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Biochemical, structural and functional study of ELIC probed with cysteine-reactive benzodiazepine analogs

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$GABA_A$ receptors belong to the class of pentameric ligand-gated ion channels and are involved in fast inhibitory transmission at synapses in the central nervous system. Benzodiazepines are positive allosteric modulators of the $GABA_A$ receptor and are widely prescribed because of their hypno-sedative, anxiolytic, anti-epileptic and muscle relaxant effects. Molecular insight into the mechanism of action of benzodiazepines has been derived from extensive mutagenesis studies. However, detailed structural knowledge is lacking due to the absence of a high-resolution crystal structure for human $GABA_A$ receptors. In this study, we take advantage of ELIC, a recently identified bacterial GABA-activated ion channel, which is also modulated by certain benzodiazepines. We studied a cysteine mutant in loop A that is homologous to H101C in $GABA_A$ receptors. Previous studies using a cysteine-reactive 7-analog of diazepam (NCS-diazepam) demonstrated that the H101C $GABA_A$ receptors are permanently potentiated after covalent reaction with the diazepam derivative (Tan et al., J. Neurochem, 2009). In ELIC, we observe that the homologous I79C is partially labelled by NCS-diazepam. In addition, we studied cysteine mutants in loop C that are homologous to S205C and T206C in $GABA_A$ receptors. Similar to I79C, it was previously demonstrated that mutant $GABA_A$ receptors are permanently potentiated after covalent reaction with a cysteine-reactive 3-analog of nitrazepam (3-NCS-nitrazepam, (Tan et al., J. Neurochem, 2009). In ELIC, we observe that the homologous D175C and H176C are completely labelled by 3-NCS-nitrazepam. To gain structural insight into binding poses of the benzodiazepine-modified ELIC mutants we have set up crystallization trials. Initial crystal hits have been obtained and X-ray diffraction data set has been collected to 3.8 Å. In parallel, we have characterized the functional properties of NCS-diazepam on the ELIC mutant I79C expressed in *Xenopus* oocytes. We observe that NCS-diazepam either inhibits or potentiates I79C depending on the level of expression. For oocytes producing more than 15 A of current (18 A ± 2, n=3) we find that NCS-diazepam reduces current to 67 ± 4 %). For oocytes producing less than 1 A of current (0.2 A ± 0.4, n=3) we observe that NCS-diazepam potentiates the current to 298 ± 53%). Together, we aim to combine structural and functional studies to provide detailed understanding on benzodiazepine recognition in $GABA_A$ receptors.

Relative positioning of diazepam in the benzodiazepine-binding-pocket of GABA receptors.

Tan KR, Baur R, Charon S, Goeldner M, Sigel E. *J Neurochem.* 2009;111(5):1264-73