Hormonal and Osmotic Regulation of NaCl Transport in Renal Distal Nephron Epithelium

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Key words: sodium channel, chloride channel, amiloride, ADH, aldosterone, osmolality.

One of the most important factors controlling blood pressure is the total body Na⁺ content, which depends upon Na⁺ intake and excretion. The kidney influences body Na⁺ content by regulating the tubular absorption of the Na⁺ filtered through the glomeruli. Thus, the regulation of Na⁺ absorption in the tubules of the kidney plays an important role in controlling blood pressure. More than 99% of the Na⁺ passing through the glomerulus is reabsorbed in the kidney. About 90% of the filtered Na⁺ through the glomerulus is reabsorbed in the proximal tubule and the ascending limb of the loop of Henle. The remainder of the Na⁺ absorption occurs in the distal nephron. This process is regulated by hormones such as aldosterone and antidiuretic hormone (ADH), and also by the osmolality of the plasma. These observations suggest that the regulation of Na⁺ transport in the distal nephron by hormones and osmolality plays an important role in the control of blood pressure.

The distal nephron is composed of two different types of epithelial cells: the principal cell and the intercalated cell. The latter is also composed of two types of cells: α and β intercalated cells. In addition to Na⁺ absorption, the distal epithelial cells also participate in K⁺ and H⁺ secretion. Na⁺ absorption is mediated through the principal cell, which also contributes to K⁺ secretion, whereas H⁺ is secreted through both types of intercalated cells, α and β, in different ways [1–3].

There are, in general, two steps in the transepithelial ion movement across the epithelium, including the distal-nephron epithelium. For example, in the case of Na⁺ absorption, one is the entry step of Na⁺ across the apical membrane and the other is the extrusion step of Na⁺ across the basolateral membrane (Fig. 1). This means that there are two major regulatory sites of transepithelial Na⁺ absorption: namely, regulation of the entry and extrusion steps of Na⁺. It is generally thought that the entry step of Na⁺ across the apical membrane is the rate-limiting step in the transepithelial Na⁺ transport and that Na⁺ channels in the apical membrane play an important role as an entry step of Na⁺ and are regulated by hormones and plasma osmolality.

In this review, we describe the regulatory mechanisms of Na⁺ absorption in renal distal-nephron epithelium by aldosterone, ADH and osmolality. Further, we will review the regulatory mechanisms of Cl⁻ transport, which also plays an important role in Na⁺ transport as a major counter ion, and discuss other roles of Cl⁻ in the active regulation of Na⁺ transport.

1. Amiloride-Sensitive Na⁺ Absorption in the Distal Nephron

 Transepithelial Na⁺ absorption in the distal nephron is characterized as amiloride-sensitive. Amiloride shows its diuretic action through the diminution of transepithelial Na⁺ absorption in the distal-nephron epithelium by blocking the Na⁺ channels located at the apical membrane. Na⁺ absorption in the distal nephron is indirectly linked to K⁺ secretion (Fig. 1). Na⁺ absorption is partly regulated through the modification of K⁺ handling, such as a change in the apical membrane potential that affects the driving force for the entry of Na⁺. In this article, however, we review only the regulation of NaCl transport, not K⁺ handling, which has been intensively reviewed in other articles [4–6].
2. Amiloride-Sensitive Na⁺ Channels

The properties of amiloride-sensitive Na⁺ transport pathways have been intensively reported for frog skin, which has been used as a model of the distal nephron because their characteristics are similar [7–10]. Pioneer works have been performed by Ussing and his colleague [7], who introduced a new technique to detect active, transepithelial, transcellular Na⁺ absorption across frog skin by measuring the so-called short-circuit current. Later, Lindemann and Van Driessche [11] studied single-channel current characteristics using fluctuation analysis of the noise in short-circuit (macroscopic) currents across frog skin through amiloride-sensitive Na⁺ channels [11, 12]. Similar studies [13, 14] have been done for the Todd urinary bladder, another model of the distal nephron. A technique directly measuring single-channel currents, the patch-clamp technique, which was originally developed using skeletal muscles by Neher, Sakmann and their colleagues [15], has also been applied to the distal-nephron epithelium [16–18].

The single-channel–current recording technique has provided us with a lot of information about the single-channel characteristics of amiloride-sensitive Na⁺ channels. A single amiloride-sensitive Na⁺ channel current recorded by the patch-clamp technique was first reported in A6 cells, which are a model cell line of the distal nephron derived from the kidney of Xenopus laeves, cultured on impermeable glass coverslips [19]; the channel has a relatively small single-channel conductance of 8 pS and a relatively low Na⁺ selectivity (the ratio of permeability to Na⁺ over K⁺ \(P_{Na}/P_K\) ≈4). Subsequently, another type of amiloride-sensitive Na⁺ channel has also been reported in the same cell (i.e., the A6 cell) cultured on porous membrane permeable supports: the channel has a single-channel conductance of 4 pS, high Na⁺ selectivity \(P_{Na}/P_K=10–20\) and high sensitivity to amiloride, the concentration of the half maximum inhibition \(IC_{50}\) <1 μM [20–22]. In the rat distal nephron, a similar type of 4-pS amiloride-sensitive highly Na⁺-selective channel has been reported [16–18]. Further, in other epithelial tissues, similar (i.e., the 4-pS Na⁺ channel) and some other types of amiloride-sensitive Na⁺ channels have been reported [23]. Consequently, amiloride-sensitive Na⁺-permeable channels are classified into three types: 1) highly Na⁺-selective \(P_{Na}/P_K≈10–20\) channel, with a small single-channel conductance of ~4 pS; 2) moderately Na⁺-selective \(P_{Na}/P_K≈4\) channel, with a moderate single-channel conductance of ~7–15 pS; and 3) nonselective cation channel \(P_{Na}/P_K≈1\), with a relatively large single-channel conductance of 23–30 pS [23–29]. The characterization of these amiloride-sensitive channels indicates an interesting relationship between Na⁺ selectivity and single-channel conductance: the single-channel conductance increases as the Na⁺ selectivity decreases. However, the molecular mechanisms regulating ion selectivity and single-channel conductance are still unclear. Figure 2 shows a typical trace of the single amiloride-sensitive Na⁺ channel, with a high selectivity for Na⁺ (the ratio
Fig. 3. The sensitivity of the channel shown in Fig. 2 to amiloride, benzamil and 6-chloro-3,5-diaminopyrazine-2-carboxamide (CDPC). Benzamil and CDPC are analogs of amiloride. The IC_{50} of benzamil, amiloride and CDPC were 0.01, 0.1 and 3 μM, respectively.

of permeability to Na\(^+\) over K\(^+\)=15) and a small conductance of about 4 pS, obtained from the apical membrane of A6 cells cultured on permeable supports. The highly Na\(^+\) selective amiloride-sensitive channel has relatively slow gating kinetics: the mean open and closed times are a couple of seconds [21, 23, 30]. This type of amiloride-sensitive Na\(^+\) channel (e.g., 4 pS) is thought to contribute mainly to the amiloride-sensitive transepithelial Na\(^+\) transport. The amiloride-sensitivity of the 4-pS Na\(^+\) channel is shown in Fig. 3. The IC_{50} of amiloride to the Na\(^+\) channel is about 0.1 μM. This channel is considered a highly amiloride-sensitive Na\(^+\) channel (called “H-type”) as compared to some other channels that are sensitive to amiloride with an IC_{50}=1 μM (the low amiloride-sensitive Na\(^+\) channel called “L-type”).

Amiloride has some other effects on ion transport, in addition to the blocking action on the Na\(^+\) channel: amiloride also blocks the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)/Ca\(^{2+}\) exchanger with an IC_{50} of 10–100 μM, depending on the tissue type [31], which is at least 10-fold higher than that blocking the highly amiloride-sensitive Na\(^+\) channel. Benzamil, an analog of amiloride, has more specific blocking effects on the Na\(^+\) channel than the Na\(^+\)/H\(^+\) exchanger or Na\(^+\)/Ca\(^{2+}\) exchanger; the IC_{50} for the Na\(^+\) channel and Na\(^+\)/H\(^+\) exchanger is ≤0.1 μM and >100 μM, respectively. Benzamil is more suitable for studying the Na\(^+\) channel with no significant effects on the Na\(^+\)/H\(^+\) exchanger or Na\(^+\)/Ca\(^{2+}\) exchanger [31].

Amiloride and benzamil are good tools for studying Na\(^+\) transport in epithelial tissues. However, the dissociation rates of these compounds from the channel are slow and they have a positive charge. These character-istics mean that it takes a relatively long time to wash out these compounds, and their inhibitory action depends upon the membrane potential. 6-Chloro-3,5-diaminopyrazine-2-carboxamide (CDPC), a neutral compound with a much faster dissociation rate than that of amiloride or benzamil [21], is a suitable compound for a study using fluctuation analysis and the voltage dependency of the channel characteristics, although its IC_{50} is not as low as that of benzamil or amiloride [21, 32–36].

Recently, an epithelial amiloride-sensitive Na\(^+\) channel (epithelial Na\(^+\) channel, ENaC) has been cloned from rat distal colon by the functional expression of amiloride-sensitive Na\(^+\) currents in Xenopus oocytes [37, 38]. Since this channel has been cloned from rats, it is called rat ENaC (i.e., rENaC). This channel is a heteromultimeric complex consisting of three homologous, α, β and γ subunits, of which the molecular weights are 79, 72 and 75 kDa, respectively. Each subunit has at least two putative transmembrane segments. The rENaC has significant sequence similarities to those of mec-4 and deg-1, members of a family of Caenorhabditis elegans genes involved in sensory-touch transduction [37, 39]. Further, more recently, a super family of ENaC has been cloned from various species (e.g., human, bovine, and Xenopus) [39–46]. β or/and γ subunits of the rENaC expressed in the Xenopus oocyte without the expression of an α subunit cannot induce significant amiloride-sensitive Na\(^+\) currents. On the other hand, an α subunit alone can induce amiloride-sensitive Na\(^+\) currents, although the amount of amiloride-sensitive Na\(^+\) current is small. All three subunits together can induce a much larger amount of amiloride-sensitive Na\(^+\) current than that induced by an α subunit alone or even with β or γ subunits. These observations imply that the α subunit alone can function as an amiloride-sensitive Na\(^+\) channel (i.e., a channel pore), whereas β and γ subunits are required for the maximal expression of amiloride-sensitive Na\(^+\) channels. Recent studies using reconstitution of the subunits of ENaC into a lipid bilayer support this idea that the α subunit functions as an ionic conductive pore and the other two subunits (β and γ) regulate the gating mechanisms of the α subunit [47, 48]. It is proposed that the amiloride-sensitive Na\(^+\) channel consists of five subunits: three α subunits that are ionic conductive pores, one β subunit, and one γ subunit, and that there are three different conductive states of one unit channel [47, 48].
3. Regulation of Na⁺ Channels

A) Aldosterone action on Na⁺ channels

It is well recognized that aldosterone, a mineralocorticoid, is secreted from the adrenal cortex and regulates the body's extracellular fluid (ECF) volume. One of the most important roles of aldosterone is to stimulate Na⁺ uptake in the colon and the distal nephron, resulting in an increase in the Na⁺ content of the body followed by an increase in the ECF volume. The aldosterone action on epithelial Na⁺ transport occurs by action at two different sites: 1) an apical amiloride-sensitive Na⁺ channel and 2) the basolateral Na⁺-K⁺-ATPase. Aldosterone also has stimulatory effects on K⁺ secretion that increases the driving force for Na⁺ entry across the apical membrane by hyperpolarizing the apical membrane, resulting in the indirect stimulation of Na⁺ absorption.

The stimulatory action of aldosterone on Na⁺ transport occurs in at least two phases: in the first (early) phase (~1–4 h), amiloride-sensitive Na⁺ channels are activated, resulting in an increase in Na⁺ conductance [49–51]; in the second (late) phase (4 h–a couple of days), no further increase in Na⁺ conductance, but an increase in Na⁺-K⁺-ATPase protein message and proteins occurs, resulting in a rise in Na⁺ transport [49, 52, 53].

It was initially proposed that the action of aldosterone is mediated by the synthesis of new Na⁺ channel and Na⁺-K⁺-ATPase proteins. Although the action of aldosterone on the amiloride-sensitive Na⁺ channel is generally thought to occur through the synthesis of new channel proteins, some other candidates to show its stimulatory action in the early phase, in addition to new protein synthesis, have been reported. The early response to aldosterone is due to the modification of preexisting apical amiloride-sensitive Na⁺ channels. This explains why the early response of Na⁺ transport to aldosterone is proportional to the basal Na⁺ conductance and the number of Na⁺ channels. This finding is supported by reports [54–56] that aldosterone induces no changes in the apical cell-surface expression of the amiloride-sensitive Na⁺ channels or cellular pools of the channels that aldosterone increases the open probability of amiloride-sensitive Na⁺ channels in A6 cells. Transmethylation, G proteins, interactions with the cytoskeleton or modification of cytosolic Ca²⁺ concentration, and the modification of its sensitivity are thought to play important roles in the early effects of aldosterone on the channel [57–61]. However, in the epithelial cells of the rat cortical collecting-duct, the effects of aldosterone, such as an increase in the open probability, are not observed, but aldosterone increases the channel number at the apical membrane [62]. Further, in rats, methylation of the channel has no effect on the channel function, which is proposed as a mediator of aldosterone to stimulate preexisting apical amiloride-sensitive Na⁺ channels [62]. These different responses might be due to the different sequences of the Na⁺ channels; Xenopus ENaC (xENaC) and rENaC [37, 38, 43].

Aldosterone has also been reported to stimulate Na⁺-K⁺-ATPase, in addition to its stimulatory action on the amiloride-sensitive Na⁺ channel. On the other hand, if as is generally thought, the rate-limiting step of transepithelial Na⁺ transport is the entry of Na⁺ from the luminal into the intracellular space irrespective of aldosterone treatment, and not the extrusion of Na⁺ by the Na⁺-K⁺-ATPase (pump), the stimulatory action of aldosterone on the amiloride-sensitive Na⁺ channel is essential for the stimulation of transepithelial Na⁺ transport. This idea suggests that the action of aldosterone on Na⁺-K⁺-ATPase might not be essential for the stimulatory action of aldosterone on transepithelial Na⁺ absorption. If so, only an increase in apical Na⁺ conductance is an essential requirement for the aldosterone-induced stimulation of Na⁺ reabsorption in the epithelium, and aldosterone can stimulate transepithelial Na⁺ transport without any stimulatory effect on Na⁺-K⁺-ATPase. However, it has recently been reported that the amount of Na⁺ entering ADH-stimulated cells exceeds the capacity of Na⁺ extrusion by Na⁺-K⁺-ATPase when aldosterone has no stimulatory effect on Na⁺-K⁺-ATPase [63]. The result indicates that the stimulatory action of aldosterone is limited in transepithelial Na⁺ transport [63]. This report clearly concludes that the stimulatory effect of aldosterone on Na⁺-K⁺-ATPase is essential to the stimulatory action of aldosterone on transepithelial Na⁺ absorption. A similar observation [64] was also reported in other types of Na⁺-absorbing epithelial cells such as epithelial cells of the rat fetal distal lung.

B) ADH action on Na⁺ channels

Vasopressin is one of the most important ADHs. Secretion of vasopressin from the posterior pituitary is stimulated by an increase in the osmotic pressure of the plasma or hypovolemia. Vasopressin acts at many sites inside and outside of the kidney through at least two different receptors, V₁ and V₂: 1) the V₁ receptor mainly locates in smooth muscle and liver, involves the phosphatidylinositol pathway, resulting in an increase in cytosolic Ca²⁺ concentration; and 2) the V₂
receptor mainly locates in renal collecting-ducts and initiates adenylyl cyclase, resulting in an increase in cytosolic cAMP concentration. Vasopressin acts as an ADH through the V$_2$ receptor and increases the water permeability of the apical membrane of the distal nephron to increase its water absorption. This leads to a decrease in plasma osmolality, of which an increase stimulates ADH secretion.

However, ADH has also recently been reported to stimulate amiloride-sensitive Na$^+$ absorption in distal-nephron epithelium [65–67]. Recent reports using the patch-clamp technique indicate that ADH or its second messenger, cAMP, increases the number of amiloride-sensitive Na$^+$ channels in the apical membrane of epithelial cells of the distal nephron treated with aldosterone [68, 69], suggesting that this increase in the number of channels leads to increased amiloride-sensitive Na$^+$ absorption (Fig. 4). Further, it has been more recently reported that the ADH-induced increase in amiloride-sensitive Na$^+$ absorption in aldosterone-treated distal-nephron epithelium is abolished by brefeldin A, which blocks intracellular protein translocation; suggesting that ADH increases Na$^+$ absorption by stimulating the translocation of the amiloride-sensitive Na$^+$ channel from intracellular pools into the apical membrane [70].

The ADH action on amiloride-sensitive Na$^+$ absorption in aldosterone-treated distal-nephron epithelium is mimicked by cAMP [68], which is thought to be an intracellular second messenger of ADH. These observations indicate that cAMP stimulates the intracellular translocation of the amiloride-sensitive Na$^+$ channels. However, it is still unclear whether the translocation of the channel is mediated through cAMP-dependent protein kinase (PKA)-induced phosphorylation. One report [71] indicated that the stimulatory action of the Cl$^-$ channel from intracellular pools into the apical membrane is mediated through a cAMP-dependent pathway, but not through PKA-induced phosphorylation. Furthermore the ADH- or cAMP-induced increase in the number of amiloride-sensitive Na$^+$ channels is not also blocked by H89, a specific inhibitor of PKA. Accordingly, there is a possibility that cAMP stimulates the translocation of the amiloride-sensitive Na$^+$ channel into the apical membrane through a PKA-independent pathway. On the other hand, in aldosterone-untreated distal-nephron-epithelial cells, the stimulatory action of ADH could not be mimicked by cAMP under some conditions.

These observations clearly indicate that aldosterone alters the intracellular signaling pathways of ADH and the mechanisms of the channel responding to ADH; namely, in aldosterone-untreated epithelial cells, either 1) cAMP does not act as the intracellular second messenger of ADH, or 2) additional intracellular second messengers are required when ADH stimulates amiloride-sensitive Na$^+$ absorption. In the latter case, aldosterone provides a second messenger of ADH in addition to cAMP.

C) Regulation of Na$^+$ channels by protein kinases

The phosphorylation of proteins plays various important roles in the regulation of cell function. cAMP, thought to be an intracellular second messenger of ADH in the renal distal nephron, regulates the amiloride-sensitive Na$^+$ transport in the epithelium, which in turn, suggests that the amiloride-sensitive Na$^+$ channel is regulated by a PKA-mediated pathway. As described above, the translocation of the amiloride-sensitive Na$^+$ channel is stimulated by cAMP, but might not be mediated through a PKA-mediated pathway. However, it is not yet argued whether the activity (open probability) of the amiloride-sensitive Na$^+$ channel is regulated by phosphorylation. When ADH or cAMP is applied, the 4-pS amiloride-sensitive highly Na$^+$-selective channel (P$_{Na}$/P$_K$>15) does not show any significant changes in open probability, but does show an increase in density at the apical membrane in response to the cAMP in A6 cells [68]. This means that although cAMP increases the
amiloride-sensitive $\text{Na}^+$ conductance of the apical membrane, the open probability of the individual channels is not modified by cAMP. This idea is supported by other studies using short-circuit measurement with current fluctuation analysis (so-called noise analysis) (e.g., Els and Helman [72]). Even if the open probability of the channel is not modified by cAMP, it has not been concluded that cAMP does not promote phosphorylation of the channel. On the other hand, a report [73] indicates that ADH (and also cAMP) increases the open probability of the 9-pS amiloride-sensitive $\text{Na}^+$ channel with low selectivity for $\text{Na}^+$ ($P_{\text{Na}}/P_K=4$). The ADH-induced increase in the open probability of the 9-pS channel is dependent on the phosphorylation of actin filaments. Therefore, it is still unclear whether the 9-pS channel itself is phosphorylated.

A reconstitution study [74] of amiloride-sensitive $\text{Na}^+$ channel obtained from bovine kidney into the lipid bilayer indicates that PKA-mediated phosphorylation of the channel increases the open probability of the channel. This finding suggests that the phosphorylation of the channel protein has a stimulatory role in open probability. This apparent discrepancy could be due to the difference in composition of channel proteins, although both 4- and 9-pS channels are sensitive to amiloride. Indeed, a reconstitution study [75] using cloned rENaC into lipid bilayer shows no increases in open probability by cAMP or cAMP-dependent protein kinase, supporting the idea that no putative phosphorylation sites exist in the amino acid sequences of rENaC [75]. xENaC, however, is expected to have a phosphorylation site of tyrosine from its amino acid sequence [43]. Indeed, it is reported that the inhibitors of protein tyrosine kinase decrease the open probability or the number of the 4-pS amiloride-sensitive $\text{Na}^+$ channels, which are believed to be xENaC [76]. These observations indicate that the regulation of the open probability of individual amiloride-sensitive $\text{Na}^+$ channels depends on the species in which the various types of amiloride-sensitive $\text{Na}^+$ channels function as an entry pathway of $\text{Na}^+$ across the apical membrane.

Another protein kinase, protein kinase C (PKC), is also reported to regulate the amiloride-sensitive 4-pS $\text{Na}^+$ channel. Palmer and Frindt [18] have reported that ionomycin diminishes the activity of the amiloride-sensitive $\text{Na}^+$ channel, but in inside-out patches, the cytosolic $\text{Ca}^{2+}$ has no effect on channel activity. This observation indicates that the cytosolic $\text{Ca}^{2+}$ diminishes the $\text{Na}^+$ channel, not directly but in a cytosolic factor-dependent manner. A later study [77] indicated that PKC is involved in $\text{Na}^+$ channel regulation. Further, another study in the same laboratory [78] reported that ADH diminishes the $\text{Na}^+$ channel activity via the $V_1$ receptor at the luminal side by increasing the cytosolic $\text{Ca}^{2+}$ concentration, which activates protein kinase C. These reports indicate that PKC could inhibit the amiloride-sensitive 4-pS $\text{Na}^+$ channel.

Another type of amiloride-sensitive channel, a nonselective cation channel, is inhibited by cGMP through two different pathways: through its direct action and through cGMP-activated protein kinase–induced phosphorylation [79]. On the other hand, cGMP activates a nonelective cation channel in A6 cells [80].

So, in general, $\text{Na}^+$ transport by the 4-pS amiloride-sensitive highly $\text{Na}^+$-selective channel is thought to be stimulated by cAMP-dependent protein kinase and inhibited by protein kinase C, although the regulatory mechanisms are still poorly understood.

D) Osmolality action on $\text{Na}^+$ channels

The distal nephron is exposed to the plasma and luminal fluid with various osmolarities. This variability of fluid osmolality has been reported to regulate amiloride-sensitive $\text{Na}^+$ absorption: low osmolality stimulates amiloride-sensitive $\text{Na}^+$ absorption, because of an increase in the number of the amiloride-sensitive $\text{Na}^+$ channels at the apical membrane, by applying fluctuation analysis to the short-circuit currents [81]. The action of the low osmolality on channel density is due to the change in the osmolality of the basolateral fluid, but not to the change in luminal osmolality [81, 82]. This increase in the number of channels is also observed using the single-channel–current recording technique [70]. The low-osmolality–induced increase in the number of channels is caused by the translocation of the channels from the basolateral pool into the apical membrane [70]. However, the mechanism of the low-osmolality action on the translocation of the channel is still unclear.

Recently, membrane stretch has been reported to activate protein tyrosine kinase (PTK) activity [83–85]. Hyposmolality also induces an increase in PTK activity [82, 84]. This hyposmolality-induced increase in PTK activity could be due to the membrane stretch caused by cell swelling. On the other hand, some studies report that the amiloride-sensitive short-circuit current is blocked by PTK inhibitors [76, 82]. These reports suggest that low osmolality could affect amiloride-sensitive $\text{Na}^+$ absorption by the activation of PTK. However, it is still unclear whether the blocking action is due to the inhibition of the translocation of the channel, the inhibition of channel activity or both. Recently, a study by measuring short-circuit and
single-channel currents [70] indicates that the hyposmolality-induced increases in the amiloride-sensitive Na⁺ absorption and a number of the amiloride-sensitive Na⁺ channels are blocked by brefeldin A, which blocks intracellular protein translocation. These findings suggest that hyposmolality stimulates Na⁺ absorption by activation of the translocation of amiloride-sensitive Na⁺ channels (Fig. 5). Further, another study [82] indicates that the hyposmolality-induced increases in Na⁺ absorption and channel number are also blocked by PTK inhibitors.

Taken together, these reports indicate that low osmolality stimulates translocation of the amiloride-sensitive Na⁺ channel and this translocation of the channel is mediated by PTK-dependent pathways. However, it is still unclear which step in the Na⁺-channel translocation is mediated through PTK. The capacitance measurement indicates that the PTK-dependent pathways are involved in both membrane-fusion and endocytosis steps [82], suggesting that at least PTK, of which activity is increased by hyposmolality [82], mediates the fusion process of the vesicle, including the amiloride-sensitive Na⁺ channel, to the apical membrane. Furthermore, PTK may play a role in some of the other steps of the translocation of amiloride-sensitive Na⁺ channel from the endoplasmic reticulum to the subapical-membrane area.

The number of amiloride-sensitive Na⁺ channels is increased by hyposmolality as a result of the stimulation of the translocation of the amiloride-sensitive Na⁺ channels into the apical membrane. The number of amiloride-sensitive Na⁺ channels staying at the apical membrane is dependent on the rates of the channels inserting into and leaving from the apical membrane. One of interesting questions is which rate is affected by osmolality: hyposmolality induces an increase in the rate of channel insertion into the apical membrane, induces decrease in the leaving rate of the channels from the apical membrane or both. Pretreatment of cells with brefeldin A almost completely blocks the stimulatory action of hyposmolality on amiloride-sensitive Na⁺ absorption, whereas brefeldin A has little effect on amiloride-sensitive Na⁺ absorption that has been stimulated by hyposmolality [70]. These observations suggest that the rate of channel insertion is affected by osmolality, although the leaving rate of the amiloride-sensitive Na⁺ channel from the apical membrane might also be influenced by osmolality. Further, the rate of the hyposmolality-induced increase in amiloride-sensitive Na⁺ absorption is much slower than that of the hyperosmolality-induced decrease in the current. This suggests that the insertion rate of the channel, to an active state from an inactive state under the hypotsmotic condition, is much slower than the leaving rate, to an inactive state from an active state under the hyperosmotic condition [82].
Fig. 7. A regulatory model of the amiloride-sensitive Na⁺ channel by the osmolality of bathing solution. (A) The channel before translocation into the apical membrane. (B) The channel at the apical membrane with high activity. (C) The channel at the apical membrane with low or no activity. (D) The channel translocated from the apical membrane into cytosolic space. Low osmolality stimulates the translocation of the channel from cytosolic pools (A) into the apical membrane with high activity (B), and decreases the translocation rate of the channel with low or no activity (C) to the cytosolic space (D). However, the most drastic difference in solutions with hyposmolality and hyperosmolality is the distribution of the channels in the states “B” and “C.” Namely, under the hyposmolar condition, the channel in state “B” is dominant, whereas under the hyperosmolar condition, the channel in state “C” is dominant.

Figure 6 shows the recovery rate of amiloride-sensitive Na⁺ absorption from exposure to the hyperosmotic condition for various time periods after reaching a stable condition under the hyposmotic condition. The recovery level decreased as the length of time the cells were exposed to the hyperosmotic solution increased. This means that hyperosmolality increases the leaving rate of the channel from the apical membrane.

In conclusion, low osmolality increases the insertion rate of the channel into the apical membrane and decreases the leaving rate of the channel from the apical membrane. Figure 7 shows a model of amiloride-sensitive Na⁺-channel trafficking that indicates that there are at least four states in which the channel stays: A) a state before the channel is inserted into the apical membrane; B) a state of the channel with high activity at the apical membrane; C) a state of the channel with no or low activity at the apical membrane or at the subapical-membrane areas; and D) a state of the channel degrading into lysosome (Niisato and Marunaka, unpublished observations). In A6 cells, the 4-pS Na⁺ channel shows a value of open probability with one Gaussian distribution as an active channel, except under some special conditions [30, 68], whereas the open probability of the 4-pS Na⁺ channel in rat distal nephron shows two different distributions (relatively low and high open probabilities) as active channels [86]. These differences would be due to the open probability of the channel staying in “C” (Fig. 7); i.e., the channel staying in “C” has low (rat distal nephron) or no (A6 cells) activity.

4. Regulation of Cl⁻ Transport

When Na⁺ absorption occurs, Cl⁻ is also absorbed as a counter ion. Two possible pathways of Cl⁻ movement across the epithelium are considered: the transcellular and paracellular pathways (see Fig. 8). The Na⁺/K⁺/2Cl⁻ cotransporter and the Cl⁻/HCO₃⁻ exchanger located at the basolateral membrane actively accumulate Cl⁻ into the intracellular space against the electrochemical gradient of Cl⁻: Cl⁻ channels located at the basolateral membrane participate in the pathway for Cl⁻ extrusion across the membrane. This means that the electrochemical potential of intracellular Cl⁻ is larger than that of Cl⁻ in the basolateral solution [67, 87–89]. The epithelium has a lumen-negative potential (Fig. 8): the apical membrane potential is less than the basolateral membrane potential. Therefore, the direction of Cl⁻ movement across the apical membrane is variable depending on the apical membrane
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potential and the Cl⁻ concentration of the luminal solution. The apical membrane potential depends upon Na⁺ and K⁺ transport across the apical membrane.

On the other hand, Cl⁻ could be absorbed through the paracellular pathway. If a large amount of Na⁺ is absorbed, Cl⁻ is possibly absorbed by two pathways: the transcellular and paracellular pathways. When K⁺ secretion is larger than Na⁺ absorption, Cl⁻ is secreted through the transcellular pathway and some parts of the secreted Cl⁻ could be reabsorbed through the paracellular pathway. These movements of Cl⁻ across the apical membrane, after Na⁺ or K⁺ movement across the membrane, contribute to keeping the apical membrane potential constant, although the Na⁺ and K⁺ movements in opposite directions compensate for each other. This constant potential of the apical membrane is essential for stable Na⁺ absorption and K⁺ secretion.

In general, it was believed that no Cl⁻ channels (or conductances) are located at the apical membrane of the principal cell of the distal nephron, based on evidence from reports measuring the intracellular potential by microelectrodes and short-circuit currents with ion-replacement experiments [90–92]. However, recent studies using the single-channel-current recording technique report apical Cl⁻ channels in distal-nephron epithelial cells [93–97]. The lack of evidence for the presence of Cl⁻ channels at the apical membrane from microelectrodes and short-circuit current measurements may be due to the resolution of the methods. However, no detectable apical Cl⁻ conductance by these methods suggests that even though the single-channel-current recording technique indicates evidence of apical Cl⁻ channels, the conductance is much smaller than the cation conductance.

In A6 cells, at least two types of Cl⁻ channels are reported; their single-channel conductances are 3 and 8 pS [71, 98–103]. These channels respond to ADH: the open probability of the 3-pS channel is increased by ADH or cAMP through PKA-dependent phosphorylation of the channel or channel-associated protein, whereas the number of the 8-pS channels is elevated by ADH or cAMP [71, 101, 104] through a brefeldin A–dependent pathway, but not through a PKA-dependent pathway. These observations indicate that ADH increases the Cl⁻ conductance of the apical membrane by at least two different mechanisms: 1) stimulation of the phosphorylation of the channel or channel-associated protein, resulting in an increase in the open probability of the 3-pS channel; and 2) stimulation of the translocation of the 8-pS channel into the apical membrane from intracellular pools not through PKA-dependent phosphorylation.

**Fig. 9.** The relationship between cell volume (V) and cytosolic Cl⁻ concentration. When KCl loss occurs isosmotically, the cytosolic Cl⁻ concentration decreases. The major membrane-permeable anion is Cl⁻, and a large number of membrane-impermeable anions, such as proteins, are located in the cytosolic space.

In addition to its role as a counter ion in Na⁺ absorption, Cl⁻ regulates the amiloride-sensitive Na⁺ channel in various tissues, including distal-nephron epithelium. In non-renal epithelial cells, cytosolic Cl⁻ has been reported to regulate the amiloride-sensitive Na⁺ channel [105]: lowering cytosolic Cl⁻ activates the amiloride-sensitive Na⁺ channel. A similar phenomenon is also reported in fetal-lung epithelium [28, 106]. Cytosolic Cl⁻ concentration is related to cell volume [28, 107, 108]: when cell shrinkage occurs isosmotically, the cytosolic Cl⁻ concentration decreases because the major membrane-permeable anion is Cl⁻. This moves as a counter ion for cation movement, causing a net salt movement followed by water movement, which causes the cell volume to change (see Fig. 9). In renal epithelium, cytosolic Cl⁻ regulates the action of ADH on amiloride-sensitive Na⁺ conductance [109–111], indicating that Cl⁻ plays important roles in amiloride-sensitive Na⁺ transport, not only as a counter ion for Na⁺ movement but also as an active regulator.
Apical

Basolateral

Nucleus

Endoplasmic reticulum

Fig. 10. A summary of the regulation of the amiloride-sensitive Na\(^+\) channel and Na\(^+\)-K\(^+\)-ATPase by aldosterone, ADH and osmolality. Aldosterone stimulates the new protein (Na\(^+\) channel and Na\(^+\)-K\(^+\)-ATPase) synthesis (a, b) as late-phase action. Furthermore, as early-phase action, aldosterone modulates the Na\(^+\) channel activity (d). ADH has a stimulatory action through an increase in the number of active Na\(^+\) channels (c, c', c'', and d; depending upon cell conditions). Low osmolality mainly stimulates the transition of the apical Na\(^+\) channel to the channel with high activity from that with no or low activity (d), stimulates the translocation process of the Na\(^+\) channel (c, c', c'') and diminishes the transition process of the Na\(^+\) channel from the apical membrane to lysosome (e, e'). ER, endoplasmic reticulum.

5. Conclusion

Transcellular NaCl transport in distal-nephron epithelium is controlled by aldosterone, ADH, and plasma osmolality (see Fig. 10). NaCl transport is actively regulated by apical Na\(^+\) conductance (amiloride-sensitive Na\(^+\) channel) and Na\(^+\)-K\(^+\)-ATPase at the basolateral membrane, which play roles in the entry and extrusion steps of Na\(^+\) as it moves across the apical and basolateral membranes, respectively, during transport. Aldosterone may alter the open probability of the amiloride-sensitive Na\(^+\) channel, in addition to new channel and Na\(^+\)-K\(^+\)-ATPase protein synthesis, which stimulates transepithelial Na\(^+\) transport. ADH increases the number of functional amiloride-sensitive Na\(^+\) channels at the apical membrane, causing an increase in Na\(^+\) transport. Hyposmolality, itself, increases the density of amiloride-sensitive Na\(^+\) channels at the apical membrane, mainly by decreasing the degradation of the channel with high activity from the apical membrane to the cytosolic space. Cytosolic Cl\(^-\) plays important roles in amiloride-sensitive Na\(^+\) transport, not only as a counter ion for Na\(^+\) movement but also as an active regulator of hormonal action on Na\(^+\) transport.

This work was supported by grants from the Kidney Foundation of Canada, the Medical Research Council of Canada (Group Grant Project No. 9 and MA134949), and the Ontario Thoracic Society (Block Term Grant) to Dr. Y. Marunaka. Dr. Y. Marunaka is a scholar at the Medical Research Council of Canada. An earlier version of this paper was prepared with the assistance of Editorial Services, The Hospital for Sick Children, Toronto, Ontario, Canada.

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Regulation of Renal NaCl Transport


