Ca²⁺ transport by the luminal membrane of the distal nephron: action and interaction of protein kinases A and C

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We previously reported that parathyroid hormone and calcitonin increase Ca²⁺ uptake by purified distal luminal membranes. This effect is mimicked by high concentrations of cAMP. However, both hormones stimulate adenylate cyclase and phospholipase C. The purpose of the present study was to investigate the role of the phospholipase C pathway in the hormone action, and the interrelationship between the two messengers. Distal tubules from rabbit kidneys were incubated with dibutyryl cAMP (dbcAMP) or PMA, or both, and Ca²⁺ uptake by purified luminal membranes was measured by the rapid filtration technique. Incubation of the distal tubules with 1 mM dbcAMP significantly increased Ca²⁺ transport by the luminal membranes. A dose–response curve showed a half-maximal stimulation with 0.82 mM dbcAMP. In contrast, treatment of the tubules with 10 nM, 100 nM or 1 μ M PMA did not influence Ca²⁺ uptake by

these membranes. However, the addition of 100 nM PMA to low concentrations of dbcAMP strongly increased this uptake. The presence of cAMP or protein kinase C inhibitors prevented the effects of either a high concentration of dbcAMP alone or a low concentration of dbcAMP combined with 100 nM PMA. Our laboratory has already reported that Ca²⁺ uptake by the distal luminal membranes displays two-component kinetics. dbcAMP increased the $V_{\max}$ of the low-affinity component, whereas a combination of the two messengers stimulated the $V_{\max}$ of both the low- and high-affinity components. From these results, we conclude that: (1) in the distal tubule cells, activation of both protein kinases A and C is necessary for the stimulation of Ca²⁺ transport by the luminal membrane; (2) the combined effect of protein kinases A and C involves both components of the Ca²⁺-transport kinetics.

INTRODUCTION

A number of studies have consistently demonstrated that cAMP, either applied on the surface of the kidney [1] or added to the peritubular medium of microperfused tubules [2–6], enhances Ca²⁺ reabsorption by the ascending limb of the loop of Henle, the distal and the connecting tubules. Since parathyroid hormone (PTH) and calcitonin stimulate adenylate cyclase activity [7–9] and Ca²⁺ reabsorption [1,6,10] in the same segments, it is assumed that the transport effect of these hormones is mediated through the main messenger cAMP. However, PTH [11–14] and calcitonin [15–17] receptors are also coupled to phosphoinositide signalling pathways, suggesting some contribution of protein kinase C (PKC) to the cellular cascade that increases Ca²⁺ entry into the cell. Indeed, recent studies reported by Friedman et al. [18] using immortalized cell cultures from mouse early distal tubules suggest complementary roles for the two messenger pathways. In these studies, however, Ca²⁺ uptake was measured with cell suspensions, i.e. in the presence of the intracellular organelles and without distinction between the two polar membrane contributions.

In an attempt to determine more precisely the effects of messengers on the luminal membrane specifically, we have examined the separate and combined influences of these two messengers on Ca²⁺ transport by the luminal membrane of the distal cortical segments of the nephron in the rabbit kidney.

MATERIALS AND METHODS

Tubule preparation

Proximal and distal tubules were prepared from fresh rabbit kidneys by collagenase digestion and Percoll-density-gradient centrifugation, as previously described [19]. The isolated proximal and distal tubule bands were washed three times in Krebs–

Henseleit buffer and finally transferred to culture medium (Dulbecco's modified Eagle's medium and Ham's nutrient MIX F-12; Sigma cell culture). Then, dibutyryl cAMP (dbcAMP) and/or PMA, or their respective carriers, were added to the suspensions for periods of 30 and 10 min respectively at 37 °C. The optimal time of incubation was determined from preliminary experiments. In some experiments, inhibitors of protein kinase A (PKA) or PKC (RP cAMP and calphostin C respectively) were added to the medium. The incubations were stopped by centrifugation, and the tubules were washed three times in Krebs–Henseleit buffer and frozen at –80 °C in 10 mM mannitol and 2 mM Tris/Hepes, pH 7.4, until the day of the experiment.

Membrane preparation

The luminal membranes from the proximal and distal tubules were purified using the MgCl₂ precipitation technique. Each tubule suspension was homogenized with a Dounce homogenizer, and then MgCl₂ was added to the medium to obtain a final

Table 1 Enzyme activities in the various preparations

Results are means $\pm$ S.E.M. for eight determinations. The values in parentheses are enrichment over the corresponding homogenate. PT, proximal tubule; DT, distal tubule.

	Alkaline phosphatase (nmol/15 min per μ g)	Na ⁺ /K ⁺ -ATPase (nmol/20 min per μ g)
Cortex homogenate	3.47 $\pm$ 0.21	1.72 $\pm$ 0.36
PT homogenate	3.36 $\pm$ 0.17	2.84 $\pm$ 0.38
PT luminal membranes	13.68 $\pm$ 0.57 ($\times$ 4.1)	1.86 $\pm$ 0.41 ($\times$ 0.65)
DT homogenate	0.45 $\pm$ 0.06	2.54 $\pm$ 0.54
DT luminal membranes	3.72 $\pm$ 0.28 ($\times$ 8.3)	2.43 $\pm$ 0.43 ($\times$ 0.96)

Abbreviations used: PTH, parathyroid hormone; PKC, protein kinase C; dbcAMP, dibutyryl cAMP; PKA, protein kinase A.

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concentration of 12 mM. The suspension was stirred on ice for 10 min (20 min for proximal tubules) and centrifuged at 3000 *g* for 20 min. The supernatant was collected and centrifuged at 28000 *g* for 20 min at 4 °C. The pellet was washed twice in 280 mM mannitol and 20 mM Tris/Hepes, pH 7.4, passed three times through a 22-gauge needle, and left for 1 h on ice to complete the vesiculization. Table 1 indicates the enrichment of the two marker enzymes, alkaline phosphatase and Na⁺/K⁺-ATPase. Their activities were measured by the techniques of Kelly and Hamilton [20] and Post and Sen [21] respectively.

Ca²⁺ uptake

Ca²⁺ uptake by the membrane vesicle was measured by the rapid filtration technique. The incubation medium contained 120 mM or no NaCl, 20 mM or 140 mM choline chloride, 20 mM Tris/Hepes, pH 7.4, and ⁴⁵CaCl₂ at the indicated concentration. The vesicles were preloaded with 280 mM mannitol and 20 mM Tris/Hepes, pH 7.4.

Incubation was initiated by adding 5 µl of the membrane suspension (15–20 µg of protein) to 25 µl of the incubation medium at 35 °C. At the time indicated, transport was stopped with 1 ml of stop solution (140 mM KCl, 20 mM Tris/Hepes, pH 7.4, and 2–5 mM EGTA, depending on the Ca²⁺ concentration of the incubation medium), the suspension was filtered and the filters were further rinsed with 6 ml of the same stop solution. All of the uptakes were measured in duplicate.

Statistical analysis and calculation of kinetics parameters

The results obtained with membrane vesicles from control and treated tubules were analysed using Student's unpaired *t* test. The kinetics of Ca²⁺ uptake by the membrane vesicles were calculated by a non-linear regression analysis using the equations described by Huntson [22]. Passive diffusion of Ca²⁺ was evaluated by the slope of Ca²⁺ uptake at saturating concentrations of Ca²⁺, and subtracted from the total uptake values.

RESULTS

Effect of dbcAMP on Ca²⁺ uptake by proximal and distal luminal membranes

Figure 1 illustrates the effect of 1 mM dbcAMP on the time

course of Ca²⁺ uptake by the luminal membranes from proximal and distal tubules. Freshly purified tubules were incubated with dbcAMP or the carrier for 30 min at 37 °C. After incubation, the suspensions were centrifuged and the tubules were washed, transferred to a hypotonic solution and frozen. On the day of the experiment, the membranes were prepared as described in the Materials and methods section. The incubation of proximal tubules with 0.2, 0.5, 1.0, 2.0 and 5 mM dbcAMP did not affect Ca²⁺ transport by the brush-border membranes; however, the same treatment for the distal tubules resulted in a strong stimulation of Ca²⁺ uptake by the luminal membranes (*P* < 0.001 at 10 s).

In these experiments, the incubation medium contained 0.5 mM CaCl₂, 120 mM NaCl and 20 mM Tris/Hepes, pH 7.4. In another series of experiments, tubules and membranes were prepared according to the same protocol, but the Ca²⁺ uptake was measured in the presence of choline chloride instead of NaCl. As previously reported, Na⁺ strongly diminishes Ca²⁺ transport by the distal luminal membrane [23]. Again, the replacement of Na⁺ by choline enhanced Ca²⁺ uptake, but abolished the effect of dbcAMP (Ca²⁺ uptake: 1.12 ± 0.05 and 1.28 ± 0.06 pmol/10 s per µg, *P* > 0.05, in vesicles from control and treated tubules).

Effect of PMA on Ca²⁺ uptake by proximal and distal luminal membranes

Since the receptors of Ca²⁺-regulating hormones are also coupled to the phosphoinositide pathway, we incubated tubules with the phorbol ester PMA alone, or in association with dbcAMP, to test the effect on Ca²⁺ transport by the luminal membranes. In a first series of experiments, we incubated proximal and distal tubules with 1 µM PMA for 10 min at 37 °C by the technique reported by Friedman et al. [18]. As in experiments with dbcAMP, incubations were stopped by centrifugation; then the tubules were washed, transferred in hypotonic solution and frozen. On the day of the experiments, Ca²⁺ uptake was measured with the membranes prepared from treated and untreated tubules. No effect of 1 µM PMA was observed on Ca²⁺ transport by either proximal or distal luminal membranes. In similar experiments, 10, 50 and 100 nM PMA all had no effect on Ca²⁺ uptake.

Figure 1 Time course of 0.5 mM Ca²⁺ uptake by luminal membrane vesicles from proximal tubules (PT) and distal tubules (DT) preincubated with 1 mM dbcAMP (●) or the carrier (○)

The incubation medium contained 120 mM NaCl, 20 mM choline, 20 mM Tris/Hepes, pH 7.4, and 0.5 mM ⁴⁵CaCl₂. * *P* < 0.05, ** *P* < 0.005, *** *P* < 0.001 compared with the control values. Results are means ± S.E.M. (*n* = 4 experiments, with duplicates in each experiment).

Figure 2 Time course of 0.5 mM Ca^{2+} uptake by distal luminal membranes from untreated tubules (○) or tubules incubated with 50 μM dbcAMP in association with 100 nM PMA (▲)

Results are means $\pm$ S.E.M. ($n = 6$). * $P < 0.05$, ** $P < 0.01$.

Combined effect of dbcAMP and PMA on Ca^{2+} uptake by the distal luminal membranes

In *in vitro* preparations, Ca^{2+} -transport-regulating hormones simultaneously stimulate cAMP and inositol trisphosphate production [11–18]. We investigated the effect of the combination of the two signalling systems, using more physiological concentrations of dbcAMP. As shown in Figure 2, incubations of distal tubules with 50 μM dbcAMP (a concentration that was ineffective alone) combined with 100 nM PMA (also ineffective alone) resulted in a significant increase in the 0.5 mM Ca^{2+} uptake (in the presence of 120 mM NaCl) by the corresponding luminal membranes: Ca^{2+} uptake was 0.49 ± 0.48 and 0.78 ± 0.14 pmol/10 s per μg for membranes from control and treated tubules respectively ($P < 0.01$, $n = 6$).

We then obtained a dose-response curve of the effect of dbcAMP on 0.5 mM Ca^{2+} transport by the distal luminal membranes in the presence of 100 nM PMA. Completely different

Figure 3 Dose-response curves of the effect of the incubation of distal tubules with dbcAMP in the absence (○) and in the presence of 100 nM PMA (▲) or 100 nM calphostin C (●) on 0.5 mM Ca^{2+} uptake by the distal luminal membranes

Results are means $\pm$ S.E.M. ($n = 4$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the data obtained with dbcAMP alone.

Table 2 Ca^{2+} uptake (pmol/10 s per μg) by luminal membranes from distal tubules treated with dbcAMP and/or PMA, in the absence and presence of RP cAMP or calphostin C

Results are means $\pm$ S.E.M. ($n = 3$ for all groups of experiments). * $P < 0.05$, ** $P < 0.02$, compared with control.

Experimental conditions	Ca^{2+} uptake
Control	0.51 ± 0.02
1 mM dbcAMP alone	$1.04 \pm 0.18^{**}$
1 mM dbcAMP + 300 μM RP cAMP	0.63 ± 0.05
1 mM dbcAMP + 100 nM calphostin C	0.56 ± 0.19
50 μM dbcAMP alone	0.57 ± 0.06
100 nM PMA alone	0.53 ± 0.007
50 μM dbcAMP + 100 nM PMA	$0.71 \pm 0.46^*$
50 μM dbcAMP + 100 nM PMA + 100 nM calphostin C	0.55 ± 0.02

curves were obtained whether or not PMA was added to dbcAMP (Figure 3): the combination of the two messengers strongly stimulated the uptake of Ca^{2+} at all of the dbcAMP concentrations used up to 1 mM.

Effects of cAMP and PKC inhibitors

The purpose of these experiments was to investigate further the interrelationship between the two signalling mechanisms involved in the regulation of Ca^{2+} transport. Distal tubules were separated into four main groups: untreated tubules; tubules incubated with 100 nM PMA; tubules incubated with 1 mM dbcAMP; tubules incubated with 50 μM dbcAMP plus 100 nM PMA. The two latter groups were each further subdivided into three subgroups of tubules: those treated with no inhibitor, those treated with 300 μM RP cAMP (a competitive inhibitor of cAMP) and those treated with 100 nM calphostin C (an inhibitor of PKC). Then the luminal membranes were prepared and the 10 s Ca^{2+} uptake by each preparation was measured. Results are presented in Table 2. As previously observed, 1 mM dbcAMP or 50 μM dbcAMP plus 100 nM PMA significantly enhanced Ca^{2+} uptake. However, in both groups, the addition of either of the two inhibitors, RP cAMP or calphostin C, abolished the stimulating effect of the messengers. The fact that the inhibition of PKC abolished the effect of 1 mM dbcAMP suggests that the action of dbcAMP requires the presence of PKC activity. Conversely, PKC stimulation had no effect unless in association with dbcAMP.

Which components of Ca^{2+} uptake kinetics do the messengers influence?

In previous studies, our laboratory reported that Ca^{2+} uptake by the luminal membranes of distal tubule preparations displayed dual kinetics [23,24]. We were therefore interested to determine the effect of dbcAMP alone or combined with PMA on these kinetic parameters. Figure 4 and Table 3 summarize the results. Incubation of distal tubules with 1 mM dbcAMP alone clearly increased the V_{max} of the low-affinity component from 1.2 ± 0.16 to 3.03 ± 0.65 pmol/10 s per μg ($P < 0.05$, $n = 3$), without any significant effect on the K_m (Table 3). The combination of 100 nM PMA with 50 μM cAMP influenced the V_{max} of both the low- and high-affinity components: V_{max} increased from 1.91 ± 0.10 to 2.75 ± 0.07 pmol/10 s per μg for the low-affinity component ($P < 0.001$), and from 0.39 ± 0.05 to 0.65 ± 0.08 pmol/10 s per μg for the high-affinity component ($P < 0.025$, $n = 3$).

Figure 4 Eadie-Hofstee plot of Ca^{2+} uptake by luminal membranes of distal tubules incubated with: (a) 1 mM dbcAMP (●) or the carrier (○) ($n = 4$); (b) 50 μM dbcAMP and 100 nM PMA (●) or the carrier (○) ($n = 3$)

Results are means $\pm$ S.E.M.

Table 3 Effect of 1 mM dbcAMP and 50 μM dbcAMP combined with 100 nM PMA on kinetic parameters of Ca^{2+} uptake by distal luminal membranes

Results are means $\pm$ S.E.M. ($n = 3$). * $P < 0.05$; ** $P < 0.025$; *** $P < 0.001$, compared with the control values.

Conditions	High affinity		Low affinity	
	K_m (mM)	V_{max} (pmol/10 s per μg)	K_m (mM)	V_{max} (pmol/10 s per μg)
Control	0.075 ± 0.016	0.30 ± 0.013	1.45 ± 0.34	1.20 ± 0.16
1 mM dbcAMP	0.064 ± 0.01	0.31 ± 0.017	2.49 ± 0.58	$3.03 \pm 0.65^*$
Control	0.06 ± 0.01	0.39 ± 0.05	1.38 ± 0.10	1.91 ± 0.10
50 μM dbcAMP + 100 nM PMA	0.06 ± 0.02	$0.65 \pm 0.08^{**}$	1.46 ± 0.02	$2.75 \pm 0.07^{***}$

DISCUSSION

Interrelationship between the two messengers

It is well established that the two peptide hormones PTH and calcitonin that regulate Ca^{2+} reabsorption by the distal nephron are coupled to adenylate cyclase and phosphoinositide pathways. However, neither the exact contribution of each of the messengers to the Ca^{2+} uptake by the distal luminal membrane nor the interrelationship of these messengers are well defined.

cAMP alone has been shown by several groups to stimulate the net Ca^{2+} reabsorption by the cortical distal nephron. Costanzo et al. [1] reported in micropuncture experiments an increase in Ca^{2+} transport by the superficial segment of the distal tubule when the renal cortex was bathing in a solution containing cAMP. Similar effects of cAMP analogues (8-bromo-cAMP, dbcAMP or 8-*p*-chlorophenylthio-AMP) on the ascending limb and the distal tubule were observed *in vitro* in microperfusion experiments [2–6]. Chase and Wong [25], Bourdeau and Eby [26], Lau et al. [27] and Siga et al. [9] also reported an increase in intracellular free Ca^{2+} content after incubation of distal and collecting tubule cells with cAMP. In all of these experiments

except one [6], millimolar ranges of cAMP were used. Our experiments also showed that high concentrations of cAMP were necessary to obtain an effect on Ca^{2+} transport *in vitro*.

Friedman and colleagues [18,28] emphasized the requirement of both PKA and PKC for the action of PTH in the kidney: in primary and immortalized distal tubule cells from mice, PTH-dependent Ca^{2+} uptake was inhibited by either PKA- or PKC-blocking substances. Although high concentrations of cAMP alone were necessary to increase intracellular Ca^{2+} concentration, the addition of a phorbol ester, which by itself had no effect, to low concentrations of cAMP resulted in a strong stimulation of Ca^{2+} uptake. Since the experiments were performed with cell suspensions, not only was the exact polar membrane responsible for Ca^{2+} influx not determined, but the eventual contribution of intracellular Ca^{2+} storage was not entirely eliminated. Finally, in contrast with the observation of Friedman et al. [18,28], Bindels et al. [29], using fresh cell cultures from rabbit cortical collecting tubules, reported that a 2 h incubation with the PKC activator PMA resulted in an inhibition of the transepithelial transport of Ca^{2+} . Our data confirmed the mutual influence of the two messengers on Ca^{2+} influx as described by Friedman and colleagues, and localized the effect to the luminal membrane, which kept the message despite the separation from the rest of the cell.

The intracellular concentration of cAMP is within the micromolar range [30]. Since, in our experiments, the tubule cells were incubated in a medium containing dbcAMP, the exact intracellular concentration of the messenger is unknown. Whatever the extracellular/intracellular ratio was, it is probable that the low concentration used that produced the effect in association with PMA (i.e. 50 μM dbcAMP) resulted in an intracellular concentration close to physiological levels.

Even when dbcAMP alone, at high concentration, was used to increase Ca^{2+} transport, an intracellular mechanism involving PKC contributed to this effect since it was abolished by calphostin C. This PKC inhibitor probably abolished the activity already present in the cell and prevented any stimulation by the high concentration of cAMP. Indeed, Anderson and Breckon [31] showed that the exposure of LLC-PK cells, an immortalized cell line from porcine renal cortex, to cAMP analogues, increased both soluble and particulate forms of PKC. Similarly, Friedman et al. [18] observed in immortalized mouse distal tubule cells that

high concentrations of forskolin significantly enhanced PKC activity. Therefore, not only is the activation of the two messenger systems implicated in the increase in Ca^{2+} transport by the distal luminal membrane, but cAMP by itself is able to generate the PKC activation necessary for the effect.

Effect of messengers on the two kinetic components of Ca^{2+} uptake

Electrophysiology experiments have clearly suggested the presence of several Ca^{2+} channels in the rabbit distal luminal membrane. In the bright segment, Poncet et al. [32] described a channel conductance of 15 pS (cell-attached condition), a value that is close to that observed by Lau et al. [27] in the porcine ascending limb, whereas in the connecting duct, Tan and Lau [33] reported a potential-dependent channel with a slope conductance of 25 pS. Our membrane preparations involved both the bright and the connecting tubules. It is probable that the two kinetic components of Ca^{2+} uptake by our membrane vesicles reflect this channel heterogeneity. The present results show that, in the presence of PKC activation, relatively physiological concentrations of dbcAMP increased the V_{max} of both the low- and high-affinity components. Therefore, although their kinetic parameters are different, their regulation appears to be similar. However, the reason why high concentrations of dbcAMP alone, which can also stimulate PKC activity, failed to enhance the high-affinity component is unclear. It is possible that the action of cAMP is biphasic and that an optimal PKA/PKC activity ratio is necessary to influence the high-affinity channel.

In conclusion, whereas high concentrations of dbcAMP alone are necessary to enhance Ca^{2+} transport by the distal luminal membrane, combination of PMA with dbcAMP strongly potentiates the effect of the messenger. Both PKA and PKC inhibitors prevent the effect of the messengers, suggesting that activation of the two messenger pathways is necessary to increase Ca^{2+} uptake. Ca^{2+} entry is mediated by channels with high and low affinities. The combination of dbcAMP and PMA increases the V_{max} of the two components.

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REFERENCES

- Costanzo, L. S. and Windhager, E. E. (1980) *Am. J. Physiol.* **239**, F478–F485
- Bourdeau, J. E. and Burg, M. B. (1980) *Am. J. of Physiol.* **239**, F121–F126
- Suki, W. N. and Rouse, D. (1981) *Am. J. Physiol.* **241**, F171–F174
- Imai, M. (1981) *Pflügers Arch.* **390**, 145–151
- Friedman, P. A. (1988) *Am. J. Physiol.* **254**, F62–F70
- Shimizu, T., Yoshitomi, K., Nakamura, M. and Imai, M. (1990) *Am. J. Physiol.* **259**, F408–F414
- Chabardes, D., Imbert, M., Clique, A., Montegut, M. and Morel, F. (1975) *Pflügers Arch.* **354**, 229–239
- Chabardes, D., Imbert-Teboul, M., Montegut, M., Clique, A. and Morel, F. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3608–3612
- Siga, E., Champigneulle, A. and Imbert-Teboul, M. (1994) *Am. J. Physiol.* **267**, F354–F365
- Shareghi, G. R. and Stoner, L. C. (1978) *Am. J. Physiol.* **235**, F367–F375
- Bidol-Lopez, P., Farese, R. V. and Sabir, M. A. (1981) *Endocrinology* **108**, 1078–1081
- Meltzer, V., Weinreb, S., Bellorin-Font, E. and Hruska, K. A. (1982) *Biochim. Biophys. Acta* **712**, 258–267
- Dunlay, R. and Hruska, K. (1990) *Am. J. Physiol.* **254**, F223–F231
- Hruska, K. A., Moskowitz, D., Esbrit, P., Civitelli, R., Westbrook, S. and Huskey, M. (1987) *J. Clin. Invest.* **79**, 230–239
- Chakraborty, M., Chatterjee, D., Kellokumpu, S., Rasmussen, H. and Baron, R. (1991) *Science Washington D.C.* **251**, 1078–1082
- Force, T., Bonventre, J. V., Flannery, M. R., Gorn, A. H., Yamin, M. and Goldring, S. R. (1992) *Am. J. Physiol.* **262**, F1110–F1115
- Horne, W. C., Shyu, J. F. and Chakraborty, M. (1994) *Trends Endocrinol. Metab.* **5**, 395–401
- Friedman, P. A., Courtermarsh, B. A., Kennedy, S. M. and Gesek, F. A. (1996) *Endocrinology* **137**, 13–20
- Ramachandran, C. and Brunette, M. G. (1989) *Biochem. J.* **257**, 259–264
- Kelly, M. H. and Hamilton, J. R. (1970) *Clin. Biochem.* **3**, 33–43
- Post, R. S. and Sen, A. K. (1967) *Methods Enzymol.* **10**, 762–768
- Huntson, D. L. (1975) *Anal. Biochem.* **63**, 99–109
- Brunette, M. G., Mailloux, J. and Lajeunesse, D. (1992) *Kidney Int.* **41**, 281–288
- Lajeunesse, D., Bouhtiauy, H. and Brunette, M. G. (1994) *Endocrinology* **134**, 35–41
- Chase, H. S. and Wong, S. M. R. (1988) *Am. J. Physiol.* **254**, F374–F384
- Bourdeau, J. E. and Eby, B. K. (1990) *Am. J. Physiol.* **258**, F751–F755
- Lau, J., Quamme, G. and Tan, S. (1991) *J. Am. Soc. Nephrol.* **2**, 775 (Abstract)
- Friedman, P. A. and Gesek, F. A. (1993) *Am. J. Physiol.* **264**, FF181–F198
- Bindels, R. J. M., Demster, J. A., Ramakers, P. L. M., Willems, P. H. G. M. and VanOs, C. H. (1993) *Kidney Int.* **43**, 295–300
- Windholz, M., Budavari, S., Stroumstos, L. Y. and Noether-Fertig, M. (1976) *The Merck Index*, 9th edn., p. 353
- Anderson, R. J. and Breckon, R. (1991) *Am. J. Physiol.* **261**, F945–F950
- Poncet, V., Merot, J. and Poujeol, P. (1992) *Pflügers Arch.* **422**, 112–119
- Tan, S. and Lau, K. (1993) *J. Clin. Invest.* **92**, 2731–2736