PHARMACOLOGICAL TARGETING OF RENAL CLC-K CHLORIDE CHANNELS: LIGANDS STRUCTURAL REQUIREMENTS AND THERAPEUTIC POTENTIAL

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CLC-Ka and CLC-Kb Cl^- channels are selectively expressed in kidney and inner ear, where they are pivotal for salt re-absorption and water balance. Loss-of-function mutations of CLCNKB were associated with marked renal salt-wasting and hypotension in Bartter syndrome type III. The elucidation of the role of these channels in kidney has brought up a growing interest toward the identification of ligands allowing pharmacological interventions aimed to modulate their activity. Recently, by using 2-(p-chlorophenoxy)-propionic acid (CPP) derivatives and fenamates, we demonstrated that CLC-K channels have an activating and a blocking extracellular drug-binding site (1). Here, we tested a series of new rationally designed molecules on heterologously expressed CLC-K channels and chemically modelled the compounds. First, constraining a pure blocker of CLC-Ka (flufenamic acid,) in a co-planar configuration (compound GF-166), converted it into an activator at low concentrations, strongly indicating that the coplanarity of the two aromatic rings of the molecules is required for an activating compound. On the contrary, channel block requires a more flexible, non-coplanar configuration. Indeed, the condensation of the chlorophenoxy moiety of 3-phenyl-CPP in a benzofuran ring (compound GF-167) enhancing the planarity distortion, led to a 4-fold increased potency in blocking CLC-Ka with respect to 3-phenyl-CPP (K_D ~ 20μM). As in the case of 3-phenyl-CPP, the CLC-Ka N68D mutant was less sensitive to GF-167 block with respect to WT CLC-Ka suggesting a common inhibitory binding site. Chemical manoeuvres on the benzofuran structure (isosteric derivatives of the oxygen atom, substitutions on phenyl rings) produced marked changes in drug potency allowing the outline of a potential pharmacophore. Interestingly, benzofuran derivatives blocked CLC-Kb with an affinity in the 20-40 μM range representing the first CLC-Kb inhibitors identified so far. Because of their relatively high affinity toward CLC-K channels, the here described molecules are good lead compounds for the development of potential new diuretics drugs or treatment for Bartter syndrome type III (MIUR- COFIN-2005).

(1) Liantonio A., Picollo A., Babini E., Carbonara G., Fracchiolla G., Loiodice F., Tortorella V., Pusch M. and Conte Camerino D. (2006) Mol. Pharmacol. 69:165-173.