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TWO POTENT CENTRAL CONVULSANT PEPTIDES, A BEE VENOM TOXIN, THE MCD PEPTIDE, AND A SNAKE VENOM TOXIN, DENDROTOXIN I , KNOWN TO BLOCK K^+ CHANNELS, HAVE INTERACTING RECEPTOR SITES

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SUMMARY. Both the bee venom toxin, mast cell degranulating peptide (MCD peptide) and the mamba toxin dendrotoxin I are potent central convulsants. The two specific receptor sites for these two types of polypeptide toxins are in allosteric interaction in brain membranes. Occupation of the dendrotoxin I binding site (K_I = 0.4 nM) prevents binding of the ^{125}I-MCD peptide to its own receptor (K_I = 0.23 nM). This inhibition is of the non-competitive type.

Autoradiography has shown that a high enough dendrotoxin I concentration (30 nM) prevented binding of ^{125}I MCD peptide to all brain structures where specific receptors had been identified. A lower concentration of the mamba toxin led to a nearly selective inhibition of MCD peptide binding to the hippocampal region which is responsible for the convulsant properties of the 2 types of polypeptide toxins.

INTRODUCTION. The mast cell degranulating peptide (MCD) is known to have a potent mast cell degranulating action (1) and a potent and selective action for the central nervous system (2). Intracerebroventricular injections in rodent brain cause stimulation of arousal at low concentrations (3) and convulsions and hyperactivity (2, 4) followed by death at higher doses (5). High affinity receptor (K_d = 150 pM) sites for MCD have been recently identified in synaptic membranes (6).

Mamba snake toxins from the dendrotoxin (DTX) family extracted from Dendroaspis polylepis and angusticeps venoms are also highly neurotoxic when injected intracerebroventricularly (7) and are potent convulsants (8, 9). A high affinity synaptosomal receptor (K_d = 0.3 - 0.5 nM) was also identified for this class of toxins (10, 11) and it has been recently demonstrated that dendrotoxin action was directly linked to blockade of one type of K^+ channel (12-15).

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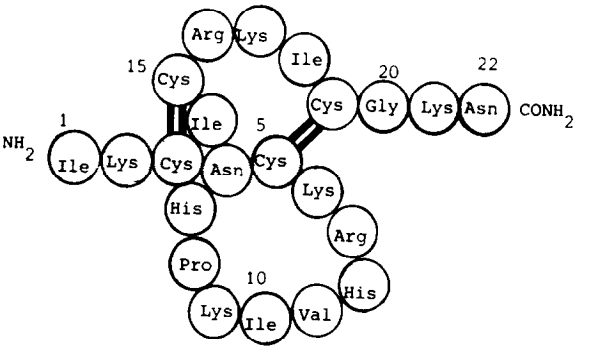
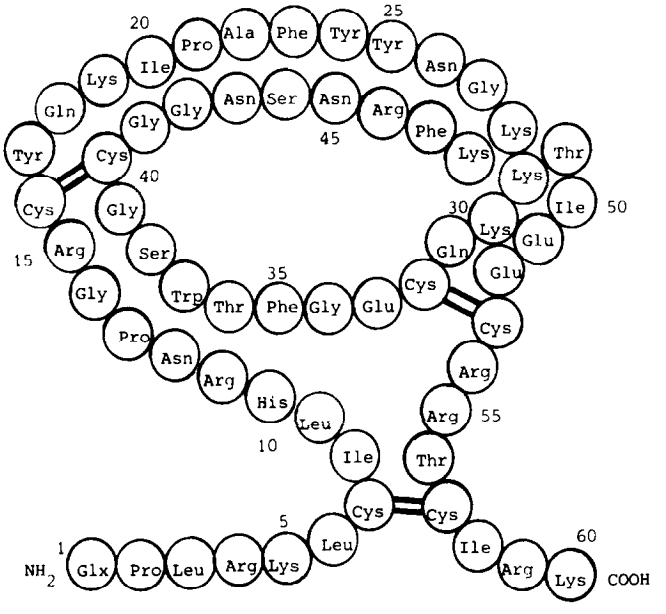


(MCD) MCD peptide from bee venom (Apis mellifera)



(DTX$_I$) Toxin I from snake venom (Dendroaspis polylepis polylepis)

Figure 1 . Structures of MCD peptide from bee venom (Apis mellifera) and of dendrotoxin I from snake venom (Dendroaspis polylepis polylepis) (21, 22).

In spite of their differences in structure (Fig. 1) both MCD and dendrotoxins produce similar symptoms of hyperexcitability following intracerebroventricular injection (2, 4, 7, 16).

The purpose of this work is to show that the bee venom toxin MCD and the most potent representative of the dendrotoxin family, in spite of their different structures, interact with the same target. They have different binding sites that are allosterically related.

MATERIALS AND METHODS. Toxin I (DTX_I) from Dendroaspis polylepis polylepis venom was a gift from Professor E. Karlsson (University of Uppsala). The mast cell degranulating peptide (MCD) and its monoiodo-labelled derivative were prepared as previously described (6). β-bungarotoxin (β-BTX) was from Serva. Synaptic brain membranes were prepared from 2-3-month-old male Wistar rats as described previously (6).

^{125}I-MCD binding to synaptic brain membranes. All binding assays of ^{125}I-MCD to brain membranes were carried out as previously described (6) using a centrifugation procedure. Bovine serum albumin (1 mg/ml) and compound 48/80 (10 µg/ml) were added in the physiological saline buffer to decrease the contribution of the non-specific binding component (measured in the presence of 100 nM MCD) down to less than 25% of the total binding.

^{125}I-MCD binding to brain sections. Whole rat brains were quickly removed and frozen in isopentane at -40°C. Serial 15 µm cryostat sections were collected and thaw-mounted onto cold chrom-alun/gelatin-coated glass slides and stored at -20°C until used. The sections were incubated for 30 min at 4°C with 33-36 pM ^{125}I-MCD in the standard buffer (described for the binding to brain membranes (6)) added with 100 µg/ml compound 48/80 and 10 µg/ml protamine chloride to reduce the non-specific binding. The non-specific binding component was determined by adding an excess of unlabelled MCD (500 nM) 15 min prior to addition of ^{125}I-MCD. Competition binding experiments were performed by adding an appropriate concentration of DTX_I 15 min prior to addition of the labelled ligand. At the end of the incubation, the sections were washed twice for 30 sec in the standard buffer and twice for 30 sec in water. A part of the slices were used to prepare and to quantify autoradiograms as previously described (17). The other labelled brain sections were removed and counted.

RESULTS AND DISCUSSION

Binding of ^{125}I-MCD to synaptosomal membranes and to brain sections in the presence of DTX_I. Specific binding of ^{125}I-MCD to synaptosomal membranes in the presence of unlabelled MCD and of the unlabelled snake toxins DTX_I and β-BTX is shown in Fig. 2A. As previously observed MCD inhibits binding of ^{125}I-MCD with a $K_{0.5}$ value of 0.25 nM. DTX_I inhibits MCD binding with a $K_{0.5}$ value of 0.16 nM. Scatchard plots for MCD and DTX_I inhibition of ^{125}I-MCD binding are also shown in Fig. 3. The data confirm the presence of a single ^{125}I-MCD binding site of high affinity (K_d = 40 pM) in these membranes. While MCD inhibition of ^{125}I-MCD binding was competitive as expected (no change in B_{max} values in the presence of increasing MCD concentrations) (Fig. 3, inset) the inhibition by DTX_I was of the non-competitive type (Fig. 3, main panel). Increasing concentrations of DTX_I did not change the slope of the Scatchard plot and only changed B_{max} values. A simple calculation using the equation $B_{max}/B_{max_I} = 1 + [I]/K_I$ where B_{max_I} is the B_{max} value at a given concentration of inhibitor ([I]) and K_I is the true dissociation constant of the DTX_I-receptor complex provides a calculated value of K_I of 0.4 nM.

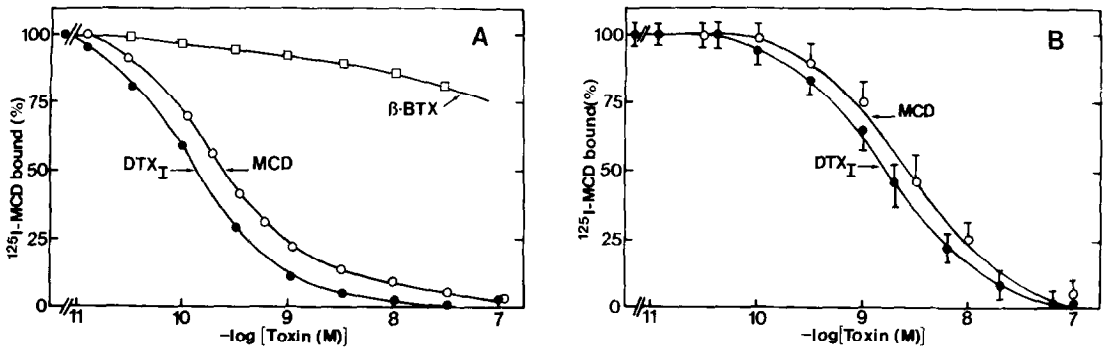


Figure 2. Inhibition by DTX$_I$ of specific ^{125}I-MCD binding to synaptosomal membranes (A) and brain sections (B). Panel (A). Membranes (150 µg protein/ml) were incubated at 4°C for 20 min in the presence of 4 pM ^{125}I-MCD and of various concentrations of β-BTX (□), DTX$_I$ (●) and MCD (○). The non-specific binding component, determined by including 100 nM MCD in the incubation medium, was subtracted from the total binding. **Panel (B).** Brain sections (240-280 µg protein) were incubated at 4°C for 30 min with 33 pM ^{125}I-MCD and various concentrations of DTX$_I$ (●) and MCD (○). Non-specific binding, determined by including 500 nM MCD in the incubation medium, was subtracted from the total binding. The average of triplicate specific ^{125}I-MCD binding (% of the maximum ± S.D.) is then plotted as a function of the toxin concentration in a semilogarithmic plot.

Specific binding of ^{125}I-MCD to brain sections was inhibited by MCD itself and by DTX$_I$ (Fig. 2B). Half-maximal inhibition was seen at 2.7 nM for MCD and at 1.7 nM for DTX$_I$.

Localization of specific receptor sites for ^{125}I-MCD in rat brain that are sensitive to DTX$_I$. Autoradiograms after equilibrium incubation of rat brain sections in the presence of ^{125}I-MCD (36 pM) show an heterogeneous localization of MCD receptor site (not shown).

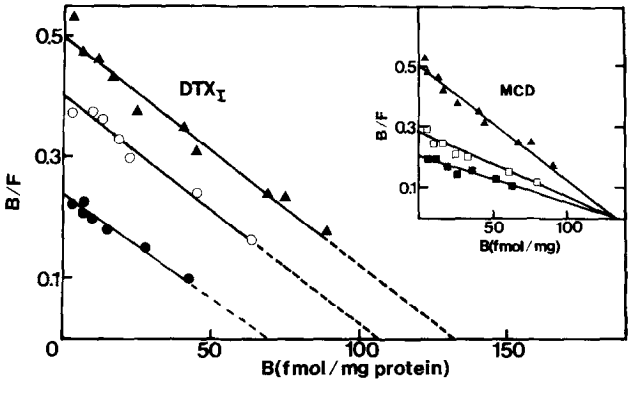


Figure 3. Scatchard analysis of saturable binding to synaptosomal membranes. Inhibition by DTX$_I$. Synaptic membranes (150 µg protein/ml) were incubated with various concentrations of ^{125}I-MCD (1-200 pM). **Main panel.** DTX$_I$ was added at concentrations (nM) 0 (▲), 0.1 (○) and 0.32 (●). **Inset.** MCD was added at concentrations (nM) 0 (▲), 0.25 (□) and 0.5 (■). Specifically bound ^{125}I-MCD was quantified for duplicate samples and the Scatchard analysis of the free (F) and bound (B) ^{125}I-MCD concentrations were calculated.

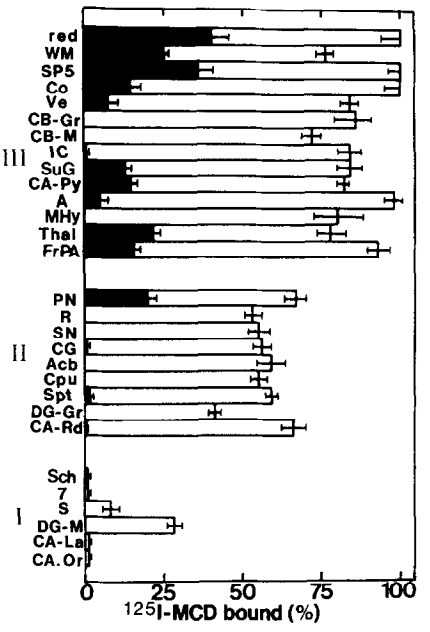


Figure 4 . Distribution of the specific ^{125}I-MCD binding inhibited by DTX_I in rat brain. Specific ^{125}I-MCD (36 pM) binding was measured in the presence of DTX_I at 2 nM (open bars) or at 6 nM (shaded bars) or at 30 nM (0% of binding, not shown). Three groups of structures have been separated depending on the extent of DTX_I inhibition of specific ^{125}I-MCD binding (I, II, III). The data are mean ± SEM of eight autoradiographic measurements. Abbreviations : red, red nucleus; WM, white matter of the cerebellum; SP5, nucleus of the spinal tract of the trigeminal nerve; Co, cochlear nucleus; Ve, vestibular nuclei; CB, cerebellum; -Gr, -granular layer; -M, -molecular layer; IC, inferior colliculus; SuG, superior colliculus; CA, Ammon's horn; -Py, pyramidal layer; A, amygdala; MHy, median hypothalamus; Thal, thalamus; FrPA, frontoparietal cortex; PN, pons; R, raphe pontis nucleus; SN, substancia nigra; CG, central grey; Acb, accumbens nucleus; Cpu, caudate putamen; Spt, septum; DG, dentate gyrus; -Rd, stratum radiatum; Sch, suprachiasmatic nucleus; 7, facial nuclei; S, subiculum; -La, -stratum lacunosum moleculare; -Or, -stratum oriens.

The autoradiographic analysis of DTX_I inhibition of ^{125}I-MCD binding is presented in Fig. 4. The extent of the prevention of ^{125}I-MCD (36 pM) binding by DTX_I (2 nM and 6 nM) is different in various brain structures. Three groups of structures can be artificially separated. In group I, DTX_I (2 nM) prevents specific ^{125}I-MCD binding by more than 70%. This is observed for the hippocampal formation. In group II, specific ^{125}I-MCD binding is prevented by DTX_I (2 nM) in an extent of 30 to 70%. In structures belonging to group III, ^{125}I-MCD binding is prevented to less than 30% by DTX_I (2 nM). When DTX_I was used at a concentration of 30 nM, all specific binding of ^{125}I-MCD to all brain regions was prevented (not shown).

A series of recent papers (12-15) indicate that snake neurotoxins extracted from Mamba venom (Dendroaspis angusticeps and Dendroaspis polylepis) block voltage-

sensitive K^+ channels. The most active representative of these dendrotoxins, DTX_I extracted from Dendroaspis polylepis, blocks one class of the different K^+ channels present in the node of Ranvier (I_{Kf}) (12). Dendroaspis angusticeps dendrotoxin blocks K^+ channels (I_A current) in CA_1 neurons of hippocampus (15) and non or slowly inactivating K^+ channels in dorsal root ganglion neurons (13) and visceral sensory neurons (14).

Symptoms observed after intracerebroventricular injection of MCD and of dendrotoxin are similar. Both peptides are potent central convulsants (2-5, 7).

The work presented in this paper indicates that DTX_I is a potent inhibitor of ^{125}I-MCD binding. Occupation of the high affinity binding site of DTX_I (K_I = 0.15-0.4 nM) clearly prevents binding of MCD at the MCD binding site (K_I = 0.23 nM). The bee and the snake toxin bind to two distinct but interacting receptor sites. This type of situation is well known for the Na^+ channel which has at least six different receptor sites for different types of toxins (polypeptides or non polypeptides), many of them being in mutual interaction (18). All the identified Na^+ channel toxin receptors are situated in the same polypeptide chain (18). Also for the slow type of Ca^{2+} channel, the 1,4-dihydropyridine receptor and the phenylalkylamine receptors are both situated on the same protein (19). It may be that both the MCD and the DTX_I receptors are situated on the same macromolecular structure responsible for K^+ channel activity.

The snake venom toxin β-bungarotoxin was found to partially inhibit (75%) the saturable binding of ^{125}I-DTX (at concentrations between 0.1 and 10 nM) and DTX also produced partial inhibition (50-80%) of [^3H] or [^{125}I] labelled β-bungarotoxin (16, 20). β-bungarotoxin is clearly without marked effect on ^{125}I-MCD binding at concentrations lower than 30 nM (Fig. 2A).

Results obtained for ^{125}I-MCD binding to intact brain slices were qualitatively similar to those obtained with synaptic membranes but they were quantitatively different. Half-maximal inhibition for prevention of ^{125}I-MCD binding are seen at concentrations of 2.7 nM for MCD and of 1.7 nM for DTX_I i.e. nearly 7 times higher than those necessary in experiments carried out with membranes. Nevertheless, the relative efficacy of MCD and DTX_I in inhibiting ^{125}I-MCD binding remained the same.

When DTX_I was used at a concentration of 2 nM (corresponding to half-maximal inhibition in Fig. 2B), it inhibited very preferentially binding of ^{125}I-MCD to the

hippocampal region : stratum oriens and lacunosum moleculare of the Ammon Horn, the molecular layer of dentate gyrus and the subiculum (Fig. 4). In addition to the hippocampal formation, drastic inhibitory effects by DTX_I were observed in the suprachiasmatic nucleus and in the facial nucleus. In other brain structures, the inhibition was less marked. These data indicate a particularly efficient interaction between MCD and DTX_I binding sites in hippocampus. This observation is of a particular interest since both toxins have a drastic action on hippocampal electrogenesis (3, 15) which is the main component of their central action (3).

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