig. 5*B*) or the diterpene alkaloid thapsigargin (*data not shown*) was abnormally low, an observation suggesting that intracellular stores were depleted. In CCD and OMCD, the fall of $[Ca^{2+}]_i$ was fully reversible upon re-adding calcium to the bath. In both segments, the pattern of recovery was biphasic, exhibiting a marked $[Ca^{2+}]_i$ overshoot.

Stepwise re-addition of calcium was carried out on some tubules to examine in more detail the relationship between $[Ca^{2+}]_e$ and $[Ca^{2+}]_i$ (Fig. 2). The results show that the sensitivity of the collecting duct to $[Ca^{2+}]_e$ variations markedly diminishes from cortex to papilla. In CCD, the relationship between $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ was linear up to 5 mM peritubular calcium (mean slope: 48 ± 9 nM · mM^{-1}, $n = 4$; $r = 0.99 \pm 0.01$), whereas in OMCD $[Ca^{2+}]_i$ rose sharply between 0 and 1 mM and, then, either did not change (2 tubules; *see* the example in Fig. 2) or only slightly increased (4 tubules). As a mean, the slope calculated from all tubules was only 12.5 ± 3.9 nM. mM^{-1} between 1 and 3 mM ($r = 0.74 \pm 0.17$, $n =$

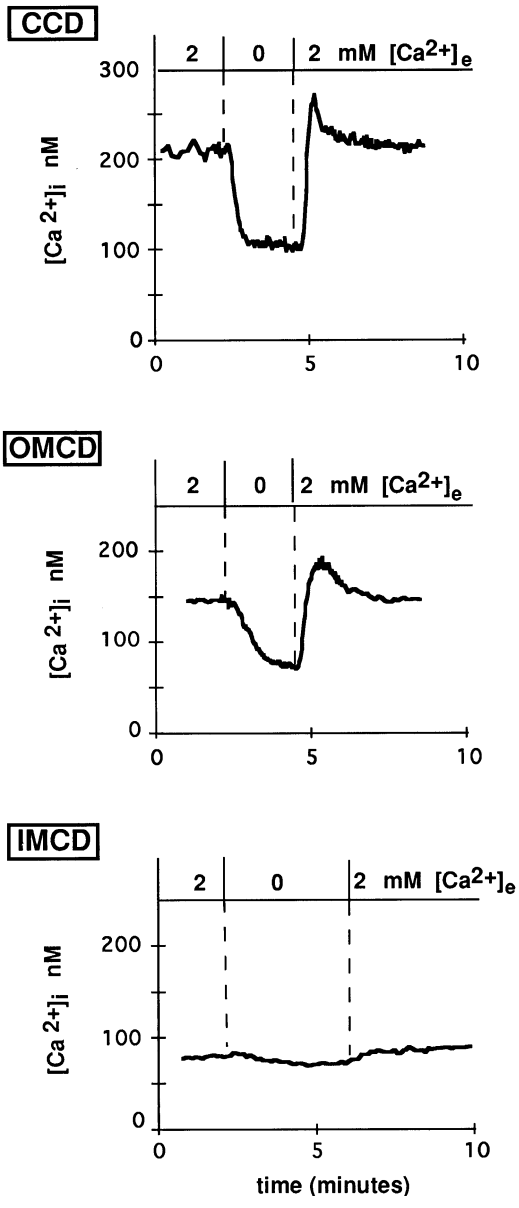

Fig. 1. Effect of removing and re-adding calcium to peritubular superfusion medium on $[Ca^{2+}]_i$. Representative traces obtained on segments superfused with standard medium (2 mM Ca^{2+}) to illustrate the different sensitivities of the cortical, outer and inner medullary portions of the rat collecting duct (CCD, OMCD, IMCD) to changes in extracellular calcium concentration ($[Ca^{2+}]_e$).

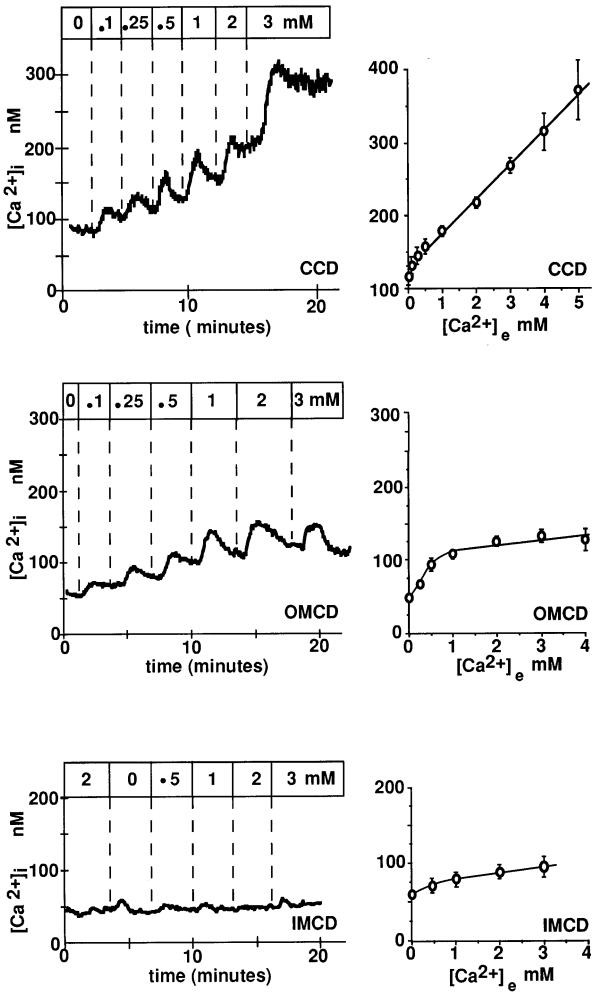

Fig. 2. Relationship between the calcium concentration of peritubular medium (mM) and $[Ca^{2+}]_i$ in the different portions of the rat collecting duct. Left hand panels: representative traces giving the time course of $[Ca^{2+}]_i$ variations induced by increasing calcium in bath medium on CCD, OMCD and IMCD. Right hand panels: relationship between $[Ca^{2+}]_e$ and mean $[Ca^{2+}]_i$ values achieved at equilibrium (±SEM). Data obtained from 4 to 6 experiments.

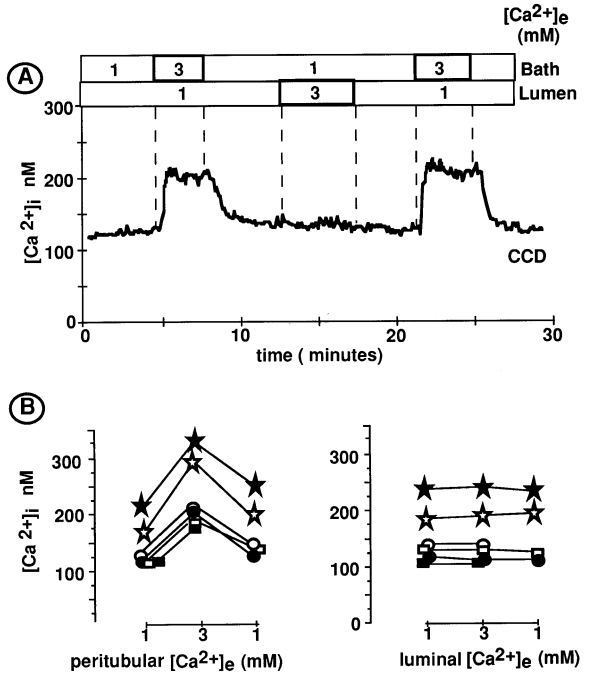

Fig. 3. Effect of increasing the luminal calcium concentration on $[Ca^{2+}]_i$ in the cortical collecting duct (CCD). Results obtained from microperfusion experiments in which extracellular calcium concentration was increased for each tubule from 1 to 3 mM, successively in basolateral (bath) and luminal medium (or conversely). Upper panel: representative trace given as an example. Lower panels: results obtained in 6 different tubules, each represented by a different symbol. Lines join $[Ca^{2+}]_i$ values measured in the same tubule during the control (1 mM Ca^{2+}), experimental (3 mM Ca^{2+}), and recovery period (1 mM Ca^{2+}).

6). In agreement with the data in Fig. 1, similar changes of $[Ca^{2+}]_e$ hardly modified $[Ca^{2+}]_i$ in IMCD. This latter observation is of obvious interest as regard to the function of IMCD cells. Indeed, it is well known that the water content and, consequently, the solute concentration of papillary interstitium are highly dependent on the diuretic state of the animal, e.g., on the amount of water reabsorbed along the collecting ducts [2]. If changes in water reabsorption alter interstitial $[Ca^{2+}]$ concentration, then the low sensitivity of IMCD to peritubular calcium may protect the cells against such modifications of their Ca^{2+} environment. In the cortex, by contrast, the tissue composition remains constant whatever the hydric state of the animal [2]. $[Ca^{2+}]_e$-induced $[Ca^{2+}]_i$ variations are thus necessarily secondary to hypo- or hypercalcemia.

Changes in Luminal Calcium

We used the in vitro microperfusion technique to analyze the effect of changing luminal calcium concentration on $[Ca^{2+}]_i$. We first observed that rat CCD segments microperfused with either standard (2 mM Ca^{2+}) or Ca^{2+}-free luminal fluid had similar basal $[Ca^{2+}]_i$ levels ($[Ca^{2+}]_i$ = 189 ± 6 nM, n = 37, and 189 ± 7, n = 32, respectively). This result suggests that the apical face of CCD cells is insensitive to variations of luminal calcium concentration. In support of this conclusion, we also found that increasing $[Ca^{2+}]_e$ from 1 to 3 mM in the lumen did not alter $[Ca^{2+}]_i$ ($[Ca^{2+}]_i$ = 154 ± 24 nM and 153 ± 22, n = 6, *see* Fig. 3), whereas the same maneuver carried out on the same tubules in peritubular medium increased $[Ca^{2+}]_i$ from 134 ± 16 to 228 ± 27 nM ($P < 0.001$). Similar results were obtained in OMCD (*data not shown*). These results demonstrate that $[Ca^{2+}]_i$ increases consecutive to elevations of extracellular calcium are exclusively of basolateral origin. From a physiological point of view, this means that there is no Ca^{2+}-receptor and no Ca^{2+} influx pathway in apical membranes of the rat

CCD. Additionally, this also indicates that, in contrast with previous data obtained in the rabbit [32], the rat CCD may not be the site of a transcellular Ca^{2+} reabsorption, at least under basal conditions.

Evidence for Extracellular Calcium Influx

The increase in $[Ca^{2+}]_i$ observed in CCD when bath calcium concentration was enhanced (Figs. 1 to 3) demonstrates net addition of calcium to cytosol but does not indicate whether calcium addition was of intra and/or extracellular origin. If Ca^{2+} influx takes place across basolateral membranes, then, specific inhibitors of calcium channels and/or transporters may be expected (1) to decrease $[Ca^{2+}]_i$ when added to basolateral superfusion solution, and (2) to reduce the rise of $[Ca^{2+}]_i$ induced by increasing peritubular calcium concentration. To test these points, we evaluated the effect of verapamil and nifedipine—two blockers of L-type voltage-gated channels—and that of La^{3+}, a nonspecific inorganic blocker of most types of calcium channels [26] (Table 1 and Fig. 4). Addition of nifedipine to standard superfusate did not alter $[Ca^{2+}]_i$, but verapamil produced in all CCDs a faint but significant $[Ca^{2+}]_i$ decrease (about 10% of the mean basal $[Ca^{2+}]_i$ value; $P < 0.001$). As shown by the figure, neither verapamil nor nifedipine modified the fall of $[Ca^{2+}]_i$ consecutive to removing calcium. Nor did they prevent the restoration of $[Ca^{2+}]_i$ upon calcium readdition, a result indicating that L-type voltage-sensitive Ca^{2+} channels, if present, do not play any significant part in the apparent permeability to Ca^{2+} of rat CCD cells. In contrast, peritubular addition of 100 μM La^{3+} induced a marked fall of $[Ca^{2+}]_i$. A further $[Ca^{2+}]_i$ decrease from 150 ± 10 to 106 ± 3 nM ($n = 6$, $P < 0.01$) occurred upon removing calcium from the bath, suggesting that La^{3+} did not totally inhibit Ca^{2+} entry into cytosol. Accordingly, the rise of $[Ca^{2+}]_i$ induced by readding calcium (2 mM) to Ca^{2+}-free superfusate was markedly dampened but not abolished in the presence of La^{3+} ($\Delta[Ca^{2+}]_i = 29 \pm 5\%$ of the control $[Ca^{2+}]_i$ variation measured in the absence of blocker on the same tubules; $P < 0.001$ *vs.* control). Taken together, these results indicate that a basolateral Ca^{2+} influx occurs in rat CCD in the absence of any added agonist. The fall of $[Ca^{2+}]_i$ consecutive to basolateral calcium removal may be explained by the suppression of this influx, the kinetics of the decay reflecting the activity of Ca^{2+} extrusion mechanisms. The rate of Ca^{2+} exchange between extracellular medium and cytosol (influx as well as efflux) is thus highest in CCD, intermediate in OMCD, and lowest in IMCD (Fig. 1).

Mechanisms of Calcium Influx

Because La^{3+} inhibits not only different types of calcium channels but also sodium-calcium exchange in intact

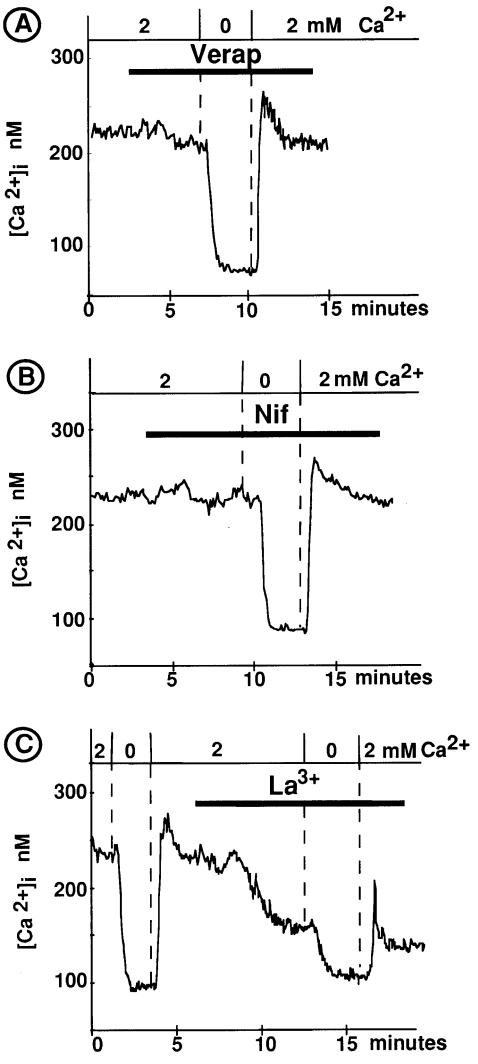

Fig. 4. Effect of calcium channel antagonists on $[Ca^{2+}]_i$ in superfused rat CCDs. These representative traces illustrate the time course of $[Ca^{2+}]_i$ variations induced by adding 10 μM verapamil (*A*), 10 μM nifedipine (*B*) and 100 μM lanthanum (*C*) to standard peritubular medium (2 mM Ca^{2+}). The effect of these blockers was evaluated, first by their ability to decrease $[Ca^{2+}]_i$; second by their ability to blunt $[Ca^{2+}]_i$ increases consecutive to re-adding calcium to Ca^{2+}-free peritubular medium. Note that only lanthanum did actually behave as an inhibitor of calcium influx in rat CCD.

cells [40], the results reported above do not give information on the nature of the transport pathway(s) involved in calcium entry. Experiments carried out by Breyer in the rabbit ascribed a primary role to the Na/Ca exchanger in Ca^{2+} entry (into) and Ca^{2+} extrusion out of CCD cells [5]. In contrast, data obtained in the rat by Taniguchi et al. rather supported the hypothesis of Ca^{2+} channels [37]. For these authors, a Ca^{2+} entry through nonvoltage-gated ''leak'' channels matched by an active extrusion via Ca^{2+}-ATPases would be the main mechanism by which CCD cells maintain a steady state $[Ca^{2+}]_i$

Table 1. Effect of different calcium channel blockers on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in the rat cortical collecting duct (CCD)

Compound	Cytosolic calcium concentration (nM)		
	Control	Experimental	*n*
Nifedipine (10 μM)	253 ± 14	252 ± 12	(8)
Verapamil (10 μM)	221 ± 9	201 ± 9*	(5)
La^{3+} (100 μM)	246 ± 15	151 ± 12**	(7)

Control: $[Ca^{2+}]_i$ values (mean ± SEM) measured in single microdissected segments superfused with standard medium (*see* Materials and Methods); Experimental: values measured at equilibrium on the same tubules after addition of the blockers to basolateral superfusion solution; *n*: number of tubules; $*P < 0.01$ *vs.* control; $**P < 0.001$. Representative traces of these experiments are given in Fig. 4.

level, under various circumstances. This conclusion rests on the three following arguments: (1) $[Ca^{2+}]_e$-induced $[Ca^{2+}]_i$ variations could be observed in tubules exhibiting a low Na/Ca exchange activity [37, 38]; (2) $[Ca^{2+}]_i$ was not increased but, on the contrary, decreased in depolarizing K^+-enriched media [37]; (3) the relationship between $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ was linear in CCD, a result compatible with a passive calcium influx down the electrochemical gradient [37]. Present results confirm these conclusions as $[Ca^{2+}]_i$ declined in CCD bathed in a high-K^+ solution (*data not shown*). Moreover, we also found a linear relationship between $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ (Fig. 2), and we demonstrated that basolateral calcium entry is insensitive to nifedipine (Fig. 4). At first glance, the slight $[Ca^{2+}]_i$ decrease induced by verapamil (Table 1) could seem at variance with these latter data. It should be noted, however, that verapamil is a potent inhibitor of basolateral K^+ channels in cortical collecting ducts of the rat kidney [31]. The $[Ca^{2+}]_i$ decrease observed in CCD may thus be indirect, resulting from membrane depolarization subsequent to blockade of basolateral K^+ channels.

As regards the Na/Ca exchanger in CCD, we confirm that it is barely active in the rat and that only drastic changes of the transmembrane sodium gradient can induce the reverse mode of functioning. This conclusion is based on the following observations: (1) in microperfused CCDs, substitution of basolateral Na^+ with TMA^+ increases $[Ca^{2+}]_i$ much less than in the rabbit under similar experimental conditions ($\Delta[Ca^{2+}]_i = 81 \pm 8$ nM, $n = 4$; *vs.* $\Delta[Ca^{2+}]_i > 400$ nM, as described in [5]); (2) we calculated that increasing $[Ca^{2+}]_e$ from 1 to 3 mM hardly modifies the driving force for Ca^{2+} entry through the exchanger[1]; finally, (3) the effect of a Na^+-free peritubular medium was tested on IMCD ($\Delta[Ca^{2+}]_i$ in IMCD $= 358 \pm 63$ nM, $n = 4$, *vs.* 106 ± 28, $n = 4$, in CCD from the same rats), a result demonstrating that the presence of a functional Na/Ca exchanger is not sufficient to generate $[Ca^{2+}]_i$ variations in response to changes in $[Ca^{2+}]_e$ (Fig. 1 and 2). On the basis of these data, we think it justified to conclude that Ca^{2+} permeable channels (rather than Na/Ca exchange) are responsible for basolateral Ca^{2+} entry in rat CCD.

As regards the mechanisms of Ca^{2+} extrusion, we found that removing basolateral Ca^{2+} from a Na^+-free superfusate (a condition expected to inhibit Na^+-dependent Ca^{2+} extrusion) did not diminish, but on the contrary tended to accelerate the exponential rate of $[Ca^{2+}]_i$ decrease ($k = 4.9 \pm 0.4$ min^{-1}, $n = 5$, *vs.* 3.7 ± 0.5, $n = 4$, in Na^+-free and standard medium, respectively). Consequently, Ca^{2+}-ATPases rather than Na/Ca exchange should account for Ca^{2+} extrusion across rat CCD basolateral membranes. This conclusion is in agreement with previous data demonstrating that the Na/Ca exchanger is preferentially expressed in nephron segments involved in transcellular Ca^{2+} reabsorption [3].

The question whether an increase in peritubular Ca^{2+} concentration can inhibit—*per se*—Ca^{2+} extrusion by the plasma membrane Ca^{2+}-ATPase (or, conversely, a $[Ca^{2+}]_e$ decrease stimulate the rate of pumping out) has not been addressed here. However, previous studies carried out by Donnadieu et al. in intact lymphocytes have shown that the activity of the plasma membrane Ca^{2+}-ATPase is highly dependent on the concentration of Ca^{2+} in cytosol, but hardly on that prevailing in extracellular medium [10].

With regard to OMCD, it is interesting to note that no $[Ca^{2+}]_i$ decrease could be observed in the presence of either verapamil or nifedipine, and that $[Ca^{2+}]_i$ fell in a high-K^+ basolateral medium, as in CCD (*results not*

[1] Assuming that the stoichiometry of the exchange is 3 Na^+/1 Ca^{2+}, as in most cell types [13], the reversion potential for the exchanger (E_R) is given by the formula: $E_R = 3\,E_{Na} - 2\,E_{Ca}$ where E_{Na} and E_{Ca} are the Nernst equilibrium potentials for Na^+ and Ca^{2+}, respectively. If V_m is the basolateral membrane voltage, the net driving force for Ca^{2+} transport through the exchanger (V_{Ca}) is given by: $V_{Ca} = V_m - E_R$ (negative values indicating Ca^{2+} exit). In CCDs superfused under the following conditions ($[Ca^{2+}]_e = 1$ mM; $[Na^+]_e = 150$ mM), mean resting $[Ca^{2+}]_i$ values amounted to about 180 nM. Assuming an intracellular concentration of Na^+ of about 16 mM [25] and a value of −80 mV for V_m [31] the calculated V_{Ca} was only slightly negative under basal conditions (−30 mV), an observation indicating that the Na/Ca exchanger functioned in the forward mode (calcium exit) but near its electrochemical equilibrium, and therefore generated small Ca^{2+} fluxes. When basolateral $[Ca^{2+}]_e$ was increased to 3 mM, V_{Ca} rose to 0 mV. It is thus clear that the Na/Ca exchanger was silent and could not account for the observed rise of $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$ in superfused tubules $= 138 \pm 15$ nM, $n = 9$). In agreement with this conclusion, we found that, in tubules superfused with a nominally Na^+-free solution (peritubular Na^+ concentration determined by flame photometry: 60 μM), the exchanger increased $[Ca^{2+}]_i$ by only 80 nM (*see above*), even though the calculated V_{Ca} value amounted to about +600 mV.

shown). The fact that the relationship between steady-state $[Ca^{2+}]_i$ values and $[Ca^{2+}]_e$ tended to saturate beyond 1 mM may be due to some regulatory process(es) and therefore does not necessarily imply that the mechanisms of Ca^{2+} entry are different in this segment.

To conclude, the basal permeability to Ca^{2+} of rat CCD cells may be ascribed to the presence of basolateral channels which are open under resting conditions. This permeability should be considered as an intrinsic specific property, since $[Ca^{2+}]_i$ was hardly decreased in proximal tubules [20] and cortical thick ascending limbs similarly superfused with Ca^{2+}-free media or La^{3+}-containing solutions (*see* Figs. 10 and 11).

Mobilization of Intracellular Calcium

Previous experiments have shown that parathyroid cells can detect small variations of extracellular Ca^{2+} concentration through a membrane receptor binding specifically divalent (and some trivalent) cations but not monovalent cations [6, 7, 23, 33]. This receptor (BoPCaR), which belongs to the superfamily of G protein-coupled receptors and activates phospholipase C, has been cloned recently from bovine parathyroid glands [7]. Further studies carried out by Riccardi et al. have established that a highly homologous isoform (RaKCaR) is present in the rat kidney [28], possibly in the cortical and medullary portions of the thick ascending limb and in the cortical portion of the collecting duct [27]. This latter observation made it necessary to investigate whether intracellular Ca^{2+} release participated—together with Ca^{2+} influx–in $[Ca^{2+}]_i$ increases subsequent to elevations of extracellular calcium, and whether the effect of a high calcium concentration could be reproduced by cations known to activate Ca^{2+}-receptors from other cell types, namely Ni^{2+}, Ba^{2+}, Mg^{2+}, Gd^{3+}, and the polycation neomycin [6, 7, 23, 28, 33, 42].

Evidence for Intracellular Calcium Release

The approach used to detect a participation of intracellular stores in the overall calcium response consisted in increasing $[Ca^{2+}]_e$ in the presence of La^{3+} to limit Ca^{2+} entry and tentatively unmask an initial peak, if present. Two different groups of tubules were studied. In the first one, CCDs were superfused with a 0.5 mM Ca^{2+} solution during the initial control period. As shown by the representative trace in Fig. 5*A*, increasing $[Ca^{2+}]_e$ from 0.5 to 5 mM triggered a clearly biphasic $[Ca^{2+}]_i$ variation involving an early spikelike component followed by a plateau. In the second group (Fig. 5*B*), tubules were first exposed to Ca^{2+}-free medium for 10–15 min, and then to vasopressin to deplete intracellular calcium pools [35], before adding La^{3+} and Ca^{2+}. Under this condition, adding 5 mM Ca^{2+} induced only a monophasic sustained

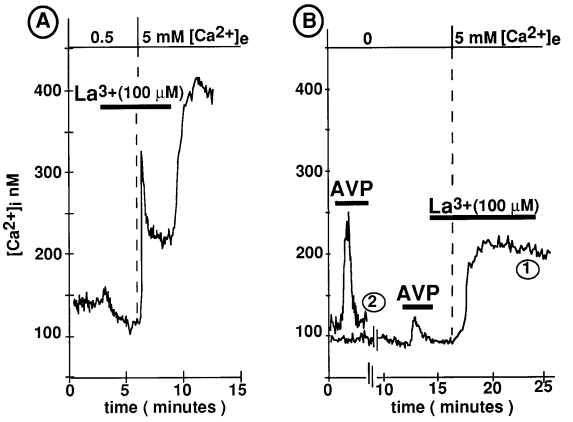

Fig. 5. Mechanisms participating in $[Ca^{2+}]_i$ variations induced by increasing extracellular calcium concentration ($[Ca^{2+}]_e$) in rat CCD. Traces compare the effect of increasing peritubular $[Ca^{2+}]_e$ in control (*A*) and Ca^{2+}-depleted tubules (*B*). Tubules of group A were superfused with a 0.5 mM Ca^{2+} solution during the equilibration period; Tubules of group *B* (trace 1), were first superfused for 10–15 min in Ca^{2+}-free medium, and then stimulated by 8-arginine vasopressin (10 nM AVP)—a peptide known to activate the phosphoinositide pathway in rat CCD [1, 35]—to achieve complete depletion of inositol phosphates-sensitive calcium stores. Comparison of the responses induced by AVP in trace 1 and in trace 2 (e.g., only 2 min after superfusing the Ca^{2+}-free medium) indicates that a substantial depletion of intracellular stores could be achieved in CCD only by superfusing the Ca^{2+}-free medium. In groups *A* and *B*, lanthanum (100 μM) was added to the bath 2 min before increasing $[Ca^{2+}]_e$ to 5 mM, to reduce extracellular calcium entry. Note that the peak of the response to 5 mM Ca^{2+} disappeared in Ca^{2+}-depleted tubules.

elevation of $[Ca^{2+}]_i$, a result suggesting that the initial peak reflected Ca^{2+} release from intracellular pools whereas the plateau (which was lower in magnitude than that observed after La^{3+} removal in control tubules (Fig. 5*A*)) reflected a residual Ca^{2+} entry persisting in the presence of La^{3+}. This entry was of extracellular origin since it could be observed in Ca^{2+}-depleted tubules as well.

Lack of Evidence for the Ca^{2+} Receptor RaKCaR in Rat CCD

Figures 6 and 7 compare the effects of Ca^{2+} and Ca^{2+}-receptor agonists on $[Ca^{2+}]_i$ in CCD. As shown in Fig. 6*A*, Ni^{2+} (5 mM)—a Ca^{2+}-channel blocker known to stimulate the osteoclast Ca^{2+}-receptor [42]—elicited a sharp calcium spike ($\Delta[Ca^{2+}]_i = 215 \pm 25$ nM above basal, $n = 8$) followed by a marked $[Ca^{2+}]_i$ decrease, when added to standard medium. In Ca^{2+}-free solution (Fig. 6*B*), the spike was still observed ($\Delta[Ca^{2+}]_i = 240 \pm 54$ nM, $n = 5$), but no further fall of $[Ca^{2+}]_i$ could be observed after reversion of the spike. As already observed with La^{3+}, restoration of $[Ca^{2+}]_i$ consecutive to re-adding Ca^{2+} was significantly blunted ($\Delta[Ca^{2+}]_i$ with $Ni^{2+} = 30 \pm 5\%$ of the control $[Ca^{2+}]_i$ variation; $P <$

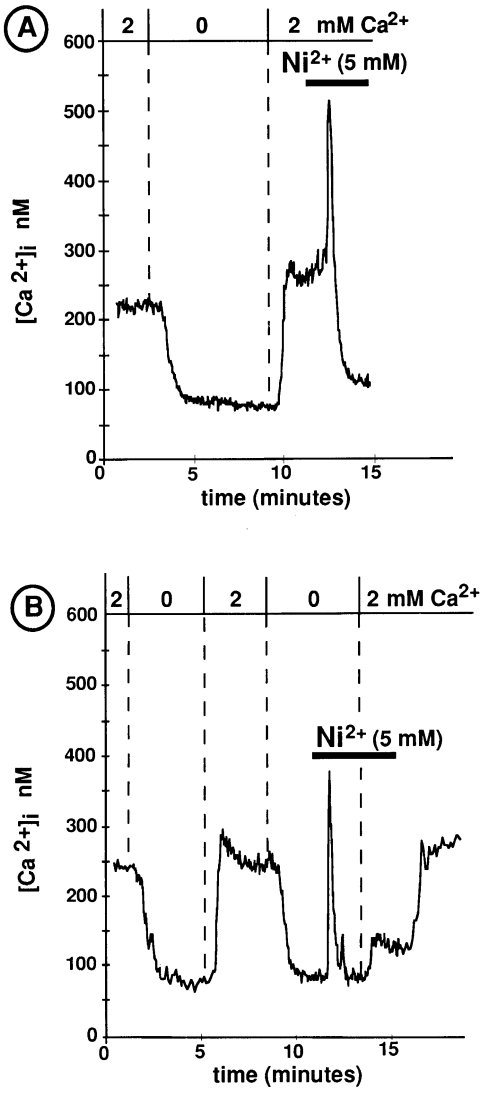

Fig. 6. Effect of Ni^{2+} (5 mM) on $[Ca^{2+}]_i$ in superfused rat CCD. (*A*): addition of Ni^{2+} to standard peritubular medium (2 mM Ca^{2+}) induced a large calcium spike before decreasing $[Ca^{2+}]_i$ to a level near that achieved in Ca^{2+}-free medium; (*B*): this spike persisted when Ni^{2+} was applied in Ca^{2+}-free medium, indicating an intracellular origin for Ca^{2+}. Note that Ni^{2+}, as La^{3+} (Figs. 4 and 5), blunted the $[Ca^{2+}]_i$ recovery usually observed upon calcium re-addition to bath medium.

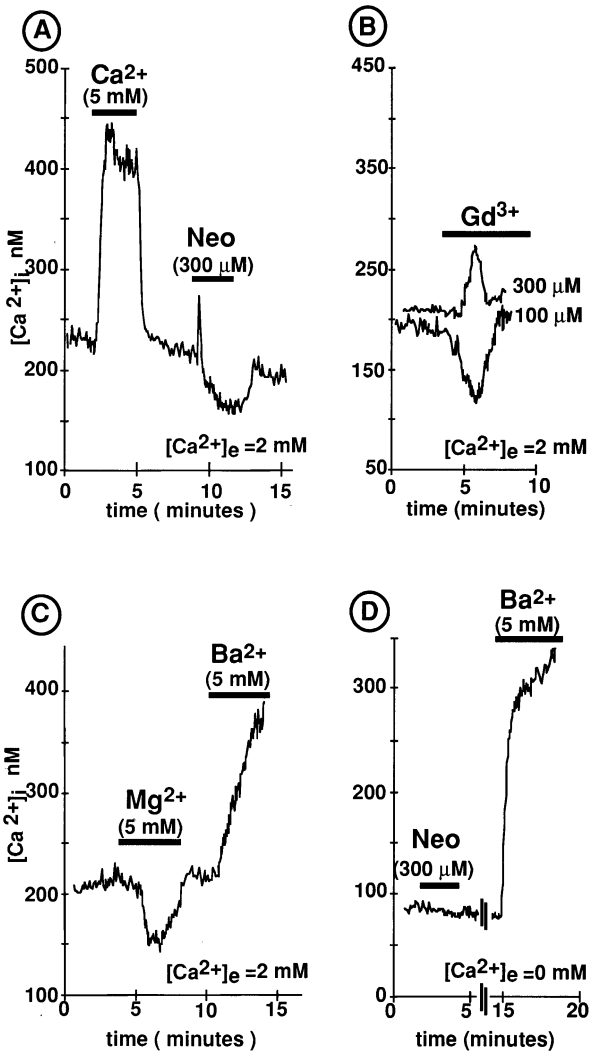

Fig. 7. Effect of Ca^{2+} and Ca^{2+}-receptor agonists on $[Ca^{2+}]_i$ in rat CCD. Traces obtained on single superfused segments to compare the time course of $[Ca^{2+}]_i$ variations induced by increasing peritubular calcium concentration, or adding neomycin (*A*), Gd^{3+} (*B*), Mg^{2+} and Ba^{2+} (*C*) to standard superfusion medium ($[Ca^{2+}]_e$ = 2 mM); Note that similar results were obtained in CCDs superfused with a 1 mM Ca^{2+} peritubular solution. (*D*): effect of neomycin and Ba^{2+} in Ca^{2+}-free medium. Note that the effect of neomycin became undetectable, whereas the effect of Ba^{2+} tested after 10 min of superfusion (when intracellular Ca^{2+} stores were depleted) was amplified. Assuming that the response to Ba^{2+} reflected Ba^{2+} and not Ca^{2+} entry within CCD cells (*see text*), the faster kinetics of the apparent $[Ca^{2+}]_i$ increase may be explained by the absence of Ca^{2+} ions which are likely to compete with Ba^{2+} to enter the cells through calcium channels.

0.001). These results demonstrate that Ni^{2+}—in addition to its inhibitory action on Ca^{2+} entry—released calcium from intracellular stores.

By contrast, no evidence for intracellular calcium mobilization was found when the same protocol was used with neomycin, Gd^{3+}, Mg^{2+} or Ba^{2+} (Fig. 7). In standard medium, the effect of neomycin (300 μM) was qualitatively similar to that of Ni^{2+} but quantitatively smaller (initial peak = 44 ± 5 nM above basal; subsequent decrease: −32 ± 8 nM, n = 6, Fig. 7*A*) and no signal could be detected in Ca^{2+}-free medium (Fig. 7*D*). Gd^{3+} (100 μM)—one of the most potent activators of the bovine parathyroid and rat renal Ca^{2+}-receptors [7, 28]—also decreased $[Ca^{2+}]_i$ (−71 ± 17 nM, n = 5; Fig. 7*B*). A transient and delayed $[Ca^{2+}]_i$ increase could be obtained in 4 out of 6 tubules (+63 ± 6 nM, n = 4) but only when the Gd^{3+} concentration was raised to 300 μM (Fig. 7*B*). Comparable results were obtained with Mg^{2+} (5 mM) which produced a discrete and transient fall of

$[Ca^{2+}]_i$ in 5 out of 12 CCDs (−34 ± 6 nM, $n = 5$, Fig. 7*C*) and a biphasic variation in the others ($\Delta[Ca^{2+}]_i$ after the initial decrease = +46 ± 13 nM, $n = 7$). Finally, Ba^{2+} (5 mM) did not elicit any calcium transient but induced in all segments tested a slow and continuous increase in the 340/380 fluorescence ratio, which was not maximal after five min of superfusion (Fig. 7*C*), and only partially reversed after Ba^{2+} removal (*not shown*).

It is well known that Gd^{3+} [24], as well as neomycin [11], and high Mg^{2+} concentrations [26] behave as blockers of Ca^{2+} channels in some cell types. Were it the case in CCD, the $[Ca^{2+}]_i$ decrease induced by these cations might be explained by an inhibition of basolateral Ca^{2+} influx. As regards Ba^{2+}, it is interesting to note that sustained, irreversible signals could be observed in Ca^{2+}-free solution on Ca^{2+}-depleted tubules (Fig. 7*D*), as in standard medium. Therefore, the response to Ba^{2+} does not result from calcium influx or calcium release, but rather reflects Ba^{2+} entry within CCD cells. Indeed, (1) Ba^{2+} is currently used to measure Ca^{2+} currents through membrane channels; (2) the interaction of Ba^{2+} with fura-2 produces the same displacement of the excitation spectrum as Ca^{2+} [18]; finally, (3) an entry of Ba^{2+} associated with an apparent increase in $[Ca^{2+}]_i$ has already been observed in bovine parathyroid [24], as well as mouse lacrimal cells [18]. From these results, it is clear that the response of CCD to Mg^{2+}, Ba^{2+}, Gd^{3+}, and neomycin reflects the interaction of these cations with basolateral Ca^{2+} channels, and that the Ca^{2+}-receptor, if present, has a cationic specificity different from that expected [28].

Evidence for a ''Ni^{2+}-Receptor'' in the Rat Collecting Duct

To account for the marked intracellular release elicited by Ni^{2+} and Ca^{2+} (but not by other cations) in CCD we hypothesized that the mRNA detected by RT-PCR in this segment [27] might encode for an isoform of RaKCaR, presenting a high degree of homology at the molecular level, but differences as regards its specificity towards polyvalent cations (in this case, an isoform more sensitive to Ca^{2+} and Ni^{2+} than to Ba^{2+}, Gd^{3+}, and neomycin). To be verified, this hypothesis implied that Ca^{2+} and Ni^{2+} interacted with a common membrane binding site to mobilize intracellular calcium, and consequently that Ni^{2+} would not release intracellular calcium in IMCD, e.g., a segment which hardly responds to variations of basolateral calcium concentration (*see* Fig. 1 and 2) and does not contain the RaKCaR mRNA [28]. In agreement with our previous data, increasing $[Ca^{2+}]_e$ from 1 to 5 mM induced only slight and transient $[Ca^{2+}]_i$ variations in this segment ($\Delta[Ca^{2+}]_i = 16 \pm 6$ nM, $n = 7$). Neomycin was also poorly active ($\Delta[Ca^{2+}]_i = 34 \pm 8$ nM, $n = 8$). In contrast, Ni^{2+} tested on the same tubules produced

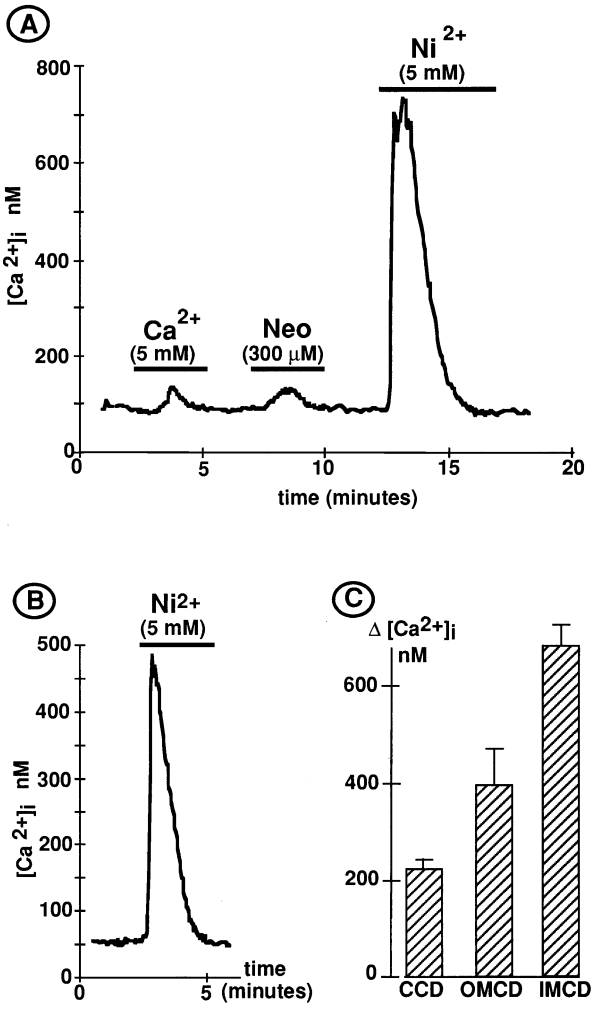

Fig. 8. Comparison of the effects of Ca^{2+}, neomycin, and Ni^{2+} on $[Ca^{2+}]_i$ in the terminal portion of the inner medullary collecting duct. (*A*): Trace obtained on a single IMCD segment, superfused with standard medium (2 mM Ca^{2+}) between the test periods; (*B*): effect of Ni^{2+} in Ca^{2+}-free medium; (*C*): mean $[Ca^{2+}]_i$ increases ± SEM induced by Ni^{2+} in CCD, OMCD and IMCD segments superfused with standard medium; IMCD: $P < 0.001$ *vs.* both CCD and OMCD; OMCD: $P < 0.025$ *vs.* CCD.

high calcium spikes amounting to 683 ± 36 nM above basal, $n = 8$, (Fig. 8*A*). These spikes persisted in Ca^{2+}-free medium ($\Delta[Ca^{2+}]_i = 331 \pm 50$ nM, $n = 3$, Fig. 8*B*), and no quenching of fura-2 fluorescence occurred upon addition of Ni^{2+} to the bath (*data not shown*). It is thus clear that Ni^{2+} behaved as a membrane impermeant cation and acted on an extracellular binding site in IMCD. This site which may be designated as a ''Ni^{2+} receptor,'' as long as its molecular identity and its function have not been identified, is distinct from RaKCaR since it is only barely sensitive to Ca^{2+} and neomycin. It is likely also distinct from the site which mobilizes intracellular calcium in CCD, since the sensitivity to Ni^{2+} decreases from IMCD to CCD (Fig. 8*C*) whereas the sensitivity to Ca^{2+}

increases (Figs. 1 and 2). Taken together, these results fail to demonstrate the presence of an isoform of RaKCaR activating PLC in the rat cortical collecting duct, but reveal the ability of Ni^{2+} to mobilize intracellular Ca^{2+} through an unknown membrane receptor, predominant in IMCD, the natural agonists of which remain to be identified.

From a functional point of view, the ''Ni^{2+}-receptor'' of IMCD shares some properties with the ''Ni^{2+}-receptor'' of osteoclasts which is highly sensitive to Ni^{2+} and only poorly sensitive to Ca^{2+} [42]. It may also be compared to the ''Cd^{2+}-receptor'' found in human fibroblasts and a number of other cell types, since this receptor stimulates inositol phosphate formation and intracellular Ca^{2+} release in response to low concentrations of Cd^{2+}, Co^{2+}, and Ni^{2+}, does not respond to Ba^{2+} and Gd^{3+}, and remains insensitive to Ca^{2+} up to 16 mM [36]. At the present time, however, none of these receptors has been cloned. It is thus not possible to conclude about their putative homologies with the ''Ni^{2+}-receptor'' of IMCD.

Expression of the Renal Ca^{2+} Receptor in CTAL

Since the renal Ca^{2+}-receptor RaKCaR mRNA is particularly abundant in the thick ascending limb of Henle's loop [27], the following experiments were carried out (1) to check whether evidence for a functional Ca^{2+}-receptor could be obtained in CTAL and MTAL; and (2) to establish whether its cationic specificity would be the same as that found in *Xenopus* oocytes transfected with the RaKCaR mRNA [28]. As shown in Fig. 9, small but detectable $[Ca^{2+}]_i$ variations could be induced by elevating $[Ca^{2+}]_e$ from 1 to 1.5 mM in CTAL, as in CCD. The pattern of response of these two segments to higher Ca^{2+} concentrations was, however, clearly different. Indeed, large, concentration-dependent $[Ca^{2+}]_i$ variations exhibiting sharp initial peaks and discrete plateaus could be obtained in CTAL upon increasing $[Ca^{2+}]_e$ from 1 to 2, 3 or 5 mM whereas the usual rectangular signals were obtained in CCD from the same rats. Data illustrated by Fig. 10 show that the effect of 5 mM Ca^{2+} on CTAL was fully reproduced by adding 300 μM neomycin, 5 mM Ba^{2+}, and (although to a lesser extent) 5 mM Mg^{2+} to bath medium. Ni^{2+} (5 mM) and Gd^{3+} (100–300 μM) also triggered sharp $[Ca^{2+}]_i$ increases but the observed responses were transient, reversing spontaneously towards initial $[Ca^{2+}]_i$ levels. Finally, La^{3+} (100 μM) was totally ineffective on its own (Fig. 10*C*), but altered the response of CTAL to an increase in $[Ca^{2+}]_e$. As shown by the trace (Fig. 10*D*), a transient spikelike $[Ca^{2+}]_i$ variation, similar to that triggered by Ni^{2+} or Gd^{3+}, persisted when $[Ca^{2+}]_e$ was increased from 0 to 3 mM in the presence of La^{3+}, but the plateau of the response to Ca^{2+} was abolished. Since La^{3+}, Gd^{3+}, and Ni^{2+} are all Ca^{2+} channel blockers,

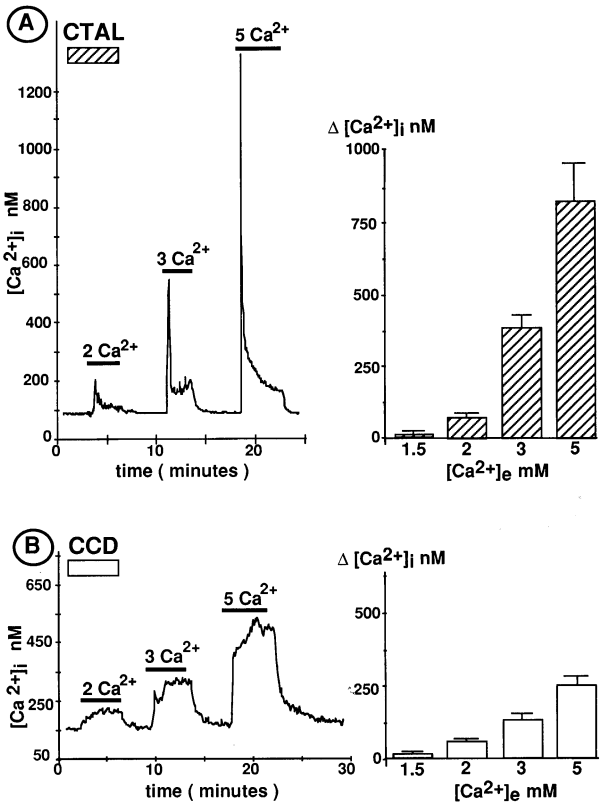

Fig. 9. Comparison of increasing peritubular Ca^{2+} concentration ($[Ca^{2+}]_e$) on $[Ca^{2+}]_i$ in the cortical portions of the thick ascending limb (CTAL) and collecting duct (CCD). Left hand panels: representative traces illustrating the pattern of $[Ca^{2+}]_i$ variations induced in these two segments by increasing $[Ca^{2+}]_e$ from 1 to 2, 3, and 5 mM; right hand panels: mean $[Ca^{2+}]_i$ increases (peak − basal) ± SEM calculated for each $[Ca^{2+}]_e$ concentration from at least 4 similar experiments.

the absence of plateau likely reflected the inhibition of extracellular calcium entry, thereby suggesting that the calcium spikes were of intracellular origin. To reinforce this latter point, we examined the effect of neomycin in the absence of Ca^{2+} in peritubular medium. As shown in Fig. 11 (*A* and *B*), $[Ca^{2+}]_i$ either did not change or only slightly decreased when CTALs were superfused with Ca^{2+}-free solutions (mean $[Ca^{2+}]_i = 88 \pm 6$ and 77 ± 6 nM, $n = 25$, with and without Ca^{2+}; $P < 0.001$). Under this condition, the peak of the response to neomycin persisted ($\Delta[Ca^{2+}]_i = 365 \pm 121$ nM above basal, $n = 7$), but the plateau disappeared. As already observed [22], angiotensin II—a peptide known to activate PLC [39]—also released intracellular calcium in CTAL (Fig. 11*B*). Interestingly, however, it was unable to promote any $[Ca^{2+}]_i$ variation when tested in Ca^{2+}-free medium after neomycin (Fig. 11*A*). Conversely (Fig. 11*B*), a first stimulation by angiotensin II in Ca^{2+}-free medium ($\Delta[Ca^{2+}]_i = 211 \pm 24$ nM, $n = 5$) markedly diminished the ability of CTAL to respond to neomycin ($\Delta[Ca^{2+}]_i = 88 \pm 45$ nM, $n = 5$). Taken together, these data demon-

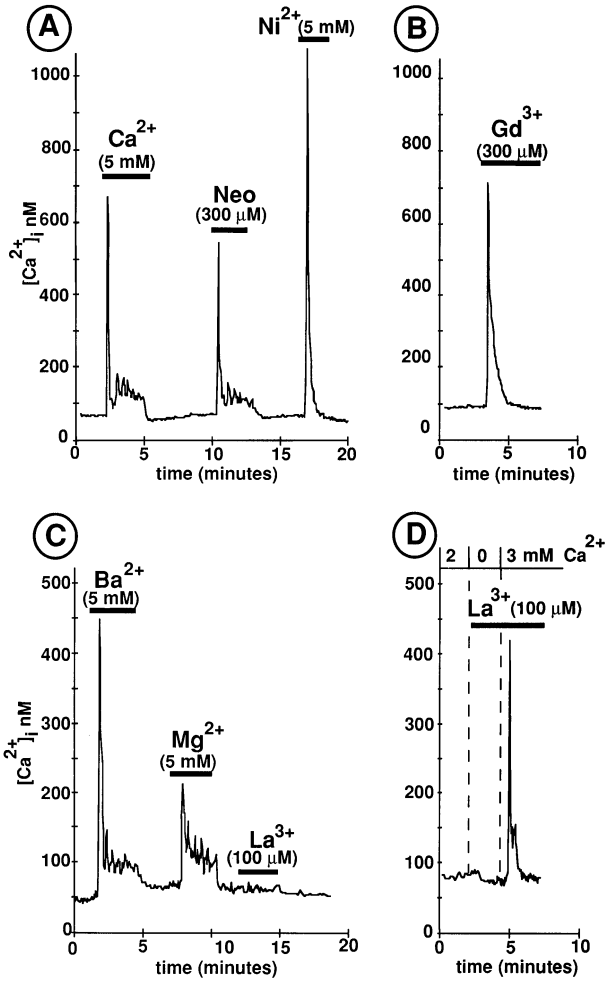

Fig. 10. Effect of Ca^{2+} and Ca^{2+}-receptor agonists on CTAL. Representative traces given to compare the time course of $[Ca^{2+}]_i$ variations induced by increasing Ca^{2+} concentration from 2 to 5 mM, and by adding neomycin and Ni^{2+} (*A*), Gd^{3+} (*B*), Ba^{2+}, Mg^{2+} and La^{3+} (*C*) to peritubular superfusion medium. (*D*): note that increasing $[Ca^{2+}]_e$ from 0 to 3 mM in the presence of La^{3+} (to blunt calcium influx) abolished the plateau but did not suppress the initial phase of the response, an observation suggesting that extracellular Ca^{2+} triggers Ca^{2+} release from intracellular stores.

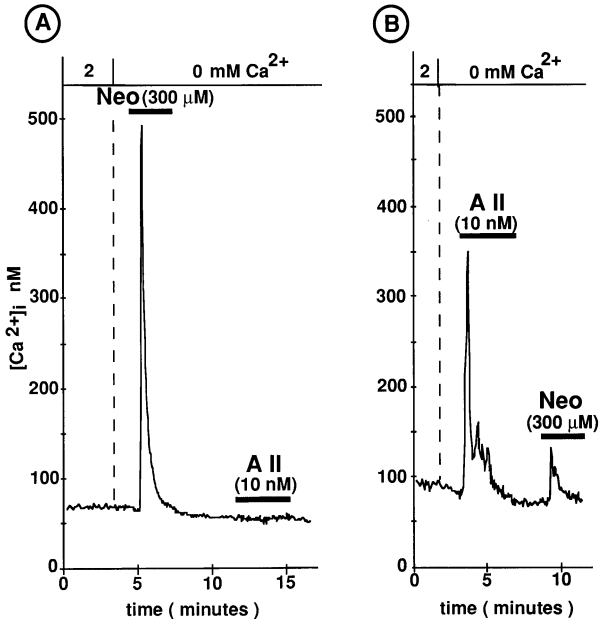

Fig. 11. Effect of neomycin in calcium-free medium in rat CTAL. Representative traces illustrating the pattern of $[Ca^{2+}]_i$ variations elicited by: (*A*), a first stimulation with neomycin (300 μM) followed by a stimulation with angiotensin II (10 nM) in Ca^{2+}-free peritubular solution; or, conversely: (*B*), a preliminary stimulation with angiotensin II followed by a stimulation with neomycin. Note that intracellular Ca^{2+} stores were not replenished between the two stimulations. We checked in a separate experimental series that a 10-min superfusion with Ca^{2+}-free medium did not alter, on its own, the ability of CTAL to respond to neomycin and angiotensin II.

strate that neomycin mobilized intracellular calcium as angiotensin II did, and that both compounds acted on the same intracellular calcium store, a store sensitive to inositol phosphates.

In MTAL superfused with standard medium, peritubular addition of Ca^{2+} and Ba^{2+} (5 mM) also induced biphasic $[Ca^{2+}]_i$ increases but the magnitude of the observed responses was significantly lower than that determined in CTAL in paired experiments (peak $\Delta[Ca^{2+}]_i$ = 146 ± 35 nM, n = 5 and 98 ± 14 nM, n = 5, with Ca^{2+} and Ba^{2+}, respectively; $P < 0.001$ *vs.* CTAL for both Ca^{2+} and Ba^{2+}).

From these results we conclude that a Ca^{2+}-receptor coupled to PLC is functionally expressed in CTAL and, although to a lesser extent, in MTAL. As judged from the magnitude of the initial Ca^{2+} peaks generated by Ca^{2+} and its agonists, and from the cation concentrations necessary to achieve these peaks (Table 2), the following approximate rank order of potency could be proposed: Gd^{3+} and neomycin > Ca^{2+}, Ba^{2+} and Ni^{2+} > Mg^{2+}. The relative ability of these cations to release intracellular Ca^{2+} in CTAL is thus similar to that described in the literature for parathyroid cells [6, 7, 23, 33] and for the renal Ca^{2+}-receptor RaKCaR expressed in *Xenopus* oocytes [28].

Conclusions

Data obtained in CTAL reinforce the conclusion that RaKCaR, if present in basolateral membranes of rat CCD, is not or poorly coupled to PLC. The presence of mRNA detected by RT-PCR in CCD cannot be explained by an apical expression of the Ca^{2+} receptor, since we show that increasing $[Ca^{2+}]_e$ in tubular lumen did not alter $[Ca^{2+}]_i$ (Fig. 3). Assuming that the Ca^{2+}-receptor protein is synthesized, an attractive hypothesis

Table 2. Effect of different activators of the calcium-receptor on $[Ca^{2+}]_i$ in the rat cortical thick ascending limb (CTAL)

Agonist	$\Delta[Ca^{2+}]_i$ nM		*n*
	peak	plateau	
Ca^{2+} (5 mM)	616 ± 57	59 ± 9	11
Ba^{2+} (5 mM)	478 ± 56	112 ± 25	10
Mg^{2+} (5 mM)	76 ± 27*	16 ± 7	6
Ni^{2+} (5 mM)	600 ± 85		9
La^{3+} (100 μM)			6
Gd^{3+} (300 μM)	525 ± 51		7
Neo. (300 μM)	453 ± 74	56 ± 17	6

Data obtained from superfusion experiments on single microdissected segments. Values are means ± SEM of absolute $[Ca^{2+}]_i$ increases ($\Delta[Ca^{2+}]_i$ above basal) induced by different Ca^{2+}-receptor agonists (final concentration in parentheses) to standard peritubular solution (2 mM Ca^{2+}); *n:* number of tubules; * significantly different from the corresponding values obtained with the other Ca^{2+}-receptor agonists, e.g., $P < 0.001$ *vs.* calcium and nickel; $P < 0.01$ *vs.* barium and gadolinium; $P < 0.05$ *vs.* neomycin. All other effects were of comparable magnitude. —: not detectable $[Ca^{2+}]_i$ variation. Representative traces illustrating the effect of these different cations on $[Ca^{2+}]_i$ are given in Fig. 10. Note that 100 μM Gd^{3+} produced a submaximal effect in CTAL ($\Delta[Ca^{2+}]_i$ = 318 ± 40 nM, n = 4, $P < 0.025$ *vs.* 300 μM Gd^{3+}), and that 300 μM Ba^{2+} and Ni^{2+} were devoid of effect (*not shown*).

would be to postulate that the Ca^{2+}-receptor expressed in CCD does not activate PLC, as usually observed in other cell types, but alters $[Ca^{2+}]_i$ through another transduction mechanism, for example via a coupling with membrane calcium channels. From a physiological point of view, the calcium influx pathway present in CCD may obviously be considered as part of a Ca^{2+}-sensing process since it allows changes of extracellular Ca^{2+} to be translated into parallel changes of $[Ca^{2+}]_i$. The question is whether this influx is regulated or not by a membrane Ca^{2+}-receptor. At the present time, two different observations would tend to support this hypothesis: (1) a passive Ca^{2+} influx pathway open at physiological extracellular Ca^{2+} concentrations has already been demonstrated in parathyroid [23, 34] and calcitonin-secreting thyroid cells [15], two cell types containing Ca^{2+}-receptor mRNAs [7, 16]; (2) the relative participation of Ca^{2+} release and Ca^{2+} entry to signals triggered by extracellular calcium seems to be variable, according to the cell preparation studied [15, 41]. Further studies will be necessary, first to investigate whether Ca^{2+} entry is a Ca^{2+}-receptor-mediated process in CCD and, if so, to re-examine the mechanisms by which an increase in $[Ca^{2+}]_e$ leads to intracellular Ca^{2+} mobilization; secondly to identify which solutes (in addition to Ni^{2+}) bind to the extracellular receptor of IMCD, and the nature of their effects on IMCD function.

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