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The renal Na^+/Ca^{2+} exchange system is located exclusively in the distal tubule

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The movement of Ca^{2+} across the basolateral plasma membrane was determined in purified preparations of this membrane isolated from rabbit proximal and distal convoluted tubules. The ATP-dependent Ca^{2+} uptake was present in basolateral membranes from both these tubular segments, but the activity was higher in the distal tubules. A very active Na^+/Ca^{2+} exchange system was also demonstrated in the distal-tubular membranes, but in proximal-tubular membranes this exchange system was not demonstrable. The presence of Na^+ outside the vesicles gradually inhibited the ATP-dependent Ca^{2+} uptake in the distal-tubular-membrane preparations, but remained without effect in those from the proximal tubules. The activity of the Na^+/Ca^{2+} exchange system in the distal-tubular membranes was a function of the imposed Na^+ gradient. These results suggest that the major differences in the characteristics of Ca^{2+} transport in the proximal and in the distal tubules are due to the high activity of a Na^+/Ca^{2+} exchange system in the distal tubule and its virtual absence in the proximal tubule.

INTRODUCTION

Extracellular Ca^{2+} homoeostasis is maintained by the concerted action of hormones on the kidney, bone and intestine. In the kidney, the results of micro-puncture, micro-injection and micro-perfusion studies indicate that most of the filtered Ca^{2+} (60%) is re-absorbed in the proximal convoluted tubule (PCT), whereas 20% is re-absorbed in the Loop of Henle, and 10% in the distal convoluted tubule (DCT).

The trans-epithelial transport of Ca^{2+} proceeds via two pathways: a transcellular route and a paracellular route. The slight increase in concentration of Ca^{2+} along the PCT (Lassiter *et al.*, 1963; Duarte & Watson, 1967; Edwards *et al.*, 1973; Legrimellec *et al.*, 1973), the variation of transport with the electrical potential gradient (Bronszky & Wright, 1982; Ng *et al.*, 1984) and the high permeability of the tubule to Ca^{2+} back-flux (Brunette & Aras, 1971; Murayama *et al.*, 1972) suggest that Ca^{2+} absorption in this segment is mostly passive, paracellular, and secondary to the absorption of Na^+ and water. However, if the amount of intracellular Ca^{2+} is of the same order of magnitude as that of other cells (0.1 μM) and the cell potential is electronegative, a certain amount of Ca^{2+} probably enters into the cell passively, which necessitates an active mechanism at the basolateral membrane (BLM) for its extrusion.

In the DCT, micro-puncture and micro-perfusion experiments revealed that Ca^{2+} is re-absorbed against a very steep transepithelial concentration gradient, independent of Na^+ and of the potential difference (Costanzo & Windhager, 1978; Shareghi & Stoner, 1978). In contrast with Ca^{2+} absorption in the PCT, which is decreased by parathyroid hormone (Agus *et al.*, 1973; Sutton *et al.*, 1976), Ca^{2+} absorption in the early DCT is enhanced by this hormone (Agus *et al.*, 1973; Greger *et al.*, 1978; Shareghi & Stoner, 1978; Costanzo & Windhager, 1980). The question arises whether the differences in the Ca^{2+}-transport characteristics in the PCT and DCT are due simply to the leaky nature of the PCT epithelium, in contrast with the tight DCT epithelium, or if the cellular transport mechanisms are of different nature at these two sites.

There is evidence for at least two different transport systems for Ca^{2+} in the renal tubular BLM: one is the secondary active Na^+/Ca^{2+} exchange (Gmaj *et al.*, 1979; Borle, 1981; Khalifa *et al.*, 1983; Van Heeswijk *et al.*, 1984; Van Os, 1987); the other is an ATP-dependent pump, presumed to be the high-affinity Ca^{2+}-ATPase (Van Heeswijk *et al.*, 1984; Gmaj *et al.*, 1985; Vieyra *et al.*, 1986; Van Os, 1987). All previous studies concerning Ca^{2+} transport through the BLM have been performed with preparations from total kidney cortex, and therefore almost exclusively from PCT. The DCT is rich in high-affinity Ca^{2+}-ATPase (Doucet & Katz, 1982; Brunette *et al.*, 1987), seemingly similar in properties to that from the PCT (Brunette *et al.*, 1987). Hence we investigated the existence of the Na^+/Ca^{2+} exchange activity and the ATP-dependent Ca^{2+}-transport mechanism in BLM isolated from purified preparations of PCT and DCT. The results suggest that, whereas the ATP-dependent Ca^{2+} pump is present in both types of membranes, the Na^+/Ca^{2+} exchange activity is located specifically in the DCT.

MATERIALS AND METHODS

Tubule preparation

Experiments were performed in New Zealand White rabbits and Sprague–Dawley rats. The tubular suspensions were prepared by a modification of techniques previously described (Vinay *et al.*, 1981; Gesek *et al.*, 1987). Kidneys from ketamine-anaesthetized animals were perfused with Krebs–Henseleit (1932) buffer, and slices of the cortex were incubated in Krebs–Henseleit buffer containing collagenase (1 mg/ml), with added 10 mM-glutamine, 1 mM-glutamic acid, 1 mM-pyruvate and 10 mM-lactate, at 37 °C in a shaking water bath.

Abbreviations used: PCT, proximal convoluted tubule; DCT, distal convoluted tubule; BLM, basolateral membrane.

After 15 min, the mixture was filtered through a strainer and a stainless-steel mesh (250 μm). The digested tubules were centrifuged ($\sim$100 *g* for 1 min), suspended in 50% (v/v) foetal-calf serum and left on ice. The undigested material was redigested for another 15 min and processed as above. The tubules were washed three times in Krebs–Henseleit buffer and resuspended in this buffer containing 45% (v/v) Percoll, and centrifuged at 27750 *g* for 30 min at 4 °C. Two bands at the top and one major band at the bottom were consistently observed in the continuous Percoll gradient. The top whitish band was routinely enriched to 70% with distal tubules; the remainder consisted of glomeruli and fragments of cortical ascending limb and collecting tubules. The lower of the top bands, reddish in colour, consisted of glomeruli. The brownish bottom band was exclusively composed of proximal tubules. The composition of the tubule suspension was monitored by microscopic examination and alkaline phosphatase activity, which is absent from the distal tubule (Brunette *et al.*, 1981).

Membrane separation

The tubules obtained were separately washed in 250 mM-sucrose/20 mM-Hepes, pH 7.5, and disrupted with a Sonifier cell disruptor (Ultrasonic Devices; 240 W). The tubular suspension was sonicated at 4 °C, three times for 10 s each at setting 8 with a 30 s interval in between sonication. The homogenates were centrifuged at 500 *g* for 10 min, and the supernatant was centrifuged at 48000 *g* for 30 min. The upper fluffy layer was suspended in 250 mM-sucrose/20 mM-Hepes, pH 7.5, containing 12% (v/v) Percoll and was centrifuged at 35600 *g* for 30 min. The top band of the gradient, consisting of BLM, was removed and washed three times in 20 mM-Hepes, pH 7.5, containing either 150 mM-NaCl or 150 mM-KCl. The method of isolation of BLM from the total cortex was similar, except that the fluffy layer was first treated with 10 mM-$MgCl_2$ (30 min, 4 °C) and then the pellet obtained after centrifugation (10000 *g* for 5 min) was used (after washing) for the Percoll-gradient centrifugation.

All the membrane preparations were stored on ice for at least 45 min before measurement of the Ca^{2+} transport. The preloading of the vesicle was accomplished during the washing procedure (Jayakumar *et al.*, 1984).

Basolateral-membrane orientation

The orientation ('inside-in' or 'inside-out') of the BLM vesicles was studied by measuring the Na^+/K^+-ATPase activity of untreated vesicles and vesicles treated with 0.4 mg of sodium deoxycholate/ml. From this we concluded that 76% and about 30% of vesicles from PCT and DCT respectively were 'inside-out'.

Ca^{2+} transport

Ca^{2+} transport was initiated by adding 20 μl of membrane suspension (60 μg of protein) to 480 μl of incubation medium. In the Na^+/Ca^{2+} exchange experiments, the incubation medium consisted of 150 mM-KCl, 20 mM-Hepes, pH 7.5, and 100 μM-$^{45}CaCl_2$ (final concn.). The vesicles were loaded in either NaCl or KCl before the incubation. In the ATP-dependent Ca^{2+}-transport experiments, the incubation medium contained 150 mM-KCl, 20 mM-Hepes, pH 7.5, 100 μM-$^{45}CaCl_2$, 2 mM-$MgCl_2$ and either no or 1.5 mM-ATP. The vesicles were loaded in KCl only. Incubation was performed at 30 °C. At times indicated, 75 μl samples of the suspension were filtered through Millipore membranes (HAWP; 0.45 μm pore size) and washed with 2 ml of stop solution (250 mM-sucrose/20 mM-Hepes, pH 7.5, plus 2 mM-EGTA). The filters were dissolved and counted for radioactivity in scintillation fluid. Non-specific binding to the filter and to the membranes was subtracted from the total radioactivity contained in the filters.

In the Ca^{2+}-release experiments, the vesicles were first loaded with KCl before the incubation. A 2 μl portion of membranes (6 μg) was incubated in 50 μl of incubation medium containing 100 μM-$^{45}CaCl_2$, 2 mM-$MgCl_2$, 1.5 mM-ATP, 20 mM-Hepes and 150 mM-KCl. After incubation for 2 min, 1 ml of 'EGTA buffer', containing 2 mM EGTA, 20 mM-Hepes and either 150 mM-KCl or -NaCl, was rapidly added. After various intervals of time, a portion of the suspension was filtered, and the filter was washed with 5 ml of the 'EGTA buffer'.

The transport of Ca^{2+} measured by the above method reflects the movement of Ca^{2+} either from the EGTA-accessible space (medium and the outside surface of the vesicle) to the EGTA-inaccessible space (inside the vesicle, either free or bound, and in the membrane not accessible to EGTA), or vice versa. This contention is supported by the ability of EGTA to cause a time-dependent release of Ca^{2+} (see the Results section).

Biochemical measurements

The protein concentrations of the homogenates, tubular suspensions and membrane suspensions were determined by the technique of Lowry *et al.* (1951). The Na^+/K^+-ATPase activity was measured by the method of Post & Sen (1967), and that of alkaline phosphatase by the technique of Kelly & Hamilton (1970).

RESULTS

Purity of the tubular suspensions and membrane preparations

The alkaline phosphatase activities in total cortex homogenate, in PCT and in DCT suspensions are shown in Table 1. There was no significant difference between the activity in the cortical homogenate and that in the PCT (2.12 ± 1.8 and 1.60 ± 0.25 μmol/mg per 15 min). In the DCT, the activity was 10–14-fold lower. The traces of enzyme present in the DCT suspension were due to contamination with very small remnants of PCT and a few glomeruli. The Na^+/K^+-ATPase contents of the BLM prepared from two tubular suspensions are also shown in Table 1. Enrichments were 4.1- and 5.6-fold in PCT and DCT membranes, compared with the respective homogenates of original tubule suspension. These enrichments were judged acceptable, considering that the non-tubule tissue, devoid of Na^+/K^+-ATPase activity such as interstitial connective tissue, had been eliminated in the tubular homogenate, with which these membranes were compared.

Two types of Ca^{2+} transport through BLM from proximal and distal tubules

The time course of the Na^+-dependent and ATP-dependent uptake of 100 μM-Ca^{2+} by BLM vesicles from PCT and DCT is depicted in Fig. 1. These vesicles were preloaded with either K^+ or Na^+ as shown. The Na^+-dependent Ca^{2+} uptake was defined as the difference in

Table 1. Characteristics of proximal and distal tubules and the basolateral membrane

Alkaline phosphatase and ouabain-sensitive Na^+/K^+-ATPase were determined as described in the Materials and methods section after solubilization of the membranes with sodium deoxycholate. The values represent means ± S.E.M. for 10–14 different preparations. Abbreviation: ND, not determined.

Tissue	Alkaline phosphatase (μmol of P_i liberated/ 15 min per mg)	Na^+/K^+-ATPase (μmol of P_i liberated/60 min per mg)
PCT/DCT mixture before separation	2.12±1.8	ND
PCT	1.60±0.25	6.43±1.0
DCT	0.188±0.12	5.84±1.8
BLM from PCT	ND	26.5±9.4
BLM from DCT	ND	32.8±17

Ca^{2+} uptake between the vesicles preloaded with K^+ and those preloaded with Na^+. The ATP-dependent Ca^{2+} uptake was defined as the difference in Ca^{2+} uptake by vesicles preloaded with K^+, incubated in medium with or without Mg^{2+}–ATP. In the PCT, the accumulation of Ca^{2+} owing to the presence of ATP progressively increased with time of incubation. After incubation for 90 s, the ATP-dependent Ca^{2+} uptake was 0.6±0.06 nmol/mg of protein. On the other hand, the Na^+-dependent transport remained negligible after a similar period of incubation. In contrast, vesicles from the DCT showed a very high activity of Na^+-dependent Ca^{2+} uptake, which was higher than the ATP-dependent Ca^{2+} transport. However, direct comparisons of the activity of the two transport systems are probably not valid, because Na^+-dependent Ca^{2+} transport occurs presumably in both inside-out and inside-in vesicles, whereas the ATP-dependent Ca^{2+} transport presumably occurs only in inside-out vesicles. The BLM from PCT and DCT are enriched to a similar extent (Table 1) and, despite the presence of a large amount of inside-out vesicles in the BLM preparation from PCT (76% in PCT compared with 30% in DCT), the ATP-dependent Ca^{2+}-transport activity in DCT was about twice as high as that of PCT. This may be due to the high concentration of the Ca^{2+}-ATPase in the DCT (Doucet & Katz, 1982; Brunette *et al.*, 1987).

Previous studies in isolated BLM have demonstrated that the Na^+/Ca^{2+} exchange is reversible. Hence, to test whether the reverse direction of the Na^+/Ca^{2+} exchange system could counteract the ATP-dependent Ca^{2+}-uptake system, the membranes were incubated in medium with (i) no ATP, no Na^+, (ii) ATP plus Na^+, and (iii) ATP but no Na^+. Presumably, if the membrane possesses a Na^+/Ca^{2+} exchange mechanism, the presence of Na^+ outside the vesicles should favour the efflux of Ca^{2+} and therefore decrease the net ATP-dependent influx. The results shown in Fig. 2 indicate that, in membranes from PCT, external Na^+ has little effect on ATP-dependent Ca^{2+} uptake, whereas in DCT external Na^+ strongly inhibits ATP-dependent Ca^{2+} uptake, again demonstrating the presence of an active Na^+/Ca^{2+} exchange system in DCT.

Na^+-dependent Ca^{2+} uptake as a function of Na^+ gradient

As shown in Fig. 3, the activity of the Na^+/Ca^{2+} exchange was a function of Na^+ gradient. Ca^{2+} uptake by vesicles loaded with 150 mM-NaCl, decreased progress-

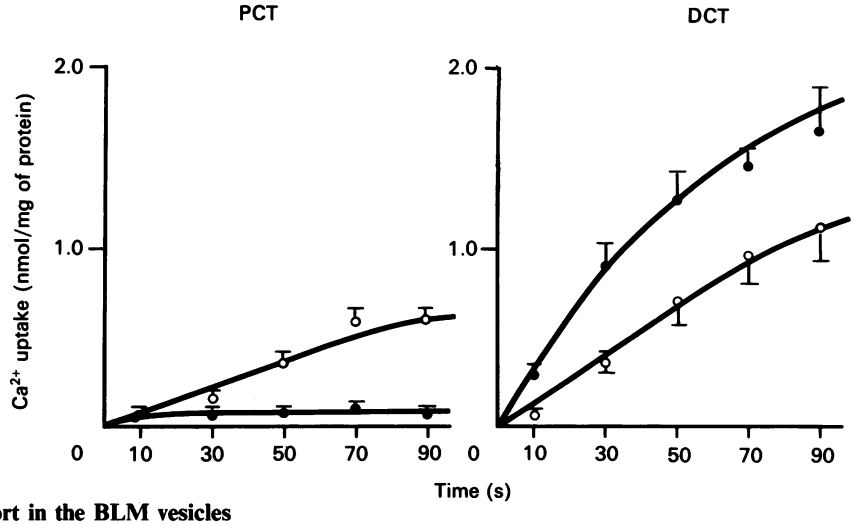

Fig. 1. Ca^{2+} transport in the BLM vesicles

Na^+-dependent (●) and ATP-dependent (○) Ca^{2+} uptakes by the BLM vesicles from rabbit PCT and DCT were determined as described in the Materials and methods section. Ca^{2+} concentration in the incubation medium was 100 μM. In Na^+-dependent experiments, vesicles were preloaded with either 150 mM-NaCl or 150 mM-KCl. Na^+-dependent uptake is the difference between the two uptakes. Experiments were performed in duplicate, in 13 and 9 membrane preparations from PCT and DCT respectively. Values are means ± S.E.M. In ATP-dependent experiments, vesicles were preloaded with 150 mM-KCl. The incubation medium contained 2 mM-$MgCl_2$ and either no or 1.5 mM-ATP. ATP-dependent uptake is the difference between the uptake in the presence and the absence of ATP. Data are means ± S.E.M. for 13 and 12 experiments with membranes from PCT and DCT respectively. The non-specific binding of Ca^{2+} to the vesicles, which was subtracted from the values given in the Figure, ranged from 33 to 62% of the ATP-dependent Ca^{2+} transport for both PCT and DCT.

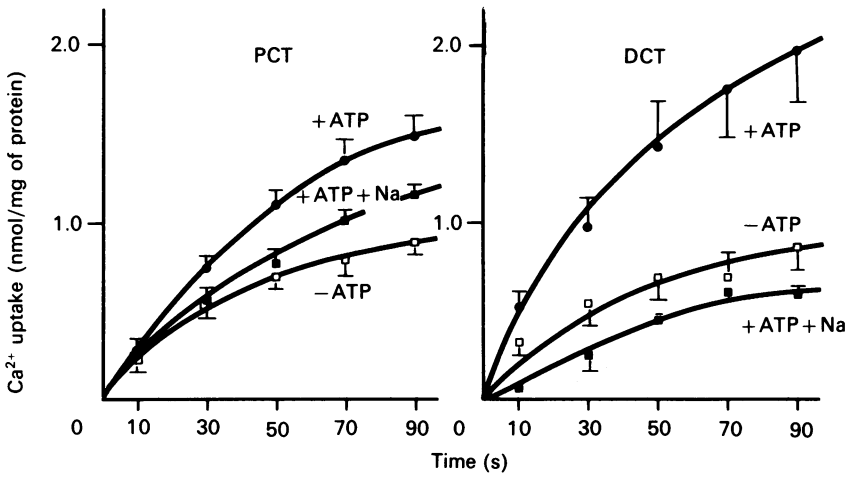

Fig. 2. Effect of external Na^+ on the ATP-dependent Ca^{2+} uptake

The vesicles were preloaded with 150 mM-KCl, and incubated (1) in the absence of Na^+ and ATP but in the presence of KCl (150 mM), or (2) in the absence of Na^+ but in the presence of KCl (150 mM), ATP (1.5 mM) and $MgCl_2$ (2 mM), or (3) in the presence of NaCl (150 mM), ATP (1.5 mM) and $MgCl_2$ (2 mM). Ca^{2+} concentration in the incubation medium was 100 μM. The Ca^{2+} uptake at zero time (0.95 nmol/mg of protein) represents non-specific binding of Ca^{2+} to the membranes. Data represent means ± S.E.M. for four measurements.

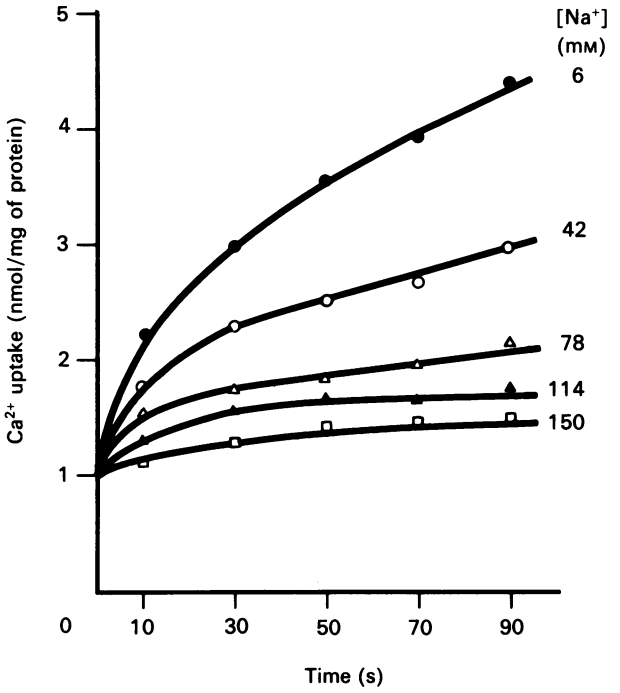

Fig. 3. Effect of Na^+ gradient on Na^+/Ca^{2+} exchange by BLM vesicles from distal tubules

The experiments were carried out as indicated in Fig. 1. Vesicles were preloaded with 150 mM-NaCl. The incubation medium contained various amounts of Na^+ (6–150 mM) as indicated in the Figure. The total concentration of the salt (NaCl + KCl) in the incubation medium was maintained constant at 150 mM.

ively when the concentration of Na^+ in the incubation medium was increased from 6 to 150 mM. Conversely (not shown in the Fig.) once Ca^{2+} is inside the vesicles, the abrupt reduction of extracellular Ca^{2+} concentration by the addition of EGTA (chelating medium), resulted in a time-dependent passive release of Ca^{2+}. However, the presence of Na^+ in the chelating medium enhanced this release in membranes from the DCT, but had no significant influence in the membranes from PCT.

Ca^{2+} transport through BLM of total kidney cortex in rat and rabbit

Since the Na^+-dependent Ca^{2+} uptake by the BLM vesicles has been described in rat renal cortex preparations predominantly consisting of PCT, a difference in species was suspected. It is possible that, in rats, the Na^+/Ca^{2+} exchange system is present in the PCT in high enough concentration to allow its measurement in BLM preparations from total cortex. Such a finding would be in contrast with the rabbit, where this system is exclusively located in the distal tubule. In Fig. 4, a comparison is provided of the two systems of Ca^{2+} transport through the BLM preparations of total cortex from rat and rabbit. The two systems are present in both preparations; the activity of each of them is about 1.5–2.0-fold higher in the rat than in the rabbit, despite the similar enrichment of the vesicles. In both species, however, the Na^+-dependent Ca^{2+} uptake was relatively very low. This raises the possibility that in rats, as in rabbits, this system is probably situated in the DCT. We have so far not been successful in obtaining enough DCT from rats to verify this. Comparison of Figs. 1 and 4 enables us to conclude that, in the rabbit, the activity of the Na^+/Ca^{2+} exchange system in membranes obtained from total cortex is due to a 10% contribution of the DCT.

DISCUSSION

The ATP-dependent Ca^{2+}-transport system and the Na^+/Ca^{2+} exchange system in renal BLM from rat kidney cortex were first described by Gmaj *et al.* (1979), who proposed that the secondary active transport of Ca^{2+} involving the Na^+/Ca^{2+} exchange represents the predominant mechanism for Ca^{2+} absorption in the tubule. Their statement was based also on the prior

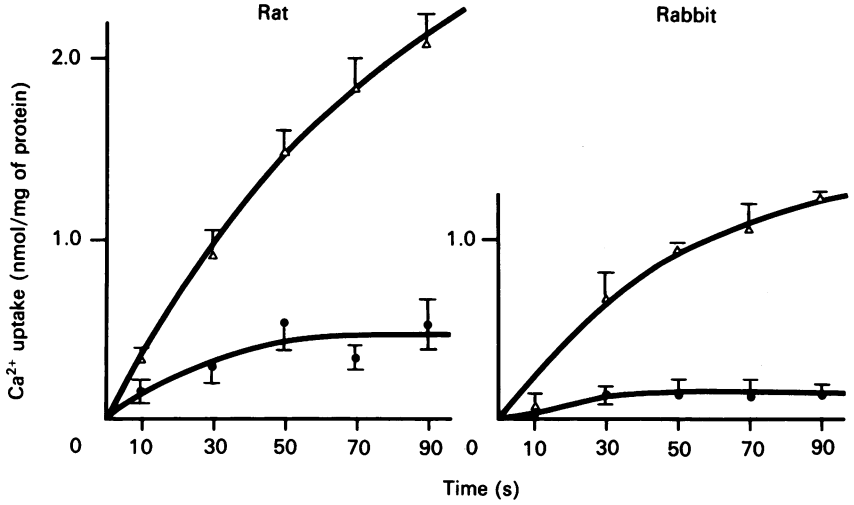

Fig. 4. Comparison of Ca^{2+} transport by BLM isolated from rat or rabbit kidney cortex

Na^+- (●) and ATP- (△) dependent Ca^{2+} uptake by BLM vesicles from total renal cortex in rat and rabbit was measured as described in Fig. 1. Values are means ± S.E.M. for four experiments for each species. The non-specific binding of Ca^{2+} to the vesicles, which was subtracted from the values given in the Figure, ranged from 29 to 54% of the ATP-dependent Ca^{2+} transport for both rat and rabbit. The enrichment of the BLM, based on Na^+/K^+-ATPase activity, was 9.7- and 7.8-fold for rat and rabbit respectively.

observation, obtained with micro-perfusion experiments, that removal of Na^+ from the perfusion fluid, or addition of ouabain to the peritubular space, inhibits active Ca^{2+} re-absorption (Ullrich *et al.*, 1976, 1977).

Van Heeswijk *et al.* (1984) compared the kinetic parameters of the Na^+-gradient-dependent Ca^{2+} uptake in BLM with those of the ATP-dependent system, and arrived at the opposite conclusion. Whereas the affinity of the two systems was comparable (0.1 μM), the maximal capacity of Ca^{2+} transport was definitely higher for the ATP-dependent system ($V_{max.}$ 7.4 nmol/min per mg) than for the Na^+/Ca^{2+} exchange mechanism ($V_{max.}$ 0.4 nmol/min per mg). It now appears that the relatively low activity of the exchange system was due to the predominance of the proximal origin of the membranes, which are poor in or devoid of Na^+/Ca^{2+} exchanger, but possess the ATP-dependent system.

The presence of the ATP-dependent system of Ca^{2+}-transport in both segments is not surprising, since this transport is presumably mediated by the high-affinity Ca^{2+}-ATPase (Gmaj *et al.*, 1979, 1982; Van Os & Ghijsen, 1981), which is present in high concentration in both segments (Doucet & Katz, 1982; Brunette *et al.*, 1987).

The results in the present paper clearly demonstrate that Na^+/Ca^{2+} exchanger is particularly abundant in the DCT BLM. The inability to demonstrate Na^+/Ca^{2+} exchanger in the PCT BLM could be due either to the absence of the exchanger from these membranes or to the differences in its modulation in the two segments. It appears unlikely that it is due to the leakiness of the PCT BLM, as substantial Ca^{2+} is transported by the ATP-dependent system.

The observation that a high-activity Na^+/Ca^{2+} exchange mechanism is located in the distal tubule is important in elucidating the mechanism of renal Ca^{2+} re-absorption. The high capacity of Ca^{2+} transport in the distal tubule probably depends on the dual system of Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger, coupled with the tightness of the epithelial cells.

The regulation of Ca^{2+} transport in kidney is of evident interest. It has been shown that parathyroid hormone enhances Ca^{2+}-ATPase activity in BLM preparations (Tsukamoto & Suki, 1984; Levy *et al.*, 1986). Whether this increase is associated with altered Ca^{2+} transport is not known. On the other hand, parathyroidectomy in rats (Jayakumar *et al.*, 1984) and in dogs (Scoble *et al.*, 1985) decreases, by 40% and 50% respectively, the Na^+/Ca^{2+} exchanger activity in the BLM vesicles. Similar results were found in isolated rat renal cells (Hanai *et al.*, 1986). The strong effect of the hormone on a system localized only in the distal tubule may explain why parathyroid hormone increases Ca^{2+} re-absorption exclusively in the distal tubule, whereas both proximal and distal segments have receptors for this hormone.

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