GHB, THIP and NCS-382 activate a subset of $GABA_A$Rs expressed in *Xenopus* oocytes

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γ-hydroxybutyrate (GHB) is a small molecule with complex pharmacology. Present in low concentrations in the mammalian brain, it acts as a neuromodulator. When taken exogenously, it is used to treat narcolepsy and to ameliorate the withdrawal effects of alcohol, and is used as a recreational drug at higher concentrations, sometimes used as a "date-rape" drug. GHB is known to activate the $GABA_B$ receptor at high concentrations, but ligand-binding studies identified "GHB receptors" that bind both GHB and the analogue NCS-382 with high affinity.

In this study, we determined the role of the interaction between GHB, NCS-382, THIP and $GABA_A$Rs in thermoregulation, and the differences in subtype selectivity that underlies these roles.

Thermoregulation was measured using radiotelemetry in wild-type and knockout mice injected intraperitoneally with GHB, NCS-382 and THIP, a molecule with selectivity for δ-containing $GABA_A$Rs. The activity of GHB, THIP and NCS-382 at $GABA_A$Rs was determined by injecting mRNA encoding the sequences of the α4, β1-3 and δ subunits of $GABA_A$Rs in various ratios and combinations into *Xenopus* oocytes and measuring currents by two-electrode voltage clamp.

GHB, THIP and NCS-382 all induced hypothermia in wild-type mice, but only THIP-mediated hypothermia was abolished in δ-knockout mice. We then investigated the pharmacology of NCS-382 at α4βδ $GABA_A$Rs by measuring concentration-response curves of THIP, GHB and NCS-382 on Xenopus oocytes injected with different combinations of α4β1-3 and α4β1-3δ RNA at different ratios. NCS-382 activated β3 homomeric receptors and these currents were inhibited with co-application of GHB. Furthermore, GHB activated α4β1 receptors injected with a 1: 10 ratio significantly more potently than when injected with a 10: 1 ratio.

These data demonstrate that THIP, but not GHB or NCS-382 induce hypothermia *via* the activation of δ-containing $GABA_A$Rs. It is likely that NCS-382 and GHB activates $GABA_A$ receptors that are expressed in *Xenopus* oocytes but not readily found on the extracellular surface of native neurons, and these receptors are most likely to contain a β-β interface. While NCS-382 has previously been reported as an antagonist of GHB receptors, the pharmacological profile of NCS-382 is considerably more complicated.