THE AFFINITY OF CYCLODEPSIPEPTIDES TO THE BRAIN CHLORIDE CHANNEL DOMAIN OF THE $GABA_A$ RECEPTOR *in Vitro*

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The γ-aminobutyric acid ($GABA_A$) receptor is part of a supramolecular complex which is responsible for gating a chloride channel in the brain.[1] Several different classes of compounds interact with this complex, *e.g.* 1,4-benzodiazepines, barbiturates, some steroids, and *t*-butylbicyclophosphorothionate (TBPS). The convulsant picrotoxin[2] and the diterpenes carnosol and carnosic acid[3] bind to the same site as TBPS. In a search for fungal metabolites that bind to the TBPS site, extracts of more than 100 species were screened for their ability to inhibit ^{35}S-TBPS binding to a rat brain membrane homogenate preparation (unpublished results), with a sensitive radio-ligand assay.[4]

One of the most potent extracts was obtained from a *Fusarium* species, and bioassay-guided fractionation yielded four active components. These were analysed by high field NMR and mass spectroscopy, and found to be identical[5] to enniatin A (**1**), A1 (**2**), B1 (**3**) and B (**4**) (see Fig. 1 for structures). The IC_{50} values for the inhibition of the binding of ^{35}S-TBPS to rat cortical membranes *in vitro* for the four compounds are given in Table 1. Enniatin A (**1**) is the most potent of the four while enniatin B (**4**) only induced 20% inhibition at the highest concentration tested (40 μg/ml). In order to investigate the selectivity of enniatin A (**1**) it was assayed for the inhibition of the binding of ligands that bind to other receptors (see Table 2). At the concentration inhibiting TBPS binding by 50% (8 μg/ml) enniatin A (**1**) has no profound effect on the binding of diazepam, muscimol or kainic acid to their respective receptors, although the binding of SCH 23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol) to the dopamine D1 receptor is slightly inhibited (by 20%).

This finding, together with the fact that the potency of the compounds is rather low, indicate that the effect of the enniatins may result from non-specific membrane disruption. Besides the non-specific membrane activity reported in this paper, the enniatins possess a number of bioactivities,[6] similar to other cyclodepsipeptides such as the destruxins and valinomycin.[7]

Table 1. Inhibition of the binding of ^{35}S-TBPS to rat cortial membranes by compounds **1**~**4** *in vitro*.

Compound	IC_{50} (μg/ml)[a]
Enniatin A (**1**)	8
Enniatin A1 (**2**)	16
Enniatin B1 (**3**)	31
Enniatin B (**4**)	>40

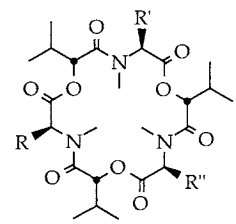
[a] The IC_{50} values were estimated by assaying 7 concentrations divided by a factor of 2 up to 40 μg/ml, single estimations.[3]

Table 2. The inhibition of enniatin A (**1**) of the binding of various labelled ligands to brain receptors *in vitro*.[a]

^{3}H Ligand	% of control
TBPS (see Table 1)	50
SCH 23390	80
Muscimol	91
Kainic acid	98
Diazepam	101

[a] 8 μg/ml, single estimations. Conditions as described in refs 3 and 4.

Fig. 1.



1 R=R′=R″=*s*-Butyl
2 R=R′=*s*-Butyl, R″=*i*-Propyl
3 R=*s*-Butyl, R′=R″=*I*-Propyl
4 R=R′=R″=*i*-Propyl

Experimental

The compounds were isolated from an EtOAc extract of the culture filtrate of a *Fusarium* sp., strain HA 43-88 collected in 1988 in Kaiserslautern from leaves of a walnut tree. The strain is deposited in the culture collection of the LB Biotechnologie, University of Kaiserslautern. The fungus was grown in a 20 liters fermentor as described previously,[8] and the culture medium was after filtration (to remove the mycelium) passed through a DIAION HP 21 polymer column. The absorbed materials were eluated with acetone that was evaporated, and the residue (containing some water) was extracted several times with EtOAc. The assay of the inhibition of binding of labelled CNS ligands was performed as described previously.[3,4]

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