A Novel Target for Diuretic Therapy

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Keywords. pendrin, sodium-chloride cotransport, volume depletion, kidney tubules, prerenal failure

The reabsorption of salt in the distal nephron is predominantly mediated via the thiazide-sensitive sodium chroride cotransporter, NCC (*SLC12A3*), and the chloride-bicarbonate exchanger pendrin (*SLC26A4*, *PDS*), with pendrin working in tandem with the epithelial sodium channel and NCC working by itself. Single deletion of *NCC* or pendrin in genetically engineered mouse models does not cause salt wasting or excessive diuresis under basal conditions. Both pendrin knockout and NCC knockout mice, however, show signs of volume depletion or develop hypotension during salt restriction. These findings have led investigators to conclude that pendrin and NCC are predominantly active during salt depletion and their contribution to salt reabsorption at baseline conditions is small. We hypothesized that pendrin may compensate for loss of NCC under basal conditions, thereby masking the role that each transporter plays in salt reabsorption. To test this hypothesis, double knockout of pendrin and sodium chloride cotransporter was generated by crossing animals with single deletion for *NCC* and pendrin. The double-knockout mice show significant salt and fluid wasting, along with severe volume depletion, metabolic alkalosis and prerenal failure under baseline conditions. Volume depletion, metabolic alkalosis and prerenal failure were significantly corrected with salt repletion. We conclude that pendrin plays an essential role in the distal tubule salt reabsorption in the setting of sodium-chloride cotransporter inactivation. We propose that pendrin could be a novel target for a new diuretic that in conjunction with thiazide can be an effective regimen for patients with fluid overload.

IJKD 2012;6:419-25
www.ijkd.org

Special Report

INTRODUCTION

Thiazides which are specific inhibitors of sodium-chloride (Na-Cl) cotransporter (NCC) in the distal tubule are the most widely used diuretic in the world, in large part because they do not cause severe salt wasting and are therefore considered mild agents.[1-3] The distal convoluted tubule (DCT) is responsible for the reabsorption of 7% to 10% of filtered salt, and published reports indicate that majority of this process is mediated via NCC.[3-5] The reabsorption of the remaining sodium in the DCT and the collecting duct—estimated at about 3% to 4% of the total filtered sodium—is thought to occur via the sodium channel working in tandem with pendrin, with the residual component being absorbed via the Na^+/H^+ exchanger.[6,7]

Pendrin is expressed on the apical membrane of intercalated cells in the connecting tubule (CNT) and cortical collecting duct (CCD) and works in conjunction with epithelial sodium channel (ENaC).[8-13] Recent investigations demonstrated that pendrin knockout (KO) mice developed significant volume depletion when injected with furosemide versus wild type littermates,[14] supporting the notion that pendrin plays an important role in compensatory salt reabsorption in response to the

loop diuretics.

Published reports indicate that pendrin expression is increased in kidneys of NCC knockout mice,[14,15] raising the possibility that pendrin may compensate for loss of NCC by upregulating its expression and activity. To test this latter possibility, double-knockout of pendrin and NCC was generated by crossing animals with single deletion for NCC and pendrin. The double pendrin-NCC KO mice show severe salt wasting, along with volume depletion, and prerenal failure under baseline conditions. The significance of the results will be discussed.

RESULTS AND DISCUSSION

The kidney plays a crucial role in the regulation of vascular volume and systemic arterial pressure predominantly through the reabsorption of filtered sodium and chloride and via highly specialized transporters within different nephron segments. The absorption of water is mediated via apical and basolateral aquaporines (AQPs) in the collecting duct and the proximal tubule.[16,17] The reabsorption and homostatic balance of salt and water are under the influence of several hormones, including aldosterone, angiotensin II, antidiuretic hormone, and atrial natriuretic factor. These hormones regulate the activity of sodium and water transporters and allow the kidney to adjust salt and water excretion to the need of body, and thus control vascular volume and blood pressure.

While the proximal tubule and thick ascending limb of Henle are responsible for reabsorption of the bulk of filtered salt, the distal convoluted tubule, connecting tubule, and the collecting duct play an important and unique role in the reabsorption of the remaining filtered salt through 2 major transport processes. The DCT reabsorbs about 7% to 10% of filtered NaCl mainly via a transcellular mechanism involving the apical NCC, and mediates the entry of NaCl in the DCT cells as depicted in Figure 1. The NCC is a secondary active transporter and utilizes the favorable chemical gradient of sodium created by the basolateral Na^+-K^+-adenosine triphosphatase (ATPase) to transport chloride into the epithelial cells against its gradient (Figure 1).[2,3] The sodium exits the cells via the Na^+-K^+ ATPase, whereas chloride passively exits through the basolateral membrane via a chloride channel (ClC-Kb; Figure 1). The NCC is regulated by aldosterone and with-no-lysine kinases (WNK1 and WNK4) pathways.[18-21]

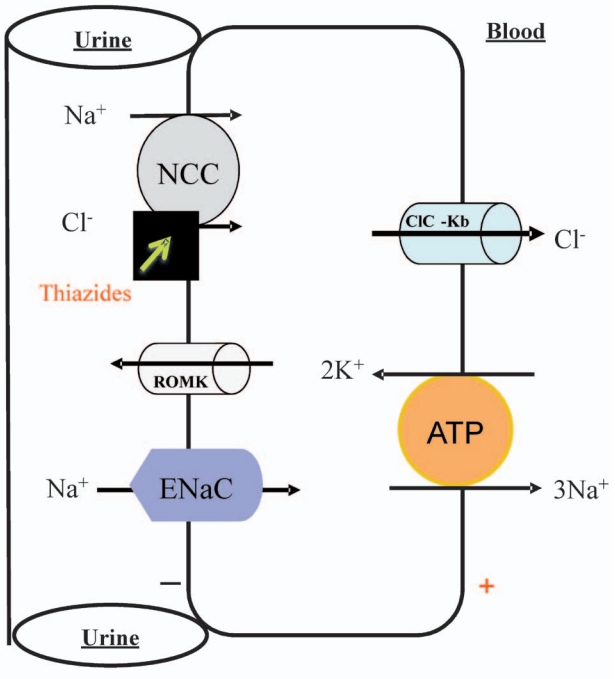

Figure 1. A schematic diagram depicting salt absorbing transporters in distal convoluted tubule cells. Early distal convoluted tubule expresses only sodium-chloride cotrasporter, whereas late distal convoluted tubule expresses both sodium-chloride cotrasporter and epithelial sodium channel on the apical membrane. ATP indicates adenosine triphosphate; NCC, sodium-chloride cotrasporter; DTC, distal convoluted tubule; ENaC, epithelial sodium channel; ClC-Kb, chloride channel; and ROMK, renal outer medullary potassium channel.

Thiazide diuretics inhibit the NCC, perhaps by competing for the chloride-binding site (Figure 1).[22]

In the connecting tubule (CNT) and the collecting duct, around 3% to 4% of filtered NaCl is reabsorbed by a rather more complex mechanism involving coordinated and parallel activities of 2 distinct transport systems expressed on the apical membranes of 2 different cell types as shown in Figure 2. These processes include pendrin and ENaC, which reabsorb chloride and sodium, respectively. The chloride-bicarbonate exchanger pendrin (SLC26A4) is expressed on the apical membrane of B-type intercalated cells in the CNT and CCD and reabsorbs chloride in exchange for bicarbonate, which is then secreted in the lumen (Figure 2).[8,9,23] The chloride exits the cell through the basolateral membrane ClC-3 chloride channel (Figure 2).[24] In parallel, sodium reabsorption is mediated via ENaC, which is expressed on the apical membrane of principal cells along the CNT and CCD (Figure 2).[25,26] The ENaC activity is energized by the inward chemical gradient of sodium generated by

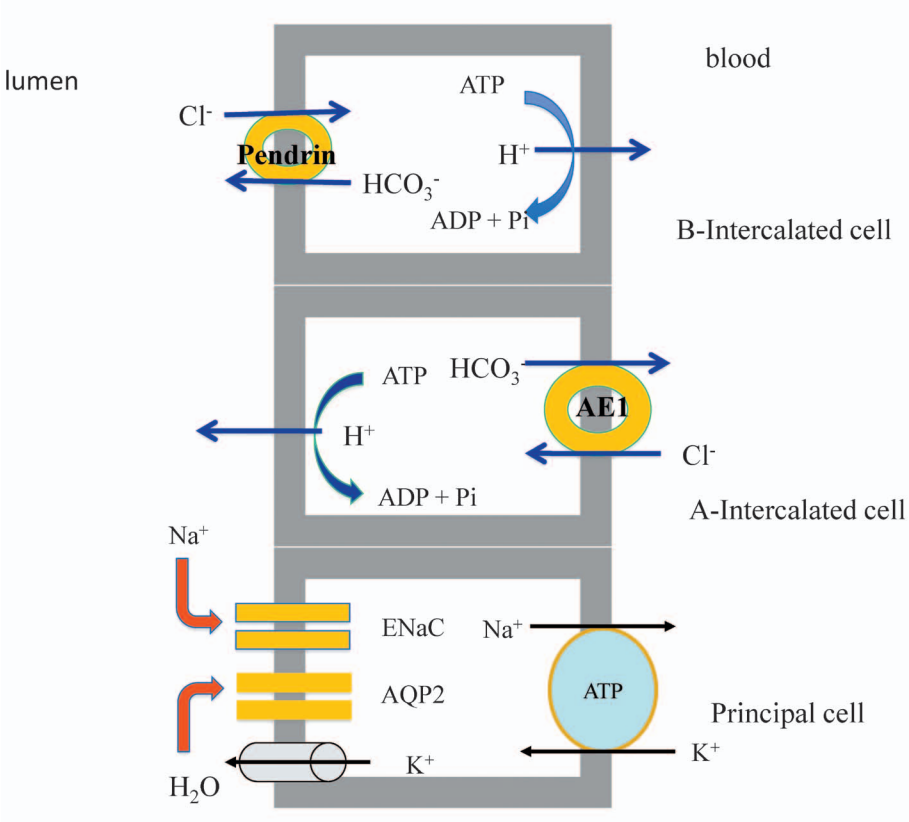

Figure 2. A schematic diagram demonstrating salt absorbing and acid-base transporters in cortical collecting duct cells. Epithelial sodium channel and aquaporin 2 are expressed on the apical membrane of principal cells and pendrin is expressed on the apical membrane of B-intercalated cells. Epithelial sodium channel indicates ENaC; AQP2, aquaporin 2; CCD, cortical collecting duct; AE1, anion exchanger 1; ATP, adenosine triphosphate; and ADP, adenosine diphosphate.

the basolateral Na^+-K^+-ATPase activity and results in the potassium secretion, mediated by potassium channels renal outer medullary potassium channel (ROMK) and Maxi K.[27,28] The activity of ROMK in the DCT and CCD is responsible for the daily obligatory losses of potassium and thus dictates the level of potassium in the blood.[29] Pendrin is sensitive to changes in acid-base status,[13-15] and both pendrin and ENaC expression and activities are regulated by aldosterone[30,31] and vasopressin.[32,33] The A-intercalated cells in CNT and CCD are mainly involved in acid secretion via H^+ ATPase (Figure 2).

It is important to note that in contrast to the collecting duct, transcellular sodium reabsorption in the DCT is directly coupled to chloride reabsorption. Therefore, disorders of salt reabsorption in the DCT affect both sodium and chloride homostasis. Because ion transport mechanisms are tightly coupled to each other, loss-of-function (either by inhibition or due to mutations) affecting one molecule results in the breakdown of the complete transport process in the respective tubular segment.

In conditions associated with fluid overload such as congestive heart failure, nephrotic syndrome, renal failure, and liver cirrhosis, loop diuretics such as furosemide have been used in conjunction with thiazide derivatives to increase salt excretion and reduce fluid retention. The schematic diagram in Figure 3 indicates that furosemide, a loop diuretic, inhibits the thick ascending limb apical Na^+-K^+-$2Cl^-$ co-transporter (NKCC2), whereas thiazide diuretics, such as hydrochlorothiazide or its derivatives, block the apical NCC in the distal convoluted tubule. This combined inhibition of salt absorption in two distinct nephron sites significantly increases salt excretion (Figure 3, right panel versus left panel). Interestingly, mice treated with furosemide show translocation of pendrin to the apical membrane in B-intercalated cells[34] as depicted by thick arrows in the schematic diagram in Figure 3 (right panel). This indicates that the pendrin/ENaC functional complex is actively involved in compensatory NaCl reabsorption in the CNT and CCD as described

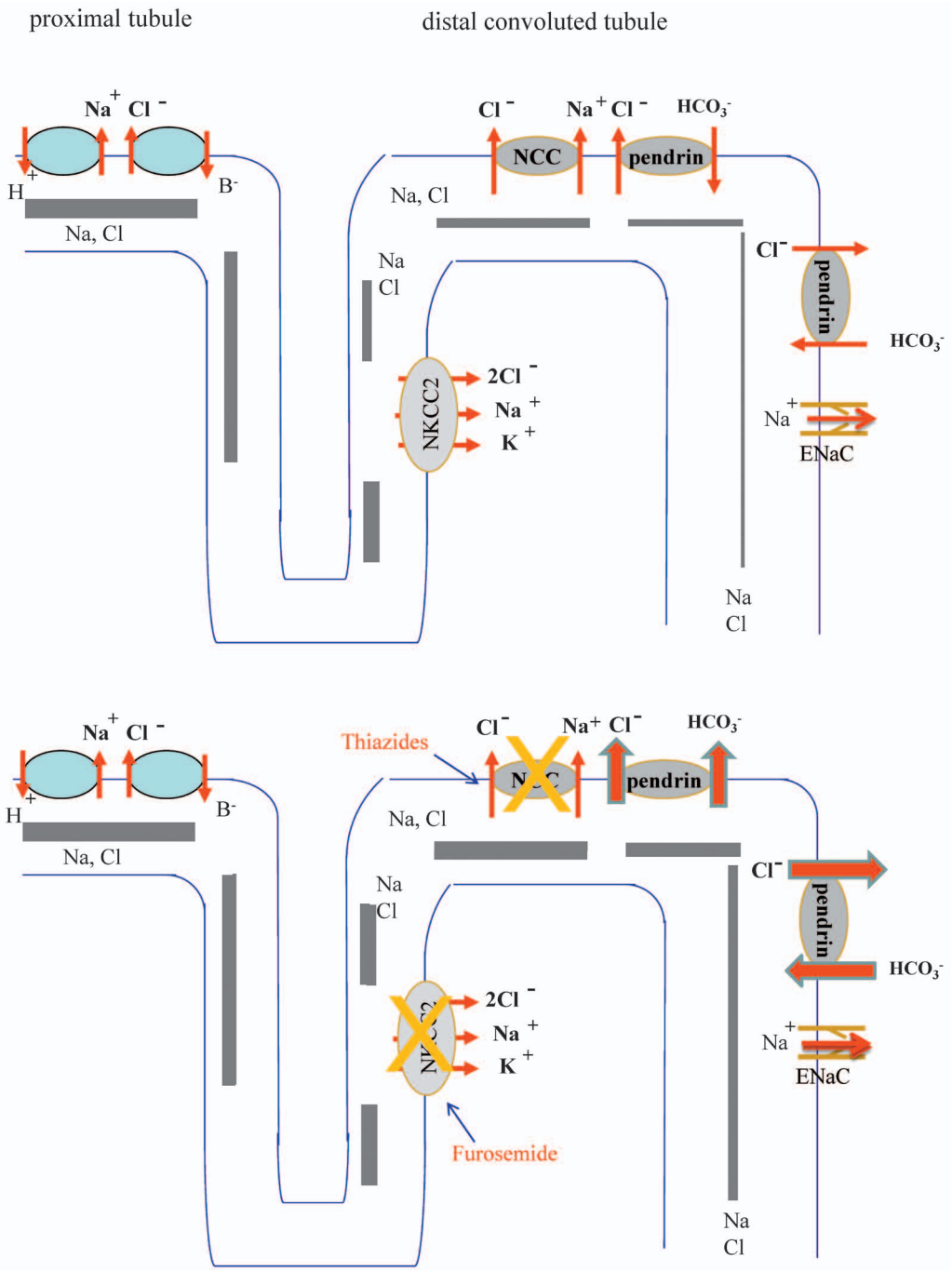

Figure 3. Top, Schematic diagram demonstrating salt-absorbing transporters in medullary thick ascending limb, distal convoluted tubules, and cortical collecting duct. The apical Na-K-2Cl cotransporter is in the medullary thick limb, whereas the apical Na-Cl cotransporter is in distal convoluted tubules, and pendrin is detected in connecting tubule and cortical collecting duct. **Bottom,** The synergistic effect of Na-K-2Cl cotransporter and Na-Cl cotransporter inhibition by furosemide and thiazides, respectively, significantly increases the magnitude of salt excretion. Pendrin expression is increased in response to increased delivery of salt to the collecting duct in order to mitigate the amount of salt wasting. Epithelial sodium channel indicates ENaC; NKCC2, Na-K-2Cl cotransporter; and NCC, Na-Cl cotrasporter.

above, raising the possibility that the complex might interfere with the diuretic effects of loop and thiazide diuretics by blunting their impact on salt and fluid excretion. Indeed, mice with the genetic deletion of pendrin showed a more significant diuresis and volume depletion versus wild type animals in response to furosemide.[14]

While combination therapy with furosemide

and thiazide is more efficient in causing salt and fluid excretion than furosemide alone,[35] thiazide alone is far less potent than furosemide as a diuretic agent. Thiazide specifically inhibits NCC and a wealth of studies demonstrates that NCC inhibition by thiazide in mouse and human or inactivation of *NCC* gene in mouse or human does not cause significant salt wasting under baseline conditions.[1-3,9,23,36,37] The mild salt-wasting effect of thiazides, which is a specific inhibitor of NCC, suggests that either the DCT, and thus NCC, is not handling a significant amount of salt reabsorption, or that other NaCl absorbing transporters such as pendrin/ENaC functional complex in the CNT and CCD are activated and thus compensate for the inactivation or inhibition of NCC in the DCT. In support of this possibility, recent published studies demonstrated that pendrin was significantly upregulated in kidneys of NCC knockout mice.[14,15]

Pendred syndrome is an autosomal recessive disorder that presents with deafness, goiter and a partial defect in the organification of iodide.[38] It is caused by biallelic mutations in the *SLC26A4* (*PDS*) gene, which encodes pendrin.[39] In addition to the thyroid and inner ear, kidney shows significant pendrin expression.[8,9] Pendrin plays an important role in compensatory salt absorption in conditions associated with increased delivery of salt to the distal nephron. Recent studies indicated that pendrin KO mice display a more profound diuresis relative to their wild-type littermates in response to furosemide.[14] These results nicely correlate with studies demonstrating that furosemide treatment of rats increased the abundance and trafficking of pendrin to the apical membrane of B-type intercalated cells.[36] Taken together, these findings support a compensatory role for the pendrin/ENaC functional complex in response to the deletion or inhibition of *NCC* or furosemide inhibition of the TAL apical NKCC2.

Single deletion of pendrin or NCC in mice does not cause any salt wasting under baseline condition.[9,23,37] Similarly, inactivating the *PDS* gene mutations in human do not lead to excessive salt and fluid wasting or changes in blood pressure under baseline conditions.[38,39] Published reports, however, indicate that mice with a single deletion of pendrin or *NCC* develop hypotension under salt-restricted conditions.[36,37] These results have been interpreted to indicate that pendrin might be only active under salt-depleted states. There is, however, evidence supporting the activation of compensatory mechanisms in kidneys of NCC KO mice. First, the CNTs show significant hypertrophy in kidneys of NCC KO mice.[40] Second, ENaC expression was shown to be increased in kidneys of NCC KO mice.[40] Lastly, the expression of pendrin was found to be increased in kidneys of NCC KO mice.[14,15] Taken together, these results support the notion that the pendrin/ENaC functional complex is activated in kidneys of NCC KO mice.

We hypothesized that pendrin and NCC are active under baseline conditions but compensate for the loss of each other, therefore minimizing the amount of salt that is wasted in pendrin or NCC KO mice. To test our hypothesis, we generated mice with double deletion of pendrin and NCC by crossing pendrin KO and NCC KO mice with each other. The results are startling. They show profound polyuria, polydipsia, and decreased urine osmolality along with a sharp increase in urinary salt excretion in double pendrin/NCC KO mice, when compared to wild-type, NCC KO or Pendrin KO mice.[41] Double pendrin/NCC KO mice show profound dehydration along with metabolic alkalosis and significant renal failure subsequent to reduced vascular volume.[41] The renin-angiotensin-aldosterone pathway showed significant upregulation in double pendrin/NCC KO mice.[41]

Volume depletion, metabolic alkalosis and pre-renal failure in pendrin/NCC double KO mice were significantly corrected with salt repletion,[41] as shown by a robust reduction in the expression of renin and concentration of blood urea nitrogen and serum bicarbonate.[41] These results strongly suggest that the profound phenotypic presentation of double pendrin/NCC KO mice was due to severe salt wasting subsequent to the combined inhibition of NCC and pendrin.

We conclude that pendrin plays an essential role in distal tubule salt absorption in the setting of NCC inactivation. We propose that pendrin could provide a novel target for a new diuretic that in conjunction with thiazide can be an effective regimen for patients with fluid overload, such as those with congestive heart failure, nephrotic syndrome, diuretic resistance or advanced chronic kidney disease. According to this proposal, the inhibition of pendrin in the setting of NCC

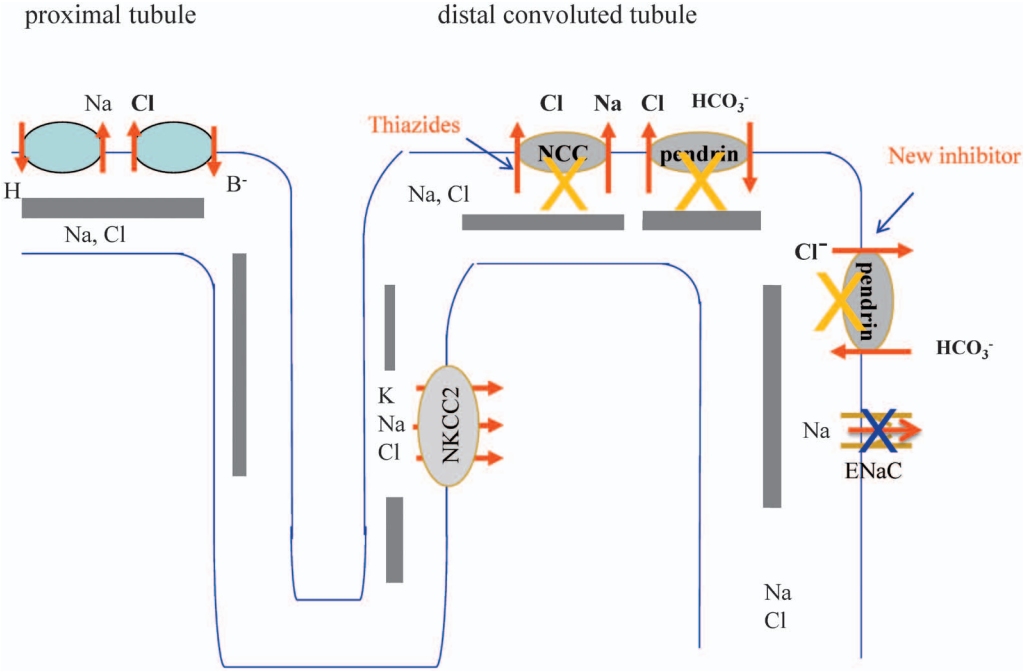

Figure 4. Schematic diagram depicting the synergistic diuretic effects of thiazides inhibitors of Na-Cl cotransporter, and a new diuretic which can inhibit pendrin. Epithelial sodium channel indicates ENaC; NKCC2, Na-K-2Cl cotransporter; and NCC, Na-Cl cotrasporter.

inactivation blunts the function of ENaC and causes severe salt wasting. The schematic diagram in Figure 4 depicts the synergistic effects of NCC and pendrin inhibitors on salt excretion.

CONFLICT OF INTEREST

None declared.

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Received September 2012