Electrostatic charge at position 552 of FMRFamide-gated Na^+ channel affects channel activation and rectification

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FMRFamide-gated Na^+ channel (FaNaC) is a ligand-gated sodium channel activated by a neuropeptide, FMRFamide. We previously reported that mutation of an aspartate (D552) in the second transmembrane domain of FaNaC affects channel activation by FMRFamide; e.g., lysine substitution induced 20-fold decrease of FMRFamide EC_{50} value. Also, some D552 mutations enhanced open channel block by FMRFamide, suggesting that D552 is located in the permeation pathway. In the present study, we altered the electrostatic charge at position 552 by treating the cysteine mutant (D552C) with charged sulfhydryl reagents, and examined channel function. FMRFamide EC_{50} for D552C mutant was close to that for the wild-type, and did not change after treatment with an anionic sulfhydryl reagent (MTSES). By contrast, a cationic sulfhydryl reagent (MTSET) decreased the EC_{50} for D552C mutant, indicating that introduction of positive charge at position 552 facilitates channel activation. We also found that the inward rectification observed in the wild-type FaNaC is weakened by D552C mutation. Following MTSES treatment, the inward rectification of D552C mutant became comparable to that of the wild-type, and the rectification was abolished after MTSET treatment. These results show that the inward rectification of FaNaC requires negative charge at position 552.