Exploration of the Basolateral Chloride Channels in the Renal Tubule using the Patch-Clamp Technique

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Key Words

CIC-K channel · Cystic fibrosis transmembrane conductance regulator · Chloride · Renal tubule · Basolateral membrane · Patch-clamp

Abstract

Chloride channels located on the basolateral membrane are known to be involved in chloride absorption in several parts of the renal tubule, and particularly in the thick ascending limb and distal convoluted tubule. The data available suggest that the CIC-K channels play the major role in this process. We provide here a description of the electrophysiological properties of these channels, still very incomplete at this stage, and we attempt to compare CIC-Ks to three chloride channels that we have identified in the basolateral membrane of microdissected fragments of the mouse renal tubule using the patch-clamp technique. Based on anion selectivity and dependence on external pH and calcium shown by the CIC-Ks, we propose candidate CIC-K1 and CIC-K2 in native tissue. We also discuss the possibility that chloride channels that do not belong to the CIC family may also be involved in the absorption of chloride across the cortical thick ascending limb.

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Current knowledge suggests that the CIC-K1 and CIC-K2 chloride channels (and their human orthologs CIC-Ka and CIC-Kb) play a major role in the basolateral step of chloride absorption in the distal nephron, i.e. in the thick ascending limb (TAL) and distal convoluted tubule (DCT). A first piece of evidence is that mutations of the gene encoding CIC-Kb are responsible for type-III Bartter syndrome (targeting TAL) and mixed Bartter-Gitelman syndrome (targeting TAL and DCT), whereas mutations of the regulatory Barttin subunit are responsible for type-IV Bartter syndrome [1–3]. Secondly, using antibodies that do not distinguish between the two isoforms, it has been shown that rat CIC-K channels are located on the basolateral membrane of the distal nephron [4, 5], where they could be involved in Cl^- absorption. In addition, CIC-K1 KO mice have been used to confirm the presence of CIC-K2 channels in the basolateral membranes of the TAL and DCT. CIC-K2 channels are also found in the intercalated cells of the collecting duct [6].

Several questions about the CIC-Ks remain to be answered. We still have not identified the corresponding channels in native tissue and we still do not know how they are regulated with regard to their supposed physiologic function or why two closely related entities (90% of sequence identity [7]) should occur in the same organ. Most of the data available about Cl^- channels in the native renal tubule derive from experiments in the rabbit or mouse. In what follows, we review some of the properties of CIC and CIC-K channels, compare cloned CIC-K channels to en-

ogenous chloride channels (focusing on the mouse renal tubule) and present some data concerning regulation.

Electrophysiological Properties of the CIC Chloride Channels

The CIC-Ks belong to the first subfamily of the CIC, voltage-dependent chloride channel family, the archetype of which is the chloride channel of the electric organ of the torpedo CIC-0 [7]. All the channels of this subfamily, which also includes CIC-1 and CIC-2, are expressed at the plasma membrane, unlike other CICs, which occur essentially on the membranes of organelles. Electrophysiological studies from the laboratories of Miller and White [8] and Ludewig et al. [9] have shown that CIC-0 is composed of two independent pores, known as protopores, and is endowed with double voltage dependence. A fast gate opens each protopore independently upon depolarization, whereas very slow, hyperpolarization-dependent activation commands a common gate. The double-pore structure is quite certainly a general characteristic of the CIC channels, since recent crystallography data have demonstrated such an arrangement for two bacterial CIC channels [10, 11]. Likewise, voltage-dependence is a common property of the CICs, but appears to vary considerably from one channel to another: for instance, CIC-2 is mainly gated by hyperpolarization [7]. Two additional properties of the CICs are worth mentioning in this context [7]: dependence on the concentration of chloride ions and on pH. It has been clearly demonstrated for both CIC-0 (fast gate) and CIC-1 that depolarization-dependent activation is shifted towards more positive voltages when the external chloride concentration is lowered [12–14] or the internal pH increased [14, 15]. In addition, lowering the intracellular chloride concentration has a complex effect on the kinetics of CIC-0: The open probability (P_o) of the fast gate is reduced at negative voltage [13] and the slow (common) gate is inhibited [16]. It is noteworthy that elevating the intracellular chloride concentration stimulates the activity of native CIC-2-like channels [17, 18]. The CIC-0, CIC-1 and CIC-2 channels all display greater activity when the external pH is lowered [7, 14, 15, 19].

Despite their having been cloned a decade ago [20–22], little is known about the functional properties of the CIC-Ks because, except for rat CIC-K1, they are not expressed functionally in the oocyte in the absence of the regulatory Barttin subunit, which was not cloned until 2001 [4]. Recent co-expression studies have demonstrated two fundamental properties. On the one hand anion permeabilities follow the sequence $\text{Cl}^- > \text{Br}^- > \text{NO}_3^- >$

I^- for CIC-K1, and $\text{Cl}^- > \text{Br}^- = \text{NO}_3^- > \text{I}^-$ for CIC-K2 [4, 23], matching the permeability sequence of other members of the first CIC subfamily. On the other hand the currents are enhanced by a basic external pH, which is the opposite of that observed for the other channels of the first subfamily, and by an elevated concentration of external calcium [4, 23]. In addition, it is likely that the CIC-Ks are endowed with other common properties of the CICs, such as the double-pore architecture, sensitivity to chloride and voltage dependence. However, voltage dependence does not seem to be prominent over the range tested [4, 23]. Finally, the unit conductance of these channels has not yet been determined.

A CIC-K2 Candidate in the Native Mouse Renal Tubule

The early DCT (DCT1) absorbs NaCl via a Na^+/Cl^- cotransporter located at the apical membrane and Na^+/K^+ -ATPase on the basolateral side. Cl^- transport across the basolateral membrane can be expected to proceed via conductive pathways, but there were no data on native DCT channels until our study. Using the patch-clamp on microdissected fragments of mouse DCT1, we detected a chloride channel of ~9 pS that exhibits several of the properties of CICs and CIC-Ks [24]. Firstly, the channel often produced a half conductance level that was reminiscent of a double-pore arrangement. However, this observation is not entirely conclusive, because transitions from half-opening to complete closure have rarely been observed. This contrasts with the behavior of CIC-0, where the channel clearly fluctuates between a non-conducting level and two equally spaced conducting levels [8, 9]. Half-amplitude openings could, therefore, just as well correspond to a conventional sub-state of the channel. Secondly, and more conclusively, like the CIC-Ks, the channel has been shown to depend on external calcium levels and pH: NP_o increased threefold when the calcium in the pipette (extracellular medium) was increased from zero to 5 mM, and fifteen-fold when the pH was increased from 6.4 to 8.0. Thirdly, setting aside I^- , the permeability sequence $\text{Cl}^- \sim \text{I}^- > \text{Br}^- \sim \text{NO}_3^- > \text{F}^-$ observed for this channel matches that for CIC-K2. Finally, single-cell RT-PCR experiments have shown that only CLC-K2 mRNA was present in the DCT1, where the investigation was done [25]. Taking all these elements into account, it is reasonable to propose that this ~9-pS chloride channel corresponds to CIC-K2. Sauve et al. [26] have reported a fairly similar Cl^- channel (conductance 14 pS) in membrane vesicles from the rabbit distal nephron incorporated into lipid bi-layers. As in our

study, the channel showed greater activity in the presence of higher extracellular pH or calcium levels.

We have investigated several regulatory properties which have not yet been investigated in cloned ClC-Ks. Channel activity is gradually inhibited in the presence of PMA, an activator of PKC, but does not seem to be modulated via the adenylyl cyclase pathway: neither forskolin in cell-attached patches nor the catalytic subunit of PKA in excised patches alter channel activity [24]. The channel is insensitive to intracellular calcium levels but, in contrast, is highly sensitive to the intracellular pH. Taking the activity at pH 7.4 as baseline, pH 6.8 decreases channel activity by 70%, whereas pH 8.0 increases it by 350%. pH could therefore be a major regulator of the ClC-K2 channel, since the activity of the channel is also lower at an acid extracellular pH.

We have detected a channel with the same unit conductance and anion selectivity in the connecting tubule (unpublished results), and also in the mouse DCT2 [25] and cortical thick ascending loop (CTAL; unpublished results), both of which are segments in which a second 9-pS Cl⁻ channel with very different anion selectivity is also present [27] (see 'A Pseudo-CFTR Chloride Channel' below).

A Possible ClC-K1 Candidate

We detected a chloride channel with a unit conductance of 45 pS in the mouse CTAL [28], and which has a permeability sequence (Cl⁻ > Br⁻ > NO₃⁻) compatible with that of ClC-K1. We did not investigate its dependence upon external pH or calcium level, which had not yet been identified as a characteristic feature of the ClC-Ks at the time of our experiments. Further information is needed before we can decide whether this corresponds to ClC-K1. The channel is activated by depolarization (like ClC-1 and the fast gate of ClC-0), but is insensitive to intracellular calcium levels and pH. Intriguingly, this channel seems to be indirectly stimulated via the adenylyl cyclase pathway: the chance of recording the channel increased eightfold (from 8 to 67%) when the tubules were pre-incubated with forskolin for about 15 min. However, application of the catalytic subunit of the PKA had no effect on inside-out patches, suggesting that direct phosphorylation is not responsible for its activation [27].

Chloride channels with similar conductance have been recorded in the rabbit kidney, but their anion selectivity was not investigated. Using the patch-clamp technique, Sansom et al. [29] reported a 49-pS Cl⁻ channel in the basolateral membrane of the principal cells of the rabbit cortical collecting duct with two open current levels. Win-

ters et al. [30] recorded Cl⁻ channels on vesicles from the rabbit renal outer medulla (MTAL channel) and basolaterally enriched vesicles from cultured mouse CTAL cells (CTAL channel) [31], incorporated into planar lipid bilayers. Both channels had a conductance of 80–90 pS in symmetrical 300 mM KCl [31, 32] (about 50 pS in 150 mM KCl [30]), and were activated by membrane depolarization, like ClC-0 and ClC-1. The activity of the MTAL channel, but not of the CTAL channel, decreased when the intracellular chloride concentration was lowered, as for ClC-0 or for ClC-2-like channels, and increased in the presence of PKA (at low intracellular concentrations of chloride). The former regulatory process is particularly relevant in the context of Cl⁻-transporting epithelia, because chloride could be the cross-talk agent that coordinates the activities of anion transporters on one membrane and of anion channels on the other.

A Pseudo-CFTR Chloride Channel

As already mentioned, there is a 9-pS chloride channel in the basolateral membrane of the mouse CTAL that has a permeability sequence I⁻ > NO₃⁻ > Br⁻ > Cl⁻ [27, 33]. This anion selectivity contrasts sharply with the permeability sequence of the ClC-Ks, and more generally with that of the other members of the first ClC subfamily. A channel with similar conductance and selectivity has also been found in the mouse DCT2 [25]. The channel is activated by intracellular ATP, pyrophosphate (in the presence of ATP; fig. 1) and the catalytic subunit of PKA. It is insensitive to intracellular calcium, but highly sensitive to intracellular pH [33]. At the time of these experiments, we were puzzled by similarities between this channel and the cystic fibrosis transmembrane conductance regulator (CFTR), which included activation by ATP, pyrophosphate and PKA, and also anion selectivity and pharmacological profile. We investigated this question using CFTR KO mice, and were able to demonstrate that these two channels are distinct entities, despite their functional similarities [34]. Thus, the molecular identity of this 'pseudo-CFTR' channel remains to be determined. Segal et al. [35] have described a Cl⁻ channel that closely resembles our pseudo-CFTR channel in terms of the conductance and regulation in the basolateral membrane of the rabbit proximal tubule. The pseudo-CFTR channel, being located at the basolateral membrane, is theoretically in the right position to contribute to the process of chloride absorption. However, this is difficult to ascertain directly in the absence of specific blockers, which would allow discrimination between this channel and the two other chloride channels also present in the CTAL basolateral membrane.

Fig. 1. Representative recording of pseudo-CFTR chloride channels on the basolateral membrane of mouse CTAL. These channels have a unit conductance of 9 pS, and are activated by intracellular ATP and pyrophosphate (PPi, in the presence of ATP). When ATP was removed from the solution, channel activity decreased progressively over time. In contrast, the ClC-K2 candidate (which has a similar conductance) was not sensitive to these factors. Channel activity is shown on the right-hand side of the traces as the product of channel number and open probability.

Table 1. A summary of the data on basolateral Cl⁻ channels derived from experiments on microdissected mouse renal tubules

g (pS)	Proposed identity	Selectivity	[Ca ²⁺] _e /basic pH _e	Basic pH _i	Tubule
45	ClC-K1	Cl ⁻ > Br ⁻ > NO ₃ ⁻	?	-	CTAL [28]
9	ClC-K2	Cl ⁻ > I ⁻ > Br ⁻ = NO ₃ ⁻	+	+	DCT1 [24], DCT2 [25]
9	Non-ClC	I ⁻ > NO ₃ ⁻ > Br ⁻ > Cl ⁻	?	+	CTAL [27, 33], DCT2 [25]

Activation by the designated agents is indicated by +, and a lack of effect by -. Not every regulating process has been demonstrated to occur in each part of the tubule. The proposed molecular identity is speculative at this stage.

Conclusions

Table 1 summarizes the properties of the three basolateral Cl⁻ channels that we have described in the mouse renal tubule. It also includes speculation about their molecular identity. We should point out that the two ClC-K candidates have several contrasting properties, including their conductance (9 vs. 45 pS), sensitivity to intracellular pH and dependence on cyclic AMP. This is rather surprising, since there is 90% sequence homology between the

two ClC-Ks. It should also be emphasized that cyclic AMP has diverse effects on the three channels: the adenylyl-cyclase pathway has no effect on the ClC-K2 candidate, it appears to increase the number of active candidate ClC-K1 channels, and stimulates the activity of the pseudo-CFTR channel via PKA. Lastly, functional diversity parallels heterogeneous distribution: we recorded all three types of channel in the CTAL, both the ClC-K2 candidate and non-ClC type in the late DCT, and the candidate ClC-K2 alone in the early DCT. This suggests that each Cl⁻ channel may make a distinct contribution to the ion-transport process, and highlights the fact that further investigation is needed to elucidate their respective physiological functions.

The CFTR-like channel is most probably not a ClC channel, and its molecular identity has yet to be determined. Additional non-ClC channels could also be involved in Cl⁻ absorption. Cyclic AMP-dependent, calcium-dependent and volume-dependent Cl⁻ currents, have been reported in cultured renal cells using the whole-cell recording technique [36–46]. Cyclic AMP-dependent Cl⁻ currents are attributed to apical CFTR, but the possible presence of volume-dependent and calcium-dependent Cl⁻ channels at the basolateral membrane remains to be investigated, especially in the native renal tubule.

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