Proposal for DRA post doc. project

Focus area within DUPS
“Structure based drug research”

Project title

“GABA<sub>A</sub> receptor ligands: Activity, selectivity and mechanism of action”

Departments primarily involved at DUPS
Department of Pharmacology and Department of Medicinal Chemistry

Senior Scientists
Associate Professor, Ph.D. Uffe Kristiansen*, Department of Pharmacology
Uffe Kristiansen has been involved in research in GABA<sub>A</sub> receptor pharmacology since 1987 and has supervised three Ph.D. students and a post-doc in the field. His expertise is mainly electrophysiology.

Associate Professor. Bente Frølund, Department. of Medicinal Chemistry, DUPS. Bente Frølund has worked with different aspects of medicinal chemistry of neurotransmitters especially in the field of γ-aminobutyric acid (GABA). Her research experience and expertise is primarily within the area of medicinal chemistry and organic synthesis.

Research Fellow Mogens Nielsen, Department. of Medicinal Chemistry, DUPS. Mogens Nielsen has for many years worked within the field of GABA<sub>A</sub> receptors focusing on the benzodiazepine site. His expertise is primarily within the area of pharmacological and biochemical studies of ligand-protein interactions.

Head of Department, Adjunct Professor Bjarke Ebert, H. Lundbeck A/S. Bjarke Ebert has a broad experience in GABA<sub>A</sub> receptor pharmacology and drug development from positions at DUPS and H. Lundbeck.

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Research hypothesis / objectives

The objective of this project is to establish a mechanistic link between structural and functional parameters for selected ligands for the GABA<sub>A</sub> receptor with efficacies ranging from full agonists to pure antagonists
Description of the project

Background: GABA, the main inhibitory neurotransmitter in the CNS, mediates a large part of its effect by activation of the GABA$_A$ receptors. These are Cl$^-$ ion channels assembled from different subunits in a pentameric composition. In the mammalian CNS a large family of subunits exist which, based on homology, are grouped into types $\alpha$1-6, $\beta$1-3, $\gamma$1-3, $\delta$, $\epsilon$, $\pi$ and $\theta$. The GABA$_A$ receptor subunits are expressed in a region- and age-specific manner (1). Different subunit combinations exhibit distinct properties that presumably underlie a precise physiological role for each subtype (2,3). Knowledge of the distribution and function of the different GABA$_A$ receptor subtypes is essential for understanding their physiological and pathophysiological roles. Development of ligands with selective action on certain subunit combinations will help to clarify these aspects and are also potential drugs.

The GABA$_A$ receptor has so far not been amenable to X-ray structure determination, and therefore no detailed 3D structural model is available. A cruder model has been obtained using the homology of the extracellular subunit domains with the acetylcholine binding protein. Mutational approaches have revealed amino acids important for ligand binding and channel gating (4). Testing ligands of varying structure have provided further information on the ligand binding site (pharmacophore model) as well as the binding and gating mechanisms. Presently, models of the GABA and benzodiazepine binding sites, including the amino acids and their approximate positions, are established (5, 6). From this, systematic variations of both ligand structure and receptor structure (mutagenesis) followed by pharmacological testing and molecular modeling will offer a more detailed picture of the interactions between specific amino acids and the ligands. This will in turn facilitate design of new selective ligands.

The group behind this project has made a substantial contribution to the present understanding of structure-activity relationships for GABA$_A$ receptors. We have previously developed a number of ligands with efficacy ranging from zero to full agonism, including the first GABA$_A$ partial agonist, 4-PIOL (6,7,8). Using single-channel electrophysiology we have determined that partial agonism on GABA$_A$ receptors is due to a reduced ability to stabilize the channel in the open conformation, while the open channel conformations are similar whether induced by full or partial agonists (9,10). However, our understanding of connection between ligand structure and receptor activity is still far from complete. Furthermore, the understanding needs to be specialized to include different combinations of subunits, so that more specific ligands can be designed. The GABA$_A$ ligand Gaboxadol, currently in clinical trials as a therapeutic in the regulation of sleep, has been shown to be functionally selective on a subpopulation of GABA$_A$ receptors and exemplifies the importance of functional selectivity in terms of in vivo activity.

In order to improve our understanding of receptor function, detailed biological data of high quality from different subunit combinations are important. Data on receptor kinetics (activation, desensitization, deactivation, single-channel data) allow construction of kinetic models, describing different states of the receptor and their interconversion.
Integration of such models with structural models will give a more complete picture of ligand-receptor interaction.

Research plan: This post. doc. project will mainly concentrate on the following subjects:

1) Electrophysiological characterization of ligands for GABA and benzodiazepine sites on different subunit combinations and already made mutants (Xenopus oocytes).
2) More detailed kinetic and/or single-channel analysis of selected ligand-receptor combinations (HEK-293 cells, cultured neurons).
3) Construction and characterization of new mutants, based on homology models of the GABA_A receptor in the presence or absence of reactive ligands.

The work will take place in constant interaction with the people working with design and synthesis of ligands as well as molecular modeling.

The compounds under study will include analogs of the partial GABA_A agonist, 4-PIOL (figure). GABA and 4-PIOL both contain a basic amino group and an acidic moiety, which are both believed to interact with specific domains of the receptor. The distance between these two groups is, however, longer in 4-PIOL than in GABA.

Several analogs of 4-PIOL and thio-4-PIOL have been synthesized, and more are on the way, which retain the low efficacy partial agonists activity (7). Addition of substituents of increasing size and lipophilicity in the 4-position of the isoxazole ring of 4-PIOL increases the affinity but decreases the efficacy gradually (6).

The substituent lipophilicity is also negatively correlated with the ligand dissociation rate from the receptor, suggesting that a lipophilic interaction is rate-limiting for dissociation. The clear correlation between ligand structural parameters and several functional parameters makes these analogs very useful for studying the mechanism of ligand receptor interaction, including the requirements for the ligands to be able to induce opening of the Cl^- channel. Moreover, these high affinity analogs will be used as a platform for the development of labeling tools, based on the structure of 4-PIOL, for studying the binding site in combination with mutagenesis.

For further description of the methods, please see appendix.
Project description to be published:

The project is part of an integrated approach to develop new selective ligands for the 
GABA_A receptor which are also potentially new drugs. The emphasis is on 
pharmacological evaluation of ligand-receptor interaction as function of both ligand 
structure and receptor composition of subunits. Starting with already synthesized ligands, 
the activity on different receptor compositions will be tested using electrophysiological 
methods. Mutation of subunits will be included to study the role of different parts of the 
receptor in receptor activity. The results will be interpreted in terms of a kinetic model for 
interconversion of the functional states of the receptor. Integration of the kinetic and 
structural models will provide a more dynamic picture of ligand-receptor interaction to be 
used as a tool to direct synthesis of new improved ligands.

Benefit of the project to

The research group at DUPS: This project will further develop the group’s expertise in 
analysis of ligand-receptor interactions and provide specific information on the function 
of GABA_A receptors.

The focus area: This project will provide information on the mechanism of interaction 
between ligands and the GABA_A receptor. Integration of this information with structural 
models of the GABA_A receptor will improve the possibilities for rational design of new 
GABA_A receptor ligands

The post doc.: This project will provide an opportunity to develop and implement 
pharmacological methods to analyze ligand receptor interactions, and to interact closely 
with experts in drug design, synthesis and computational chemistry. In this way the post 
doc. will gain experience with the integrated process of development of new receptor 
ligands.

Relevant journal for advertising

Nature
Signature of senior scientists

Date

Uffe Kristiansen

Date

Bente Frølund

Date

Mogens Nielsen

Date

Bjarke Ebert
Confirmation from department

The present project is supported by Institute of Pharmacology, DUPS, where the necessary space and equipment are available

Date: Erik Wind Hansen

Head of Department of Pharmacology
References


Appendix: Methods

GABA$_A$ ligands: Structurally diverse analogs of GABA and 4-PIOL with a broad spectrum of affinity and efficacy has been synthesized at Dept. of Medicinal Chemistry, DUPS$^{(5,6)}$. Design and synthesis of new ligands are ongoing, guided by the results from pharmacological testing and molecular modeling.

Electrophysiology: The Cl$^-$ current flowing through the open channels is the most direct measure of GABA$_A$ receptor activation. This can be measured in real-time using electrophysiological methods. For screening purposes the two-electrode voltage-clamp on Xenopus oocytes will be employed. The method has a comparatively large capacity but, because of a limited rate of ligand exchange, the measurements will be influenced by desensitization, and true kinetic measurements are not feasible. For more detailed investigations (e.g., kinetic measurements) patch-clamp recording on cultured neurons or transfected HEK-293 cells is superior. The technique is more cumbersome, but the measurements have higher sensitivity and time resolution. In combination with a piezo-electric system for ligand exchange, a submillisecond time resolution is achieved, which is a prerequisite for measurement of the true rate of conformational transitions involved in channel gating.

Recombinant GABA$_A$ receptors: Xenopus oocytes are injected with mRNA coding for relevant GABA$_A$ receptor subunits while HEK-293 cells are transfected with cDNA coding for the relevant subunits. In this way the subunit composition of the receptors can be (to some degree) be controlled. Co-transfection of the HEK-293 cells with green fluorescent protein allows visualization of the successfully transfected cells.

Naturally occurring GABA$_A$ receptors: Individual neurons in primary cultures of cerebral cortical neurons and cerebellar granule neurons exhibit considerable variation in composition of GABA$_A$ receptors expressed. Subsequent to electrophysiological recording, the cell cytoplasm can be aspirated into the recording pipette and analyzed for the content of mRNA for GABA$_A$ receptor subunits using RT-PCR$^{(7)}$. Currently we have procedures for testing 13 of the GABA$_A$ receptor subunits$^{(8)}$. Combined electrophysiological and RT-PCR data are analyzed for correlations between receptor function and mRNA expression, including interactions between individual subunits. RT-PCR is performed in collaboration with Dr. Flemming F. Johansen, Laboratory of Neuropathology, University of Copenhagen.

Receptor kinetic modeling: In order to evaluate different kinetic models for GABA$_A$ receptor function, computer simulation of the models are performed and the results compared to the experiments.

Mutagenesis: Standard molecular biological techniques will be used to create relevant single-point mutations in GABA$_A$ receptor subunits.
Curriculum vitae for Uffe Kristiansen

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1/12 1992 til 31/11 1996: Adjunk på Institut for Biologi, DFH. Forskningsprojekt: Molekylærfarmakologiske undersøgelser af GABA_α receptor aktivering i neuronale og gliale cellekulturer.

Forskningsophold på Laboratory of Neurophysiology, National Institute of Neurological Disorders and Stroke, NIH, USA i fire perioder: 1/6 - 31/7 1993, 1/1 - 5/2 1994, 1/8 - 9/9-1994 og 18/11 - 18/12 1995.

1/12 1996: Lektor på Institut for Biologi, nu Institut for Farmakologi, DFU,
Bibliography for Uffe Kristiansen

Uffe Kristiansen has 32 publications, (28 research papers and 4 book chapters).

Publications 2000 -


