Unraveling the function of GABAA receptor subtypes

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GABAA receptors are Cl– channels that can be opened by GABA and are the major inhibitory neurotransmitter receptors in the CNS. A variety of pharmacologically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants, produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on GABAA receptors1.

Multiplicity of GABAA receptor subtypes

GABAA receptors are composed of five subunits that can belong to different subunit classes. So far, at least six α-, three β-, three γ-, one δ-, one ε-, one π-, one θ- and three ρ-subunits have been cloned from the mammalian nervous system2–5, and depending on their subunit composition, receptors exhibit distinct pharmacological and electrophysiological properties1,2. Recent immunocytochemical studies have indicated that individual subunits exhibit a distinct and often widespread distribution throughout the nervous system6,7. The resulting expression of multiple subunits in the same neurons suggest the existence of a large variety of GABAA receptor subtypes in the brain. Individual receptor subunits not only exhibit an expression pattern that is specific for certain neurons8,5, but also a distinct subcellular localization9,10, which suggests that different receptor subtypes might have specific functions.

Owing to the promiscuity of the individual subunits, the actual identification of receptor subtypes has proved difficult. Recently, a subtractive purification method was established, which for the first time allowed determination of the subunit composition of native GABAA receptor subtypes7,8. Combined with studies investigating the subunit stoichiometry9, results indicated that the majority of GABAA receptors comprise two α-, two β-, and one γ-subunit10. Minor receptors appear to be composed of αβδ, αβε, αβγ, αβς or homo- and heterooligomeric p-subunits10. Because two different α- and/or two different β-subunits can be present in the same receptor, probably more than 500 distinct GABAA receptor subtypes exist in the brain. Owing to the widespread distribution and quantitative importance of the GABA system, even minor GABAA receptor subtypes probably exhibit an abundance that is comparable with that of some monoamine, 5-HT or peptide receptors.

Pharmacological and gene-knockout approaches for studying the function of GABAA receptor subtypes

What is the function of individual GABAA receptor subtypes in the brain? One way to answer this question is to study the effects of a selective pharmacological modulation of a certain receptor subtype. Although a large variety of allosteric binding sites have been identified on GABAA receptors1,11, only a few compounds have been discovered so far that exhibit a certain subtype selectivity. Most of these compounds interact with the benzodiazepine binding site of GABAA receptors. Because this site is located at the interface of α- and γ-subunits11, its binding properties are influenced by the exact types of these subunits. The classical benzodiazepines such as diazepam exhibit comparable affinities for all GABAA receptors that contain a γ2-, a β- and an α1-, α2-, α3- or α5-subunit (benzodiazepine-sensitive receptors)11, but they do not interact with receptor subtypes that contain an α4- or α6-subunit (benzodiazepine-insensitive receptors). By contrast, some newer benzodiazepine-site ligands have been identified that preferentially interact with receptors that contain α1- or α3-subunits12,12. Their selectivity, however, is not sufficient to exclude a co-activation of other receptor subtypes.

Several attempts have also been made to identify the function of receptors by generating mouse lines in which the genes encoding certain GABAA receptor subunits were inactivated. A knockout of γ2-subunits was lethal13 and that of the α6-subunit did not result in an overt phenotype14. Although disruption of the gene encoding the β3- or δ-subunit produced mice with an epileptic phenotype15 or an attenuated sensitivity to neuroactive steroids16, respectively, a possible change in the development and function of the brain caused by the lack of these receptors could not be excluded.

A novel strategy for investigating the function of GABAA receptor subtypes

In an attempt to identify the function of receptors that contain specific α-subunits, a new genetic approach was developed recently by Rudolph et al.17 This approach was based on introducing a point mutation (His101Arg) into the α1-subunit of GABAA receptors, rendering α1-containing receptors insensitive to allosteric modulation by diazepam without altering their GABA sensitivity. In the absence of any change in signal intensity produced by the mutated receptors, animals possessing this mutation developed normally and the cellular and subcellular location of receptors was unchanged17. In these animals, therefore, diazepam mediated its effects through only the α2-, α3-, and α5-subunit-containing receptors.

A comparison of drug-induced behavioral responses in α1(His101Arg) mutant and wild-type mice thus allowed the investigation of the contribution of α1-containing receptors to the effects of diazepam. It was demonstrated17 that α1(His101Arg) mutant mice failed to show the sedative, amnesic and, in part, the anticonvulsive actions of diazepam. By contrast, the anxiolytic-like, myorelaxant and ethanol-potentiating effects were fully retained, which indicates that these effects are produced via the non-mutated GABAA receptors that contain α2-, α3- or α5-subunits.

Using a similar approach in a subsequent study most of these findings were confirmed by McKernan et al.18
Some discrepancies between the studies\textsuperscript{17,18}, concerning the sensitivity of mutants towards the ataxic effects of diazepam or the existence of a diazepam-induced paradoxical hyperactivity in the \(\alpha_1\)(His101Arg) mutants, could have been a result of multiple factors such as differences in genetic background, age of the animals used, extent of handling, exact experimental conditions and the diurnal period during which testing was performed. Such inter-laboratory differences in experimental procedures highlight the need for standardization in behaviorial pharmacology studies with mutant mice.

Together, these studies, for the first time, demonstrated that the various benzodiazepine actions are mediated by different GABA\(_A\) receptor subtypes. The sedative, amnesic and possibly ataxic and, in part, anticonvulsive actions appear to be mediated by \(\alpha_1\)-subunit-containing receptors, whereas anxiolytic actions seem to be mediated by receptors that contain \(\alpha_2\), \(\alpha_3\) or \(\alpha_5\)-subunits. This conclusion was supported by experiments using L838417, a novel benzodiazepine-site ligand with a so far unique GABA\(_A\) receptor subtype selectivity\textsuperscript{19}. Although this compound weakly enhances GABA-evoked currents at GABA\(_A\) receptors that contain \(\alpha_2\), \(\alpha_3\) or \(\alpha_5\)-subunits, it does not alter such currents in receptors that contain the \(\alpha_1\)-subunit.

Indeed, L838417 in wild-type animals produced anxiolytic-like activity in the elevated plus maze and fear-potentiated startle test without causing sedation or ataxia and thus pharmacologically reproduced the \(\alpha_1\)(His101Arg) mutant phenotype\textsuperscript{18}. Partial agonists at the benzodiazepine binding site have been shown previously to produce anxiolytic but no sedative actions in animal experiments, but under clinical conditions the sedative actions were still present\textsuperscript{19}. It remains to be determined whether the anxiolytic actions of L838417 will be devoid of sedative activity in human patients.

**Studying effects of diazepam mediated by other GABA\(_A\) receptor subtypes**

Studies investigating the function of \(\alpha_2\)-, \(\alpha_3\)- or \(\alpha_5\)-subunit-containing receptors are underway in both laboratories\textsuperscript{17,18}, using mice that carry the appropriate point mutation that renders the respective receptor subtypes diazepam-insensitive. Localization of \(\alpha_2\) and \(\alpha_3\) receptors in the amygdala and cortical regions point to these subtypes mediating the anxiolytic properties of the benzodiazepines, whereas \(\alpha_5\) receptors located in the hippocampus\textsuperscript{4,5} might be involved in the memory-impairing effects of benzodiazepines. The latter conclusion is supported by the recent demonstration that \(\alpha_5\)-subunit-deficient mice exhibit increased abilities in learning and memory tasks\textsuperscript{20}.

However, it should be kept in mind that the currently used approach\textsuperscript{17,18} requires the measurement of a probable small reduction of a large behavioral effect of diazepam in the mutants. Differences between large numbers are always difficult to measure and can be reliably quantified only when the reduction is sufficiently large. In addition, part of the behavioral effects of diazepam mediated by one receptor subtype might be counteracted by other GABA\(_A\) receptor subtypes. Thus, to clearly delineate all effects that are mediated by a specific type of receptor, it would be necessary to eliminate the benzodiazepine enhancement in all the other receptor subtypes. Effects of diazepam mediated exclusively by one type of receptor can then be studied easily. In addition, it should be noted that the approach of these two laboratories\textsuperscript{17,18} cannot distinguish between receptors that contain different \(\beta\)-subunits and cannot provide information on the function of receptors that contain \(\gamma_1\)-, \(\gamma_3\)-, \(\delta\)-, \(\epsilon\)- or \(\pi\)-subunits. Nevertheless, the extension of this approach to other \(\alpha_2\)-, \(\alpha_3\)- or \(\alpha_5\)-subunit-containing receptors will provide an important overview of some of the functions of these receptor subtypes and of the possible clinical use of the respective subtype-selective drugs.

**Concluding remarks**

Recent progress in GABA\(_A\) receptor research suggested the existence of far more receptor subtypes than previously assumed. These subtypes can now be identified by a newly developed method\textsuperscript{7} and their regional, cellular and subcellular distribution can be studied by available subunit-specific antibodies\textsuperscript{4,5}. The distinct cellular and subcellular location of individual receptor subtypes\textsuperscript{4–6} suggests that they exhibit specific functions in the brain. This conclusion is supported by the recent demonstration that different effects of benzodiazepines not only are produced in different brain regions but are also mediated by different GABA\(_A\) receptor subtypes\textsuperscript{17,18}. These results should cause a revival of GABA\(_A\) receptor research and strongly stimulate the development of drugs with a higher \(\alpha_2\)-, \(\alpha_3\)- or \(\alpha_5\)-receptor subtype selectivity that might exhibit selective clinical effects.

**References**

8. Bencsics, E. et al. (1999) A significant part of native \(\gamma\)-aminobutyric acid type A receptors containing \(\alpha_4\) subunits do not contain \(\delta\) or \(\beta\) subunits. J. Biol. Chem. 274, 19613–19616
Gastric habitation by Helicobacter pylori: insights into acid adaptation

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Bacteria have developed remarkable mechanisms to withstand hostile environments and, on occasion, select hostile environments to avoid competition. The habitation of the human stomach by Helicobacter pylori is an example of the latter. This organism is of medical interest because it causes peptic ulcer disease and predisposes to gastric cancer. The means whereby peptic ulcer disease and predisposes to example of the latter. This organism is selective for Helicobacter pylori is still a concept of a mucus barrier that protects the gastric surface. It is thought that acidic pH is highly injurious to the epithelium and therefore the actual pH at the gastric surface, the major habitat of H. pylori, is still a matter of debate. pH-mediated injury requires the presence of proton pathways across the cell membrane. Within the lumen of the gastric glands that secrete acid, the concentration of H+ might reach as high as 160 mM, a pH of 0.8. This is readily demonstrated in the chicken stomach, which has papillae that can be cannulated with a microproppet and uncontaminated acid secretion aspired (G. Sachs, unpublished). Clearly, the luminal-facing membranes of parietal cells, peptic cells and mucous neck cells in these glands survive this pH and the apical membranes are therefore without proton transport pathways. A confluent monolayer of dog peptic cells resists a luminal pH of 2.0. Studies using open-tip microelectrodes suggest that there is a large pH gradient from the gastric lumen to the gastric surface such that until the luminal pH reaches 2.0, the gastric surface is maintained at ~pH 6.0. When the luminal pH decreases to below pH 2.0, the surface pH becomes equal to the luminal pH (Ref. 5). These observations have given rise to the concept of a mucus barrier that protects the gastric surface. Some hypotheses suggest that mucus transports H+ to the lumen of the stomach, a concept that is, however, not supported by current awareness

Note added in proof
A study of mice carrying a point mutation in α2- or α3-subunits has recently been reported in Science.15

Chemical name

L838417: 3-(2,5-dihydrophenyl)-7-(1,1-dimethylpropyl)-6-(2-methyl-2H-1,2,4-triazol-3-ylmethyl)-1,2,4-triazolo[4,3-b]pyridazine

15 DeLorey, T.M. et al. (1998) Mice lacking the β3 subunit of the GABA<sub>β</sub> receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. J. Neurosci. 18, 8505–8514
18 McKernan, R.M. et al. (2000) Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA<sub>γ</sub> receptor α<sub>1</sub> subtype. Nature Neurosci. 3, 587–592

What is the range of intragastric pH in which H. pylori survives and grows? It is thought that acidic pH is highly injurious to the epithelium and therefore the actual pH at the gastric surface, the major habitat of H. pylori, is still a matter of debate. pH-mediated injury requires the presence of proton pathways across the cell membrane. Within the lumen of the gastric glands that secrete acid, the concentration of H+ might reach as high as 160 mM, a pH of 0.8. This is readily demonstrated in the chicken stomach, which has papillae that can be cannulated with a micropipette and uncontaminated acid secretion aspired (G. Sachs, unpublished). Clearly, the luminal-facing membranes of parietal cells, peptic cells and mucous neck cells in these glands survive this pH and the apical membranes are therefore without proton transport pathways. A confluent monolayer of dog peptic cells resists a luminal pH of 2.0. Studies using open-tip microelectrodes suggest that there is a large pH gradient from the gastric lumen to the gastric surface such that until the luminal pH reaches 2.0, the gastric surface is maintained at ~pH 6.0. When the luminal pH decreases to below pH 2.0, the surface pH becomes equal to the luminal pH (Ref. 5). These observations have given rise to the concept of a mucus barrier that protects the gastric surface. Some hypotheses suggest that mucus transports H+ to the lumen of the stomach, a concept that is, however, not supported by digestive phase where buffering by meal contents often occurs. The period of highest sustained acidity is at night, but the volume of acid secretion is low.