'[Poster 41]'

EFFECTS OF CHRONIC DIAZEPAM ON ABSENCE-LIKE PHENOMENA IN THE EEG OF THE WAG/Rij RAT

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We evaluated the effects of chronic diazepam during 21 days on spike-wave discharges (SWD's) in the EEG of the WAG/Rij rat, an animal model for absence epilepsy.
The animals subcutaneously received silastic tubes either empty or filled with diazepam (50 mg/kg/day) resulting in constant blood concentrations (± 200 ng/ml). EEG's were recorded during 2 hours. Two baselines were recorded. During the treatment we registered the EEG 5 times. A post-drug recording was taken 9 days after tube removal.
In the control group a linear increase in the number of the SWD's was found (0.25/h/day), whereas the mean duration remained constant. Diazepam reduced both the number and the duration of SWD's during the entire treatment. These effects gradually diminished during the treatment however. The number of SWD's could be described by a limited exponential growth ($t_{½}$ of 3 days) superposed on a linear increase (0.25/h/day) suggesting a fast partial loss of diazepam effect. The mean duration was described by a linear increase only (0.08 s/day) suggesting a gradual loss of the diazepam effect with a $t_{½}$ of about 20 days. On the post treatment measurement no group differences were found.
Tolerance develops to the anti-absence effect of diazepam, but different $t_{½}$'s were found for the frequency of the absences and their duration. This observation is in agreement with the hypothesis that different molecular mechanisms underlie the genesis and the termination of epileptic phenomena.

'[Poster 42]'

Site directed mutagenesis of the 5-HT2C receptor: Identification of residues involved in ligand binding

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In order to investigate the molecular basis for ligand binding to the human 5-HT2C receptor we have constructed a three-dimensional model for this receptor using the atomic coordinates of the structurally related bacteriorhodopsin as a template. To validate our model we used an approach in which we investigated the binding of several structurally related ligands on wild-type receptor and on a panel of mutant receptors in which single residues presumed to be involved in ligand binding were systematically mutated. To investigate the role of two conserved aromatic residues in TM6 (Phe 327 and Phe328 in ligand binding, these residues were substituted for alanines.
Additional mutations were performed for two serine residues in TM4 (Ser182 and Ser186), and one in TM5 (Ser219). Wild-type and mutant receptors were stable expressed in NIH3T3 cells and characterized by saturation and competition binding assays with 5-HT and with a number of structurally related compounds as well with the antagonists mesulergine and mianserin. As proposed by our model, Phe327 supports the electrostatic interaction between the protonated amine group of 5-HT and the carboxylate group of the conserved Asp in TMR3. Phe328 seems to play a pivotal role in the binding of the aromatic ring of 5-HT and mesulergine. Ser186 in TM4 has a small but significant contribution in the binding of most of the tested compounds. In contrast, our experiments did not support a role for the indole NH of 5-HT in binding.

'[Poster 43]'

Perspectives and pitfalls of cloned receptors as tools for pharmacological studies

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One of the most fundamental advances in drug screening is the ability to express cloned human receptors in micro organisms or eukaryotic cells for pharmacological studies. Traditional receptor binding assays in animal tissue are hampered by the existence of multiple subtypes of a receptor, as well as possible species differences. Fermentor-scale cell cultures now allow the production of homogeneous preparations of a single human receptor for use in receptor binding studies. Cloned human receptors often couple to endogenously present signaling pathways in the host cell. This allows the measurement of second messenger pathways, thus providing a sensitive assay to determine the agonistic or antagonistic properties of ligands. However, our recent experience is that careful interpretation of data obtained using recombinant receptors is warranted as the system may have a number of pitfalls. For example, in a number of cases, high receptor densities have been shown to cause ligand-independent signaling which may not only affect cell morphology and proliferation, but may also change pharmacological parameters. Overexpression of the receptor may result in the activation of signaling pathways that not necessarily reflect the physiological situation. E.g. differences in G protein content between brain tissue and the host cells have been found. Overexpression of the receptor may also result in overestimation of potency (pEC_{50}) and intrinsic efficacy. This phenomenon seems to depend on the receptor subtype and the host cell used. To get more insight into these processes we generated a panel of single cell clones expressing different amounts of 5-HT_{1A} and 5-HT_{2C} receptors. The present study deals a number of these issues.

'[Poster 45]'

G-PROTEIN ACTIVATION OF CLONED HUMAN MUSCARINIC M1 AND M2 RECEPTORS EXPRESSED IN CHO CELLS

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Human M1 and M2 muscarinic receptor were cloned and stably transfected in CHO-K1 cells. For each receptor subtype a clone was selected with similar receptor density as measured by saturation binding of [^{3}H]-QNB. The pharmacological profile was confirmed by [^{3}H]-QNB displacement curves with reference antagonists and is in agreement with literature data.
High affinity binding of the aselective agonist [^{3}H]-OXOM binding (Kd=1.4 nM, Bmax=1.23 pmol/mg) to the M2 clone represented 18% of the total receptor population, whereas no such binding could be detected to the M1 clone. Agonist [^{3}H]-QNB displacement curves to M2 receptors revealed the presence of high and low affinity sites, whereas [^{3}H]-OXOM displacement curves were monophasic with Ki values corresponding with high affinity sites only. Agonist high affinity sites for M1 receptors could hardly be detected.
Furthermore, we measured the ability of agonists to increase the binding of [^{35}S]-GTPγS. In case of the M2 receptor, the obtained EC_{50} values correlated with the Ki values for the agonist high affinity sites. Pertussis toxin pretreatment of the cells impaired agonist high affinity binding as well as the increase of [^{35}S]-GTPγS binding illustrating the functional coupling of M2 receptors to Gi-proteins. Dose respons curves of agonist M1 receptor mediated increase of [^{35}S]-GTPγS binding were shallow. Interestingly these effects were partially blocked by pertussis toxin, indicating additional Gi-coupling of these receptors.