# IDENTIFICATION OF THE INVERSE BENZODIAZEPINE RECEPTOR AGONIST METHYL-BETA-CARBOLINE-3-CARBOXYLATE (BETA-CCM) IN ARUM-MACULATUM, WHEAT AND IN RAT ADRENALS

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**Abstracts**

## LECTURES
(according to the programme)

C.A.O. Stuermer, M. Bastmeyer, J. Vielmetter and K. Wehner

### Growth associated cell surface molecules in the fish visual system

With one known monoclonal antibody (Mab $D_3$, Schlosshauer, 1989) and three newly created Mabs (E 587, E 21, M 802) we detected four cell surface associated proteins on goldfish retinal axons which are selectively expressed during retinal axonal growth. Accordingly, all retinal axons are stained in fish embryos. In larger fish, however, only a small group, the new axons derived from newborn ganglion cells at the retinal peripheral margin are labeled. After sectioning of the optic nerve and during retinal axonal regeneration, all regenerating axons re-express the four antigens. Mab staining disappears from the regenerated axons over time, and for each antigen with a different time course.

Mab $D_3$ recognizes NCAM 180, E 587 a molecule, related to the cell adhesion molecules L1/G4 in mouse and chick, E 21 a 84 kd glycoprotein (GP), possessing the HNK1 epitope, M 802 a 50 kd GP. E 587 also binds in vitro to fish optic nerve derived oligodendrocyte-like cells, which proved to be optimal substrates for retinal axonal regeneration. On these glial cells the E 587-antigen appears to condense at cell-cell and axon-cell contact sites, suggesting a role of this molecule in axon-oligodendrocyte interaction. The distribution of these molecules in the context of axonal pathfinding and regeneration will be discussed.

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### A monoclonal antibody recognizes a putative axonal guidance molecule

Ganglion cell axons grow from the retina through optic nerve and tract to their target, the optic tectum. There they form a topographic projection. Nasal axons project to the posterior, temporal axons to the anterior pole of the optic tectum. Molecules on surfaces of neurons or glial cells are probably involved in guiding the axons to their final position. Our laboratory is trying to characterize and to isolate such guiding molecules.
One candidate is a 33 KD glycoprotein which is capable of guiding temporal growth cones in an in vitro assay (the in vitro system will be discussed in detail). After treating tectal membranes with the enzyme phosphatidylinositol-specific phospholipase C the 33 KD protein is removed from the membranes and is found in the supernatant after centrifugation. Supernatants containing the 33 KD component were used to immunize mice. We isolated a hybridoma cell line which produces an antibody recognizing the 33 KD protein.
On Western-blots the antibody binds to two proteins with a molecular weight of 33 and 35 KD. Some staining is also observed in the higher molecular weight range. Both, the 33 and the 35 KD proteins are present at higher concentrations in posterior than in anterior tectum. In cryostat-sections antibody-staining of the stratum opticum declines gradually from the posterior to the anterior pole of the optic tectum.
This is in good agreement with the hypothesis that in vivo temporal retinal axons are guided by gradients of guiding molecules.

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understanding its mechanism of action. A model has been proposed for the ion channel (the Helix M2-model) on the basis of photoaffinity labelling and protein sequencing experiments (1).

By a similar approach, the signal recognition domain, i.e., the binding site for cholinergic ligands, has been analyzed: Photoaffinity derivatives of α-neurotoxin II from the cobra Naja naja oxiana, which carry the photoactivatable group in known positions, are cross-linked to the membrane-bound nAChR from Torpedo californica electric tissue. The receptor subunits then are separated by preparative electrophoresis. After cleavage of the cross-linked subunit-toxin complex (e.g. by cyanogen bromide) the cross-linked peptides are purified by HPLC and subjected to Edman sequencing.

Two toxin-photoaffinity derivatives are applied. Using the oxiana II toxin which carries the photoactivatable group in position Lys 46, the nAChR α-subunits are labelled almost exclusively. The β-, Γ- and δ-subunits of the receptor are labelled with the corresponding Lys 26 derivative as well (2). This indicates that the binding pocket for this neurotoxin is large, extending beyond the α-subunits which are assumed to be the ligand binding subunits.

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## Non-Electrolyte Transport in Astroglial Cells

Astroglia-rich primary cultures derived from the brains of newborn rats can be cultivated in the absence of glucose as long as the medium contains either sorbitol or fructose. To a lesser extent, also xylitol or mannose can sustain the primary cultures. Utilization of energy substrates is dependent on uptake as well as metabolism of the respective compound. Therefore, in extension of our work on sorbitol (1), the transport of various sugars and polyols was investigated in astroglial cultures with the radioactive tracer method in order to characterize the first step in substrate utilization.

The results suggest the existence of a variety of transport mechanisms for non-electrolytes in astroglial cells. Open-chain polyols as well as fructose enter the cell by simple diffusion, maybe through a protein structure (1). Facilitated diffusion via the hexose carrier determines the uptake of glucose ($K_m$= 0.5 mM), mannose ($K_m$= 8 mM), and galactose ($K_m$= 13 mM). Uptake of myo-inositol is saturable only in the presence of $Na^+$ with a $K_m$ of 72 μM. Transport mechanism for this cyclic polyol is distinct from that of glucose and probably is an active one.

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## Identification of the Inverse Benzodiazepine Receptor Agonist Methyl-β-carboline-3-carboxylate (β-CCM) in Arum maculatum, wheat and in rat adrenals

Methanolic and acetic acid extracts of the berries of Lords and Ladies (Arum maculatum L.) contained compounds competing at the central benzodiazepine (BZ) binding site. The most active fraction exhibited decreased receptor binding in the presence of $10^{-4}$M GABA and coeluted together with synthetic β-CCM in 4 different HPLC systems. In addition the dose-activity ratio of BZ receptor binding competition and mass spectrometrical analysis combined with gas chromatography identified the plant constituent as the inverse BZ receptor agonist β-CCM. In A. maculatum the β-carboline quantity present (10-20 μg/g fresh weight) may represent or at least contribute to the toxicity of this plant.

In wheat grains and in rat adrenals β-CCM could be also detected although in lower amounts. Recently in rat brain the structurally closely related compound butyl-β-carboline-3-carboxylate has been identified[1]. This β-carboline derivative acts as a partial inverse BZ-receptor agonist showing a receptor affinity ($IC_{50}$ 29 nM) below that of β-CCM (2,5 nM). Interestingly besides some classical agonistic BZ such as diazepam or nordiazepam, lately by various groups found to occur naturally in plants, animals and man[2], now the coexistence with an inverse BZ receptor agonist is demonstrated in wheat and rat adrenals.

The origin and possible physiological function of the natural BZ receptor ligands are unclear. A possible meaning will be discussed.

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Antioxidants improve the *in vitro* survival of external granule layer cells of postnatal rats

Early postmitotic to premigratorial external granule layer cells were dissected from thick-slices of postnatal rats (P7), dissociated and cultivated in serum-free, chemically defined media. In order to improve the culture conditions we tested several oxygen-reducing measures because of the low expression of natural antioxidants in these cells occurring in situ.

The cells were sown into 24-well culture plates. After 2 days *in vitro* we determined the number of viable cells employing a very sensitive Fluorescein-assay, in which the amount of intracellularly trapped Fluorescein is a measure of the number of viable cells in the well.
With this assay we were able to overcome the statistical problems of taking random cell counts in dye-exclusion assays.
- The sandwich-culture technique was very useful with low cell densities (5.000 - 50.000 cells/$cm^2$) especially for morphological studies.
- By enriching the cell culture atmosphere with nitrogen and thereby reducing the oxygen concentration over the cells, it was possible to enhance the granule cell survival up to high cell densities (100.000 cells/$cm^2$).
- Supplementing the media with natural-occurring (in mature neurons) oxygen-detoxifying agents, such as superoxide-dismutase, catalase, reduced glutathione, vitamin E and others, we almost reached the viability-promoting "sandwich-effect", but in a wider cell-density range.
- Combining nitrogenizing and antioxidants we received the highest viabilities in our study.

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