Several challenges to this concept remain, mostly why the transition from slit diaphragm to tight junction in nephrosis produces an increase and not, as one would predict, a decrease in permeability for macromolecules. For nephrologists, understanding these events and their associated signaling cascades is clinically relevant, because novel approaches and innovative treatment strategies for glomerular failure may be deduced from this knowledge. Five decades after the first work by Dr. Farquhar and her colleagues, the podocyte slit diaphragm remains a fascinating and promising area of research.

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DISCLOSURES

None.

REFERENCES


Type II Calcimimetics and Polycystic Kidney Disease: Unanswered Questions

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The proteins involved in polycystic kidney disease (PKD) detect extracellular cues at primary cilia, cell–cell and cell–matrix contacts, and affect signaling pathways essential to the three-dimensional structure of tubular epithelium. Reduction in one of these proteins below a critical threshold results in a phenotypic switch characterized by defects in planar cell polarity, increased rates of proliferation and apoptosis, expression of a secretory phenotype, remodeling of the extracellular matrix, and cyst development.1,2 Evidence in the past decade strongly suggests that alterations in two major interacting second messengers, intracellular calcium and cyclic adenosine monophosphate (cAMP), play a central role in the pathogenesis of increased cell proliferation and fluid secretion.3,4

Polycystin-1 and -2, the proteins encoded by the genes mutated in autosomal dominant PKD (ADPKD), constitute a family (TRPP) of transient receptor potential (TRP) channels.5 Polycystin-2 (TRPP2) exhibits characteristic structural features of a TRP channel. Polycystin-1 (TRPP1) is a distant TRP family (TRPP) of transient receptor potential (TRP) channels.5

Type II calcimimetics have been developed primarily to treat autosomal recessive PKD (ARPKD) indirectly interacts with polycystin-2

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and regulate their channel function. Polycystin-1, polycystin-2, and fibrocytin localize to primary cilia and are required for the rise in intracellular calcium triggered by physical and/or chemical stimulation of these organelles. Polycystin-2 interacts with other TRP channels (TRPC1, TRPC4, or TRPV4). Polycystin-2 is also found in the endoplasmic reticulum, functionally interacts with inositol 1,4,5-triphosphate and ryanodine receptors, and modulates intracellular calcium signaling. ADPKD cyst cells lack flow-sensitive calcium signaling and exhibit reduced endoplasmic reticulum calcium stores, store depletion–operated entry, and, under certain conditions, intracellular calcium concentrations. cAMP levels reflect a balance between synthesis by adenyl cyclases (activated by Gs protein–coupled receptors [GsPCR] and inhibited by Gi protein–coupled receptors [GiPCR]) and degradation by phosphodiesterases. Stimulation of calcium-inhibitable adenyl cyclases and inhibition of calcium–dependent cAMP phosphodiesterases likely account for increased levels of cAMP and expression of cAMP-dependent genes in kidneys of cpk, jck, pcy, Ksp.Cre;HNF1Blox, Pkd2−/−/JWST5 and γGT.Cre;Pkd1lox mice and PCK rats; liver of PCK rats; vascular smooth muscle of Pkd2+−/− mice; and choroid plexus of Tg737opk mice. cAMP stimulates cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride–driven fluid secretion. In addition, cAMP stimulates mitogen-activated protein kinase, extracellularly regulated kinase signaling, and cell proliferation in ADPKD renal epithelial cells, whereas it has an inhibitory effect in wild-type cells. This proliferative response links to alterations in intracellular calcium because it is reproduced in wild-type cells by lowering calcium from intracellular stores. By coupling to Gi proteins, CaSR activates phospholipase C–protein kinase C (PLC-PKC) signaling and mobilizes calcium from intracellular stores. By coupling to Gi proteins, it inhibits adenyl cyclase-cAMP (AC-cAMP) signaling. Calcium activates the CaSR by binding to the extracellular domain. Other inorganic divalent cations and organic polycations bind to the CaSR, act as classic agonists, and are referred to as type I calcimimetics. Type II calcimimetics interact with the CaSR at transmembrane domains, increase its sensitivity to extracellular calcium, and act as positive allosteric modulators. R-568, which was initially tested in clinical trials, has been replaced by cinacalcet, which has better bioavailability.

CaSRs are expressed in organs involved in extracellular calcium homeostasis, namely the parathyroid gland; C cells of the thyroid gland, the kidney, and the intestinal tract; and in many other tissues. CaSR activation inhibits PTH and stimulates calcitonin secretion. In the kidney, CaSR protein is expressed in all nephron segments (excluding the glomerulus but including the macula densa) and in the collecting duct. In the proximal tubule, activation of the CaSR results in increased phosphate reabsorption by antagonizing the effects of PTH. In the thick ascending limb of Henle, it inhibits calcium reabsorption by two mechanisms. The first is by eliciting phospholipase A2–mediated arachidonic acid release and production of PGE2 and 20-hydroxyeicosatetraenoic acid (20-HETE), which inhibit recycling of potassium by apical potassium channels. The second is by inhibiting the vasopressin-dependent stimulation of adenyl cyclase and downregulating the Na-K-2Cl co-transporter. Whereas the second mechanism may exert a protective effect on the development of PKD, the first could be detrimental, because PGE2 and 20-HETE promote cystogenesis. In the macula densa, CaSR activation suppresses cAMP formation and renin release. In the inner medullary collecting duct, it enhances aquaporin 2 degradation and induces polyuria.

CaSR activation may also affect indirectly the development of PKD by inhibiting PTH secretion and stimulating calcitonin secretion. By both mechanisms, it exerts a hypocalcemic effect that partially offsets the direct effects of CaSR activation on the tubular epithelium. PTH acts on the parathyroid receptor 1 (PTH1R), which is found in proximal tubules, cortical thick ascending limb, distal tubules, and cortical collecting duct. Like the CaSR, PTH1R is a GPCR. By linking to Gq andGs proteins, it activates PLC-PKC and AC-cAMP signaling, respectively. In the proximal tubule, PTH acts on apical receptors activating PLC-PKC and on basolateral receptors activating AC-PKA. By both mechanisms, it downregulates sodium-dependent phosphate co-transporters and phosphate reabsorption. Also in the proximal tubule, PTH
stimulates transcription of 25-hydroxyvitamin D 1α-hydroxylase and conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3.46 In the distal tubule, PTH stimulates calcium reabsorption by activating TRPV5 by PLC-PKC–dependent pathways.47 Two studies have investigated whether type 2 calcimimetic drug attenuates or prevents the development of PKD.49,50 In the study published in this issue of JASN, Gattone et al.49 treated male cy/+ rats starting at 20 wk of age with R-568 or 2% calcium gluconate added to the drinking water or both. The three interventions lower serum PTH levels but have no effect on the severity of the cystic disease or renal function at 34 wk of age. They are very effective, however, in preventing the rapid progression of the cystic disease, development of interstitial fibrosis, and deterioration of renal function that occurs in the untreated animals between 34 and 38 wk of age, coinciding with a three-fold increase in serum PTH levels. R-568 (alone or with calcium) is marginally more effective than calcium alone in preventing the cystic expansion, suggesting that R-568 has beneficial effects on cyst growth independent from PTH suppression. This effect is observed despite significant hypocalcemia in rats treated with R-568 alone. The three treatments are equally effective in inhibiting interstitial fibrosis and protecting renal function.

In a second recent study, Wang et al.50 administered R-568 to PCK rats from 3 to 10 wk and to Pkd2−/WS25 mice from 3 to 16 wk of age. The administration of R-568 produces hypocalcemia and hyperphosphatemia; increases urine output and osmolar clearance; but fails to lower renal cAMP, inhibit PKD development, or protect renal function. The authors suggest that hypocalcemia associated with the administration of R-568 might offset the calcimimetic effects on intracellular calcium and cAMP.

Neither of the two studies is able to demonstrate a calcimimetic effect at early phases of PKD. Because its administration results in significant hypocalcemia, the possibility that a lower dosage without hypocalcemia would be protective cannot be ruled out. R-568 effectively suppresses cyst expansion at an advanced stage in cy/+ rats. Whether it would have also been effective in the PCK rat or Pkd2−/WS25 mouse at similarly advanced stages was not studied. Gattone et al. suggest administration of calcimimetics to patients with PKD at late stages of the disease may slow or halt cystic disease progression and renal function deterioration.

The development of PKD in the cy/+ rat, however, is different from that in human ADPKD; therefore, extrapolations from this animal model should be done cautiously. The cystic transformation in this model begins at the S3 segment and gradually extends to other segments of the proximal tubule.51,52 Cystic enlargement peaks at 10 wk of age and is followed by a reduction in size accompanied by development of interstitial fibrosis. At more advanced stages, renal enlargement resumes and a small proportion of cysts derive from distal tubules or cortical collecting ducts. The natural history of cystic disease in human ADPKD is different. As in the PCK rat and Pkd2−/WS25 mouse, cysts derive predominantly from collecting ducts and distal nephron, renal enlargement is progressive and relatively constant, and renal function starts declining only after marked enlargement of the kidneys.53–56 Progression of cystic disease in the cy/+ rat is more reminiscent of human acquired renal cystic disease, a disease that affects mostly proximal tubules, than of ADPKD.57,58

The effect of R-568 on progression of renal insufficiency may be independent from that on cystic disease. Two studies have ascertained the effect of R-568 on the progression of chronic kidney disease (CKD) in subtotally nephrectomized rats.59,60 The first found that R-568 or parathyroidectomy (with 5% calcium gluconate added to the drinking water to prevent hypocalcemia) are equally effective in reducing tubulointerstitial damage, glomerulosclerosis, urine albumin excretion, and serum creatinine.59 The second found that R-568 and calcitriol reduce PTH levels, remnant kidney weight, tubular dilation and atrophy, interstitial fibrosis, glomerulosclerosis, proteinuria, and serum creatinine to the same extent.60 These studies suggest the major, if not the only, factor explaining the effect of R-568 on progression of CKD is the decrease in the serum PTH concentration. PTH is also implicated in the development of myocardial fibrosis after subtotal nephrectomy.51

Interestingly, in the studies by Gattone et al.49 and Wang et al.,50 the administration of R-568 reduces the extent of the renal fibrosis. This is marked in the cy/+ rat and more moderate in the PCK rat. In Pkd2−/WS25 mice, an effect on fibrosis is insignificant. That cy/+ rats were studied at an advanced stage of the disease when interstitial fibrosis is prominent, whereas PCK rats and Pkd2−/WS25 mice were studied at an early stage of the disease when interstitial fibrosis is very mild, may account for the differences.

In summary, many unanswered questions remain regarding use of calcimimetics in PKD. Two studies suggest their administration does not affect cystogenesis at early stages of the disease. The study by Gattone et al.49 finds a beneficial effect at advanced stages, but it is uncertain whether mechanisms other than suppression of PTH are responsible. The possibility that calcimimetics prevent the development of acquired renal cystic disease is intriguing. Potential risks and benefits relative to other treatments that control secondary hyperparathyroidism need further evaluation before their use in patients with advanced ADPKD is considered. Similar reservations have been raised regarding their use in patients with stages 3 to 4 CKD for their effects on mineral metabolism.52–54 Phases 2 and 3 cinacalcet studies in stages 3 and 4 CKD also raise whether a reduction in PTH at the expense of worsening hyperphosphatemia and hypocalcemia with corresponding increases in urinary calcium excretion and decreases in urinary phosphorus excretion is in the best interest of the patient.64

DISCLOSURES

None.
REFERENCES

44. Park F, Sweeney WE, Jia G, Roman RJ, Avner ED: 20-HETE mediates mediating the effect of extracellular Ca2+/H11001 in a small-molecule screen blocks toxin-induced intestinal fluid secretion. Clin Pract Endocrinol Metab
45. Ortiz-Capisano MC, Ortiz PA, Garvin JL, Harding P, Beierwaltes WH: Specificity of calcimimetic Inhibits Late-Stage Cyst Growth in ADPKD,
50. Bajwa A, Forster MN, Maiti A, Woolbright BL, Beckman MJ: Specificity of calcimimetic Inhibits Late-Stage Cyst Growth in ADPKD,

The Promise of Well-Being: Stay in Shape with N(i)ck

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Just more than a decade ago, a report of a rare human genetic disorder initiated a new spurt of research that profoundly changed our understanding of the glomerular filtration barrier. In that landmark article,1 mutations in the gene encoding the adhesion protein nephrin identified the cause of congenital nephrotic syndrome of the Finnish type. Subsequent studies localized nephrin to the podocyte slit diaphragm, a specialized...

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