Unraveling the function of $GABA_A$ receptor subtypes

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$GABA_A$ 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 $GABA_A$ receptors[1].

Multiplicity of $GABA_A$ receptor subtypes

$GABA_A$ 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 system[2,3], and depending on their subunit composition, receptors exhibit distinct pharmacological and electrophysiological properties[1,2]. Recent immunocytochemical studies have indicated that individual subunits exhibit a distinct and often widespread distribution throughout the nervous system[4,5]. The resulting expression of multiple subunits in the same neurons suggest the existence of a large variety of $GABA_A$ receptor subtypes in the brain. Individual receptor subunits not only exhibit an expression pattern that is specific for certain neurons[4,5], but also a distinct subcellular localization[4,6], 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 $GABA_A$ receptor subtypes[7,8]. Combined with studies investigating the subunit stoichiometry[9], results indicated that the majority of $GABA_A$ receptors comprise two α-, two β-, and one γ-subunit[10]. Minor receptors appear to be composed of αβδ, αβϵ, αβπ, αβ or homo- and hetero-oligomeric ρ-subunits[10]. Because two different α- and/or two different β-subunits can be present in the same receptor, probably more than 500 distinct $GABA_A$ receptor subtypes exist in the brain. Owing to the widespread distribution and quantitative importance of the GABA system, even minor $GABA_A$ 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 $GABA_A$ receptor subtypes

What is the function of individual $GABA_A$ 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 $GABA_A$ receptors[1], 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 $GABA_A$ receptors. Because this site is located at the interface of α- and γ-subunits[11], its binding properties are influenced by the exact types of these subunits. The classical benzodiazepines such as diazepam exhibit comparable affinities for all $GABA_A$ receptors that contain a γ2-, a β- and an α1-, α2-, α3- or α5-subunit (benzodiazepine-sensitive receptors)[1], 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 α5-subunits[1,12]. 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 $GABA_A$ receptor subunits were inactivated. A knockout of γ2-subunits was lethal[13] and that of the α6-subunit did not result in an overt phenotype[14]. Although disruption of the gene encoding the β3- or δ-subunit produced mice with an epileptic phenotype[15] or an attenuated sensitivity to neuroactive steroids[16], 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 $GABA_A$ 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 $GABA_A$ 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 unchanged[17]. 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 demonstrated[17] that α1(His101Arg) mutant mice failed to show the sedative, amnesic and, in part, the anticonvulsant 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 $GABA_A$ 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]

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Some discrepancies between the studies[17,18], concerning the sensitivity of mutants towards the ataxic effects of diazepam or the existence of a diazepam-induced paradoxical hyperactivity in the α1(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 behavioral 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 α1-subunit-containing receptors, whereas anxiolytic actions seem to be mediated by receptors that contain α2-, α3- or α5-subunits. This conclusion was supported by experiments using L838417, a novel benzodiazepine-site ligand with a so far unique $GABA_A$ receptor subtype selectivity[18]. Although this compound weakly enhances GABA-evoked currents at $GABA_A$ receptors that contain α2-, α3- or α5-subunits, it does not alter such currents in receptors that contain the α1-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 α1(His101Arg) mutant phenotype[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[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 α2-, α3- or α5-subunit-containing receptors are underway in both laboratories[17,18], using mice that carry the appropriate point mutation that renders the respective receptor subtypes diazepam-insensitive. Localization of α2 and α3 receptors in the amygdala and cortical regions point to these subtypes mediating the anxiolytic properties of the benzodiazepines, whereas α5 receptors located in the hippocampus[4,5] might be involved in the memory-impairing effects of benzodiazepines. The latter conclusion is supported by the recent demonstration that α5-subunit-deficient mice exhibit increased abilities in learning and memory tasks[20].

However, it should be kept in mind that the currently used approach[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[17,18] cannot distinguish between receptors that contain different β-subunits and cannot provide information on the function of receptors that contain γ1-, γ3-, δ-, ϵ- or π-subunits. Nevertheless, the extension of this approach to α2-, α3- or α5-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[7] and their regional, cellular and subcellular distribution can be studied by available subunit-specific antibodies[4,5]. The distinct cellular and subcellular location of individual receptor subtypes[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[17,18]. These results should cause a revival of $GABA_A$ receptor research and strongly stimulate the development of drugs with a higher α2-, α3- or α5-receptor subtype selectivity that might exhibit selective clinical effects.

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Note added in proof

A study of mice carrying a point mutation in α2- or α3-subunits has recently been reported in *Science*[21].

Chemical name

L838417: 3-(2,5-difluorophenyl)-7-(1,1-dimethylethyl)-6-(2-methyl-2*H*-1,2,4-triazol-3-ylmethoxy)-1,2,4-triazolo[4,3-*b*]pyridazine

Gastric habitation by *Helicobacter pylori*: insights into acid adaptation

George Sachs, David Scott, David Weeks and Klaus Melchers

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[1,2]. The means whereby this bacterium resists gastric acidity is not only of biological interest but provides a natural pharmacological and therapeutic target for its eradication. Recent studies have shown that acid-induced activation of a urea channel regulates intra-bacterial urease activity, which enables maintenance of the pH of the periplasm of the bacteria within viable limits during gastric acidity. This appears to be an adaptation mechanism that is unique to *Helicobacter* species. Because no other organism inhabits the normal human stomach, targeting of drugs to these acid resistance mechanisms should provide an antibiotic that is selective for *H. pylori*.

Neutralophiles live within a pH range of ~4.0–8.0 and grow best at a pH of between 6.0 and 8.0. Acidophiles can be divided into obligatory acidophiles that require a pH ~2.0 for sustenance or facultative acidophiles that can grow, if necessary, at highly acidic pH. Alkalophiles select an environment of pH 9.0 or greater for their habitation. Habitation of any of these pH environments depends on, as for all bacteria, maintenance of a viable proton motive force, the sum of the proton and potential gradients (i.e. the electrochemical proton gradient)[3]. This proton motive force is required for synthesis of ATP and H^+-coupled transport of solutes. In the case of neutralophiles, the proton motive force is maintained at ~−200 mV. The redox pumps in the cytoplasmic membrane generate the electrical potential and at neutral pH there is a small inward pH gradient in the case of neutralophiles. As their environment becomes more acidic, the inward pH gradient rises and is accompanied by a fall in the interior negative membrane potential, perhaps as a result of a proton leak. In the case of acidophiles at acidic pH, an internal positive potential is generated to prevent excessive rates of proton entry whereas alkalophiles generate large interior negative potentials to maintain a driving force for protons across the F_1F_0 ATPase because there is then an absence of a potential gradient[4].

The median intragastric pH of the human stomach is 1.4. However, there are periods in which gastric contents are largely neutral, such as during the 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.

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 canulated with a micropipette and uncontaminated acid secretion aspirated (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

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