The effect of mutation E232Q and clofibric acid derivatives on gating in the human skeletal muscle chloride ion channel, ClC-1

J.M.E. Cederholm,[1] G.Y. Rychkov[1,2] and A.H. Bretag,[1] [1]Sansom Institute, University of South Australia, Adelaide, SA 5000, Australia and [2]Discipline of Physiology, University of Adelaide, SA 5005, Australia.

The chloride ion channel, ClC-1, is predominantly found in skeletal muscles. A decreased chloride conductance due to mutations in the gene, CLCN1, induces hyperexcitability in the muscle and is the cause of the disease myotonia congenita. The ClC-1 protein is a homodimer with each monomer containing 18 α-helices (labeled A-R), which are variable in length, tilted in relation to the vertical transmembrane axis (Dutzler *et al.,* 2002) and wrapped around a common centre. This orientation allows each monomer to form its own protopore with multiple chloride binding sites, its own selectivity filter and its own independent fast gate by bringing together appropriate amino acids at the ends of several different α-helices. Both fast gates are then simultaneously regulated by the slower, common gating, with unknown structural basis. When the X-ray crystal of a prokaryotic CLC became available, it was proposed that the side chain of E148 (equivalent to E232 in ClC-1) works as the fast gate (Dutzler *et al.,* 2002; Dutzler *et al.,* 2003), by swinging in and out of the pore and thereby controlling the chloride conductance. Studies on ClC-0 support this view (Dutzler *et al.,* 2003; Traverso *et al.,* 2003), but also show that fast gating might involve global conformational changes (Accardi & Pusch, 2003; Traverso *et al.,* 2003). Dominant negative effects on common gating suggest intersubunit allostery and it is possible that an interaction pathway between fast gates could constitute this "common gate". We sought to investigate whether common gating would be affected by mutation E232Q of the putative fast gate. Neither of the two gating processes has been studied before in this mutant of ClC-1. Using site-directed mutagenesis the glutamate at position 232 was substituted by a glutamine and the mutated protein was then expressed in HEK293 cells. In ClC-0, the equivalent mutation (E166Q) has been shown to abolish fast gating. As expected, whole-cell patch-clamping of mutant E232Q revealed that in ClC-1 fast gating was also eliminated. Furthermore, by using a method of patch-clamp analysis that allows us to separate fast from common gating, we observed that common gating was simultaneously absent. Application of the divalent cation, Zn^{2+}, which has been shown to stabilise a closed substate of common gating and thereby block the channels (Duffield *et al.,* 2005), was no longer effective at blocking chloride currents which confirmed the absence of common gating. In a further effort to elucidate the nature of common gating and its relation to the fast gate, we used additional known CLC channel inhibitors, 2(-4-chlorophenoxy) propionic acid (CPP) and trichlorophenoxy acetic acid (TCPAA), to test whether they could restore gating, as previously shown for the E166A mutant of ClC-0 (Traverso *et al.,* 2003). We expected a block of E232Q currents that could be fitted by a single exponential (*i.e.*, recovery of the fast gate), as seen in the E166A mutant (Traverso *et al.,* 2003). However, E232Q currents subjected to either inhibitor revealed a two exponential block. To test if this second, slower, exponential corresponded to the common gate, mutant channels were then exposed to Zn^{2+}. If, indeed, common gating had been recovered, we expected Zn^{2+} to bind to the channel and block its currents, but the results we obtained were both complex and inconclusive. We observed that large currents were 40-50% blocked, while smaller currents only showed a 10-15% block. Furthermore, block of currents was voltage independent. Therefore, the second, slow, exponential observed in the mutant in the presence of either inhibitor can not, at this stage, be definitely ascribed to the common gate. Further experiments are needed to explain the presence of the second exponential.

Accardi A & Pusch M. (2003) *Journal of General Physiology,* **122:** 277-93.
Duffield MD, Rychkov GY, Bretag AH & Roberts ML. (2005) *Journal of Physiology,* **568:** 5-12.
Dutzler R, Campbell EB, Cadene M, Chait BT & MacKinnon R. (2002) *Nature,* **415:** 287-94.
Dutzler R, Campbell EB & MacKinnon R. (2003) *Science,* **300:** 108-12.
Traverso S, Elia L & Pusch M. (2003) *Journal of General Physiology,* **122:** 295-306.