The pharmacological profile of kidney CLC-K channels depends on the expression system: comparison of CLC-K chloride currents in a mammalian cell line and Xenopus oocytes

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Human CLC-Ka and CLC-Kb chloride channels are located in the Henle loop, distal convoluted tubule and cortical collecting ducts of the nephron where they govern chloride absorption and urine concentration (Kramer et al., 2008). Both channels require battin as accessory subunit for full expression (Estevez et al., 2001). In humans, loss-of-function mutations of the genes encoding CLC-Kb and barttin are responsible for rare human diseases called type-III and type-IV Bartter syndrome, characterized by impairment of urinary concentration ability (Birkenhager et al., 2001). Furthermore, CLCNKA and CLCNKB polymorphisms have been associated with salt sensitive hypertension (Sanada et al., 2011). Therefore, novel therapeutic approaches activating CLC-K channels are highly appealing for treating these diseases which lack specific drugs. At the same time, molecules capable of inhibiting CLC-K channels could be potential drugs for hypertension. We have recently identified the molecular determinants that distinguish CLC-K activators from blockers using the *X. laevis* oocyte expression system. Indeed, we recognized niflumic acid (NFA) as a powerful activator and phenyl-benzofuran carboxylic acid analogs as potent inhibitors (Liantonio et al., 2008). Here, by using these molecules as lead compounds and the patch-clamp technique, we explored the pharmacological profile of human CLC-K/barttin expressed in mammalian HEK-293 cells, a more physiological expression system allowing a more reliable translation of *in vitro* results to the clinical practice. Differently from channels expressed in oocytes, CLC-Ka and CLC-Kb channels expressed in HEK293 gave rise to currents with neither voltage-dependent gating nor kinetics. In addition, CLC-K channels expressed in HEK cells displayed a reduced pH and calcium sensitivity compared to oocytes. Similarly to what observed in oocytes, benzofuran derivatives resulted efficacious blockers of CLC-Ka currents. A rational drug design allowed us also to ameliorate drug potency, finally identifying a newly synthesized benzofuran derivative (SRA-36) with an inhibitory affinity of 4 microM. Therefore, independently from the biochemical environment surrounding CLC-K channels, the blocking binding site is active and exposed to high affinity compounds applied to the extracellular side of the membrane. Surprisingly, NFA failed to increase CLC-Ka and CLC-Kb currents, producing only an inhibitory effect in the 1-1000 microM range. This unexpected finding suggests that when CLC-K channels are expressed in HEK 293 cells the activating binding site is not easily accessible as in oocytes. In order to gain insight into this relevant difference between the two expression systems, we evaluated NFA effect on CLC-Ka mutants that drastically affect NFA potentiation in oocytes. Actually, neither the most potentiating mutant nor the most inhibiting one were successful to unmask the activating binding site. We are currently testing the hypothesis that different CLC-K/barttin interaction and/or diverse protein-lipid hydrophobic contact occur in HEK cells with respect to oocytes and might contribute to NFA modulation. These results emphasize the importance of the cellular expression environment in efforts to develop compounds with therapeutic potential as well as to correlate endogenous chloride currents with heterologously expressed channels. In addition, the finding that CLC-K channels show distinct pharmacological profiles in different cells suggests that cell-type-specific folding or associations with endogenous CLC-K or accessory subunits and/or post-translational processing play roles in determining the properties of functional chloride channels (MIUR- COFIN-2009; Telethon GGP10101).

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