Elucidating functions of GABA(A) receptor subtypes in neocortical circuits by specifically designed benzodiazepine-site agonists

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The Problem Subtype-selective benzodiazepine site agonists are useful tools for investigating the function of specific GABA(A) receptor subtypes in vivo and in brain slices. In the ideal case these agents potentiate GABA-induced activation of one GABA(A) receptor subtype without affecting others. Unfortunately, subtype selectivity is limited to a narrow range of concentrations and "big effects" cannot be simply produced by applying high drug concentrations. One solution to this catch 22 situation is offered by the use of specifically designed "high efficacy" benzodiazepine site agonists in combination with knock-in mutations.

Methods

- Specifically designed benzodiazepine site agonists.
- 1(H101R) and 5(H105R) knock-in mice.
- Organotypic slice cultures of the neocortex of wild-type and mutant mice.
- Extracellular voltage recording of multi unit activity.

Results

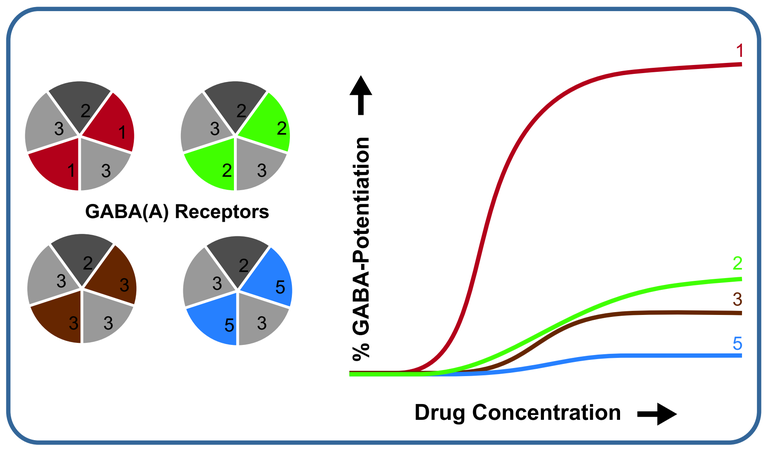
- The newly designed benzodiazepine site agonists SH-I-S66 and SH-053-2'F-R-CH3 are sensitive to knock -in mutations.
- Receptor selectivity is largely maintained up to a concentration of about 10 µM.
- Our results provide proof of principle that newly designed high-efficacy benzodiazepine site agonists can be combined with specific knock- in mutations.

Conclusions Application of newly designed high efficacy benzodiazepine site agonists in triple knock-in mice offers the opportunity to evoke a strong pharmacological response, which is, however, still selective for a specific GABA(A) receptor subtype.

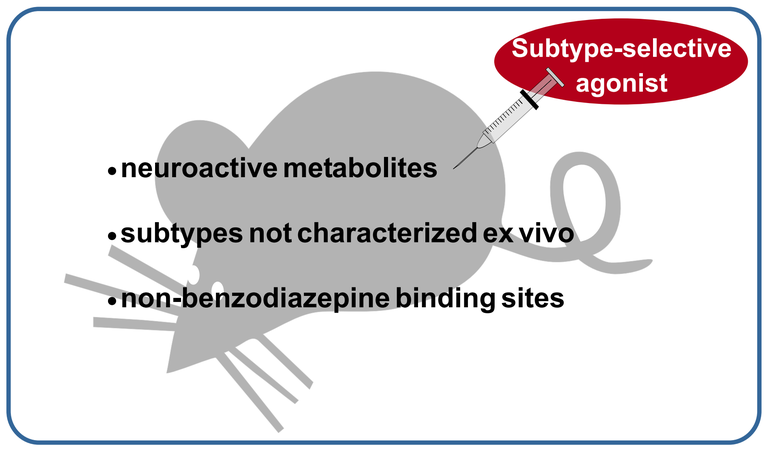
This experimental approach is equally applicable to behavioral and brain slice studies.

The Common Approach: Subtype-Selective Benzodiazepine-Site Agonists

Oocyte Profiles



Behavioral Testing



Conflicting Results

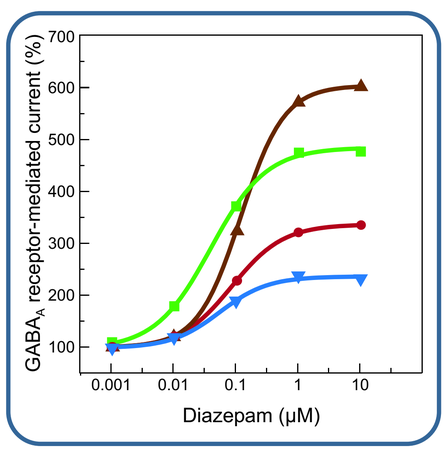
Anxiolysis:

- …**is mediated via 1** (*Ocinaplon, Lippa A et al; Proc Natl Acad Sci USA 2005 102:7380*)
- …**is mediated via 2** (*Löw K et al; Science 2000 290:131*)
- …**is mediated via 3** (*Dias R et al; J Neurosci 2005 25:10682*)

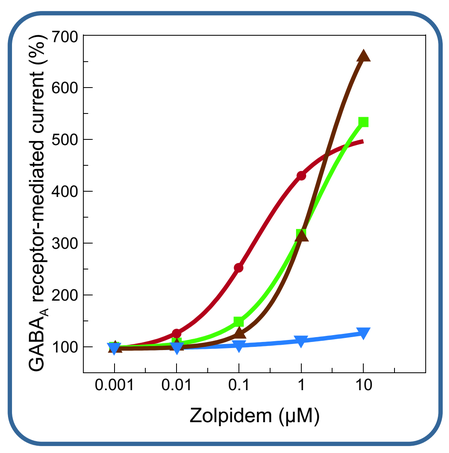
There is need for critical testing what receptors are involved!

Benzodiazepine-Site Agonists

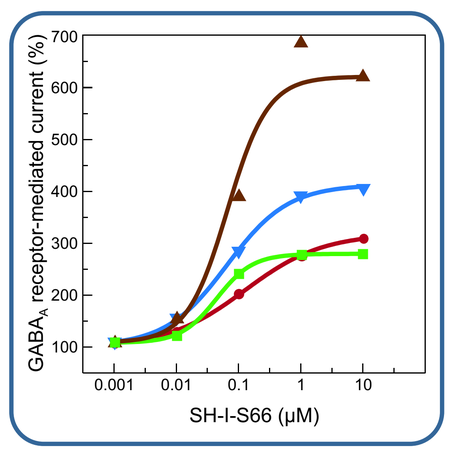
Diazepam



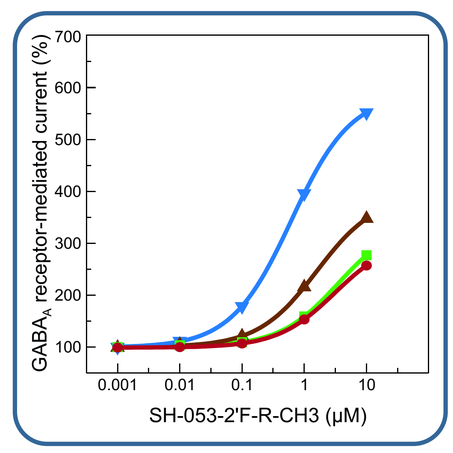
Zolpidem



SH-I-S66

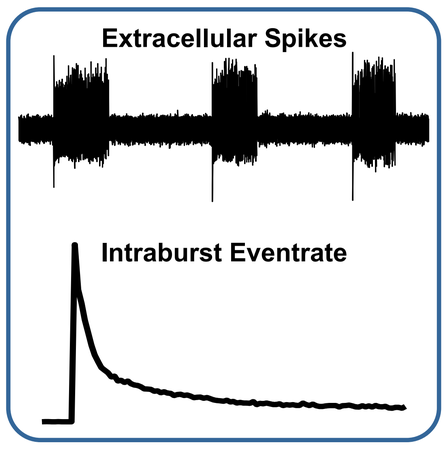


SH-053-2'F-R-CH3

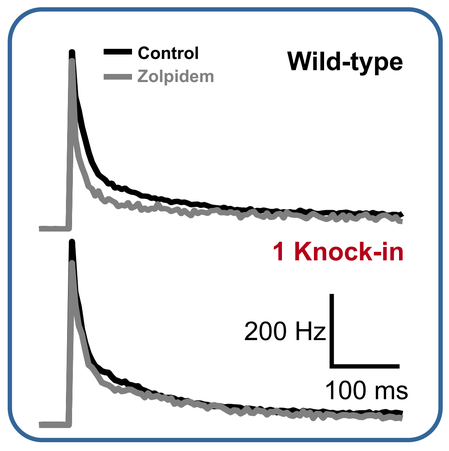


Proof of Principle: Single Knock-in

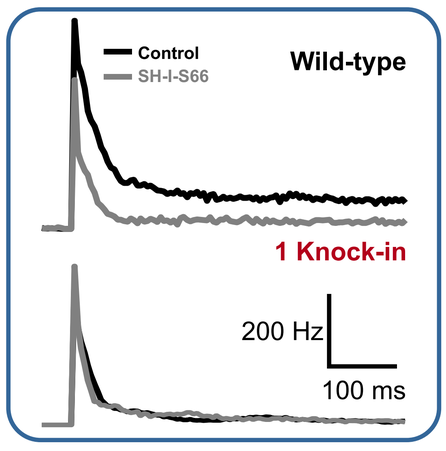
Brain Slice Recording



Zolpidem (3 µM)



SH-I-S66 (1 µM)



SH-053-2'F-R-CH3 (7.5 µM)