The ubiquitin ligase Nedd4-2: a novel regulator of the heteromeric KCNQ2/3 potassium channel

J.A.K. Ekberg,[1] S. Conroy,[1] F.S. Schuetz,[1] N. Boase,[2] S. Kumar,[2] D.J. Adams[1] and P. Poronnik, [1]School of Biomedical Sciences, The University of Queensland, Brisbane, QLD 4072, Australia and [2]Hanson Institute, IMVS, Frome Road, Adelaide, SA 5000, Australia.

The muscarine-sensitive K^+ current (M-current) regulates neuronal excitability by stabilising the resting membrane potential. Changes in its activity/regulation are implicated in clinical states such as epilepsy. The M-current is mediated by a heteromeric channel consisting of KCNQ2 and KCNQ3 subunits. The C-terminal tail of KCNQ3 exhibits a proline-rich motif (PPXPPY), a target for the ubiquitin-protein ligase Nedd4/4-2. Ubiquitination of target proteins by Nedd4/4-2 typically leads to their internalization and destruction, thus reducing their cell surface density. This study was undertaken to determine whether KCNQ2/3 was regulated by Nedd4-2. When KCNQ2/3 was co-expressed with Nedd4-2 in *Xenopus* oocytes, the K^+ current amplitude was down-regulated to 53.7 ± 5.2 % (n = 70, $p<0.01$) of control levels. In contrast, a ligase deficient Nedd4-2 mutant was without effect. However, mutating the PY motif in KCNQ3 had no effect on the down-regulation of the heteromeric channel by Nedd4-2. To determine whether Nedd4-2 physically interacted with KCNQ2/3, GST-fusion pulldown and co-immunoprecipitation experiments using rat brain lysates were performed. These results showed that Nedd4-2 interacted with both KCNQ2 and KCNQ3 and that the interaction was not mediated by the PY motif. Ubiquitination experiments using the KCNQ2 and KCNQ3 subunits were then performed in HEK293 cells. It was found that KCNQ2, KCNQ3 and the PY mutant of KCNQ3 were all ubiquitinated in the presence of Nedd4-2. Taken together, the data from this study show that Nedd4-2 functionally interacts with the KCNQ2/3 heteromer, but not *via* the PY motif. It is possible that Nedd4-2 binds to a different intracellular motif in KCNQ2/3, or alternatively interacts with the channel *via* an accessory protein.