5′-GTATGTTG-3′ (wt) → 5′-GTATATTG-3′ (Ps) → 5′-GTATAATG-3′ (UV-5)
3′-CATACAAC-5′ 3′-CATATAAC-5′ 3′-CATATTAC-5′

Another interesting observation from our data set in this regard is that adjacent sequences occasionally act to abolish what should be a preferred binding site. One such region (Figure 2, right) may assume the Z conformation in supercoiled plasmids (Barton & Raphael, 1985). In general, however, the interactions of $[(Phen)_2Cu^I]^+$ can best be rationalized in terms of present knowledge of effects of sequence on the local conformation of B-form DNA.

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The Receptor Site for the Bee Venom Mast Cell Degranulating Peptide. Affinity Labeling and Evidence for a Common Molecular Target for Mast Cell Degranulating Peptide and Dendrotoxin I, a Snake Toxin Active on K^+ Channels†

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ABSTRACT: The mast cell degranulating peptide (MCD) and dendrotoxin I (DTX_I) are two toxins, one extracted from bee venom, the other one from snake venom, that are thought to act on voltage-sensitive K^+ channels. Binding sites for the two toxins have been solubilized. The solubilized sites were stable and retained their high affinity for ^{125}I-DTX_I and ^{125}I-MCD ($K_d \simeq 100$ pM). Interactions were found between MCD and DTX_I binding sites in the solubilized state, establishing that the two different toxins act on the same protein complex. This conclusion was strengthened by the observations (i) that conditions of solubilization that eliminated ^{125}I-MCD binding activity also eliminated ^{125}I-DTX binding activity while both types of activities were preserved in the presence of K^+ or Rb^+ and (ii) that binding components for the two types of toxins had similar sedimentation coefficients and copurified in partial purifications. A component of the receptor protein for ^{125}I-MCD has been identified; it has a M_r of 77 000 ± 2000. This polypeptide was similar to or identical in molecular weight with that which serves as a receptor for DTX_I (M_r 76 000 ± 2000).

The mast cell degranulating peptide (MCD)[1] is a basic toxin of 22 amino acids (Habermann, 1972). Aside from its mast cell degranulating activity, the peptide also acts in the central nervous system. Depending on the concentrations injected, MCD induces hippocampal θ rhythms associated with arousal

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[1] Abbreviations: MCD, mast cell degranulating peptide; DTX_I, dendrotoxin I; DMS, dimethyl suberimidate; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; WGA, wheat germ agglutinin.

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or/and convulsions and epileptogenic crisis (Bidard et al., 1987a). Recent electrophysiological evidence has shown that the peptide induces hippocampal long-term potentiation, which is usually associated with information storage and memory (Cherubini et al., 1987).

Dendrotoxins are neurotoxins of the venom of *Dendroaspis* snakes (Harvey et al., 1984). These basic polypeptides (M_r ~7000) are homologous to protease inhibitors and facilitate evoked neurotransmitter release (Harvey & Karlsson, 1982; Harvey & Anderson, 1985). Recent research has provided data strongly suggesting that blockade of K^+ channels accounts for the facilitatory effects of the toxins (Penner et al., 1986; Weller et al., 1985). DTX_I[1] is the most potent member of the dendrotoxin family known until now.

High-affinity MCD binding sites (Taylor et al., 1984) have been identified in brain membranes, and it has been shown recently that DTX_I was capable of inhibiting ^{125}I-MCD binding to both brain membranes and slices (Bidard et al., 1987b). The inhibition was particularly potent at the level of hippocampus (Bidard et al., 1987b).

The purpose of this paper is to establish definitely by use of the solubilized toxin receptor that the snake toxin DTX_I and the bee venom toxin MCD are both binding to the same protein complex, which is presumably closely associated with a K^+ channel. The polypeptide chains bearing the MCD and DTX_I binding sites have been identified in cross-linking experiments.

Experimental Procedures

Materials. MCD was prepared as previously described (Taylor et al., 1984; Bidard et al., 1987b). DTX_I was purified from the venom of *Dendroaspis polylepis* by gel filtration on Sephadex G-50, followed by ion-exchange chromatography on a Spherogel TSK SP 5PW column. Minor impurities were eliminated by reverse-phase chromatography on a Lichrosorb RP-18 column from Merck, with a linear gradient between 10% and 40% acetonitrile. The biological activity of the DTX_I preparation was checked by its toxicity after intraventricular injection into mouse brain (lethal dose of 4 ng/g of body weight) (Schweitz, 1984). The polyamine compound 48/80 that stimulates mastocytes degranulation was from Sigma.

Iodination of MCD and DTX_I. ^{125}I-MCD was prepared as previously described (Taylor et al., 1984). DTX_I (3 nmol) was incubated for 15 min at room temperature with radiolabeled ^{125}INa (1 nmol) in an Eppendorf tube containing Iodogen (Pierce) (3 nmol) and 100 mM Tris-HCl buffer at pH 7.5. The mixture was loaded on a SP-Sephadex C-25 column (5 mL) equilibrated with 20 mM Tris-HCl buffer at pH 9.4 containing 1 mg/mL bovine serum albumin, 3 mM sodium azide, and 80 mM NaCl. The column was eluted stepwise with 30 mL of the equilibration buffer and then with a buffer containing 355 mM NaCl instead of 80 mM NaCl. The procedure separated native and iodinated peptides. LD_{50} values determined by intracisternal injections were nearly identical for ^{125}I-DTX_I and unlabeled DTX_I.

Preparation of Detergent Extract. P3 membranes from rat brain prepared as described by Taylor et al. (1984) were suspended in a 10-fold volume of 20 mM NaHepes buffer at pH 7.4 containing 120 mM KCl, 2 mM $CaCl_2$, 2 mM $MgSO_4$, 10% (v/v) glycerol, 1% (w/v) Triton X-100, and 0.2% (w/v) soybean phospholipid (buffer A) to a protein concentration of 4–5 mg/mL. A volume ($^1/_{10}$) of 20% (w/v) Triton X-100 containing 0.1 mM EDTA, pH 7.4, was then added and the suspension incubated for 1 h at 4 °C. The suspension was centrifuged for 15 min at 10000*g* and the supernatant for another 60 min at 150000*g*. The supernatant of the latter centrifugation is referred to as detergent extract and was used for binding assays. Protein concentrations were determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Binding Assays. ^{125}I-DTX_I and ^{125}I-MCD binding to detergent extracts was determined by a modification of the filter assay described by Bruns et al. (1983). A sample (2–50 μL) of detergent extract was adjusted to a volume of 190 μL with buffer A. ^{125}I-DTX_I or ^{125}I-MCD peptide was added in 10 μL of buffer A, and the mixture was incubated for 20 min at 4 °C. To measure nondisplaceable binding (hereafter referred to as nonspecific binding), parallel incubation mixtures contained in addition 100 nM unlabeled DTX_I or 1 μM unlabeled MCD for ^{125}I-DTX_I or ^{125}I-MCD peptide binding, respectively. Incubation mixtures (total volume 200 μL) were then diluted with 5–6 mL of ice-cold buffer A and filtrated through GF/B filters coated with 0.5% poly(ethylenimine) at pH 7.5. The filters were then counted in a γ-counter. All binding data were corrected for nonspecific binding and represent the mean of triplicate determinations.

Sucrose Density Centrifugation. Linear sucrose gradients [3–20% (w/v)] were prepared in buffer A or in buffer A containing only 4% (v/v) glycerol. The detergent extract (800 μL) was layered on top, and the gradients were centrifuged in a Spinco SW41 Ti rotor at 35000 rpm and 4 °C for 17 h. Fractions of 0.8 mL were collected and assayed for ^{125}I-DTX_I and ^{125}I-MCD peptide binding. Bovine liver catalase (11.3 S) was detected by enzymatic assay.

Partial Purification of DTX_I and MCD Binding Sites. A column of DEAE-Affi-Gel blue (0.5 × 8.8 cm) was equilibrated with buffer A containing 1 mM EDTA instead of $CaCl_2$ and $MgSO_4$ (buffer B). The detergent extract (40–100 mL) was then loaded onto the column. The column was washed with 100 mL of buffer B and eluted with buffer B containing 180 mM KCl instead of 120 mM.

Fractions containing the binding activity were pooled and loaded onto a column of 8 mg/mL wheat germ agglutinin (WGA) coupled to Affi-Gel 10, which had been equilibrated with buffer B. The column was washed with 15 mL of buffer B and then eluted with buffer B containing 50 mM *N*-acetylglucosamine and 0.1% (w/v) Triton X-100 instead of 1% (w/v).

Cross-Linking of Iodinated Toxins to Rat Brain Synaptic Membrane. ^{125}I-MCD (2 nM, 500 Ci/mmol) or ^{125}I-DTX_I (0.2 nM, 2000 Ci/mmol) was incubated for 30 min at 4 °C with P_3 membranes (0.5 mg of protein) in the absence or in the presence of various concentrations of unlabeled MCD or DTX_I in a total buffer volume of 500 μL. The buffer used for ^{125}I-MCD was 20 mM Tris-HCl, pH 7.5, containing 140 mM NaCl, 1.3 mM $MgSO_4$, 5 mM KCl, 2.8 mM $CaCl_2$, 1 mg/mL bovine serum albumin, and 10 μg/mL compound 48/80. The buffer used for cross-linking experiments with ^{125}I-DTX_I was 50 mM Tris-HCl, pH 7.5, containing 140 mM NaCl, 1.3 mM $MgSO_4$, 5 mM KCl, and 1 mg/mL bovine serum albumin. The samples were then centrifuged at 10000*g* for 5 min and washed twice at 4 °C with 500 μL of 50 mM phosphate buffer, pH 8.5. The membranes were resuspended in 500 μL of the latter buffer containing the indicated concentrations of dimethyl suberimidate (DMS)[1] (usually at a final concentration of 0.5 mg/mL). Following incubation at 4 °C for 20 min, the reaction was stopped by washing the membranes repeatedly with 100 mM Tris-HCl buffer at pH 7.4. The membranes were then solubilized in 50 μL of solubilizer (Laemmli, 1970) containing 10 μg/mL soybean trypsin inhibitor, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM

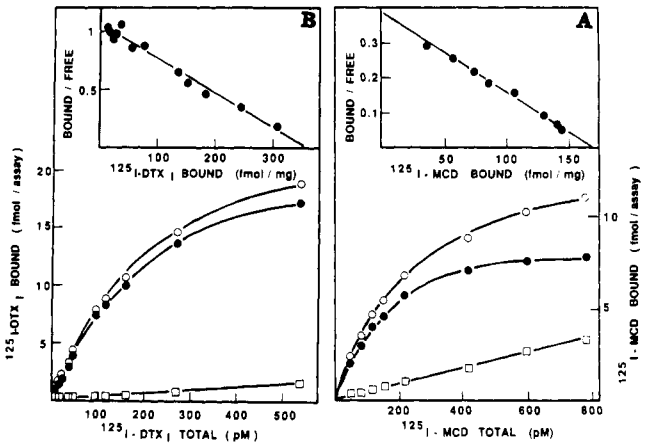

FIGURE 1: Binding of ^{125}I-DTX$_I$ and ^{125}I-MCD peptide to a detergent extract (40–50 μg of protein) of rat brain membranes. (A) Binding of ^{125}I-MCD peptide. Specific binding (●) is the difference between total binding (○) and nonspecific binding (□). Inset: Scatchard plot of specific binding. (B) Binding of ^{125}I-DTX$_I$. Legend as for (A).

o-phenanthroline, and 1 mM iodoacetic acid and analyzed by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) (4% stacking gel, 8% separating gel). Molecular weight standards were carbonic anhydrase (31 000), ovalbumin (45 000), bovine serum albumin (66 200), phosphorylase B (92 500), β-galactosidase (116 250), and myosin (200 000). After being stained with Coomassie blue R250 (Bio-Rad), gel slabs were dried and autoradiographed with X-Omat AR Kodak diagnostic film.

RESULTS

Binding of ^{125}I-DTX$_I$ and ^{125}I-MCD Peptide to Detergent Extract of Brain Membranes. Evidence for a Maintained Interaction between the Snake and the Bee Venom Toxin Receptor Sites in the Solubilized State. Triton X-100 extracts of rat brain membranes, prepared in the presence of K^+ and glycerol, showed saturable binding of ^{125}I-DTX$_I$ and ^{125}I-MCD peptide (Figure 1). The binding activity of the detergent extract was stable with a half-life of 3–4 days at 4 °C (not shown). Scatchard analysis (Figure 1, insets) of the binding revealed, for each peptide, a single class of high-affinity binding sites (K_D = 96 pM for ^{125}I-DTX$_I$ and K_D = 115 pM for ^{125}I-MCD peptide). The maximal number of toxin binding sites in the detergent extract was 356 fmol/mg for ^{125}I-DTX$_I$ and 168 fmol/mg for ^{125}I-MCD peptide. These data suggest the presence of about two DTX$_I$ binding sites per MCD binding site.

Association and dissociation kinetics of ^{125}I-DTX$_I$ and ^{125}I-MCD binding to its solubilized binding sites were also determined. ^{125}I-DTX$_I$ (180 pM) bound to its solubilized site (0.355 mg/mL protein) with a half-time of association of 38 s. Binding was essentially complete after 6 min. Dissociation of ^{125}I-DTX$_I$ from its solubilized site, in the presence of an excess of unlabeled toxin, had a half-time of 7.7 min. Kinetics of ^{125}I-MCD binding were faster with a half-time of association of 12 s (21 pM ^{125}I-MCD, 0.171 mg/mL protein) and a half-time of dissociation of 45 s.

Figure 2 shows that DTX$_I$, as MCD itself, inhibits ^{125}I-MCD binding to the detergent extract and that MCD, as DTX$_I$ itself, inhibits ^{125}I-DTX$_I$ binding. Half-maximal inhibitions of ^{125}I-MCD binding were observed at $K_{0.5}$ = 3.2 nM for DTX$_I$ and at $K_{0.5}$ = 0.56 nM for MCD. Half-maximum inhibitions of ^{125}I-DTX$_I$ binding were observed at $K_{0.5}$ = 0.35 nM for DTX$_I$ and $K_{0.5}$ = 7.5 nM for MCD. Other basic toxins that block other types of ionic channels such as apamin, which blocks Ca^{2+}-activated K^+ channels (Hugues et al., 1982), or

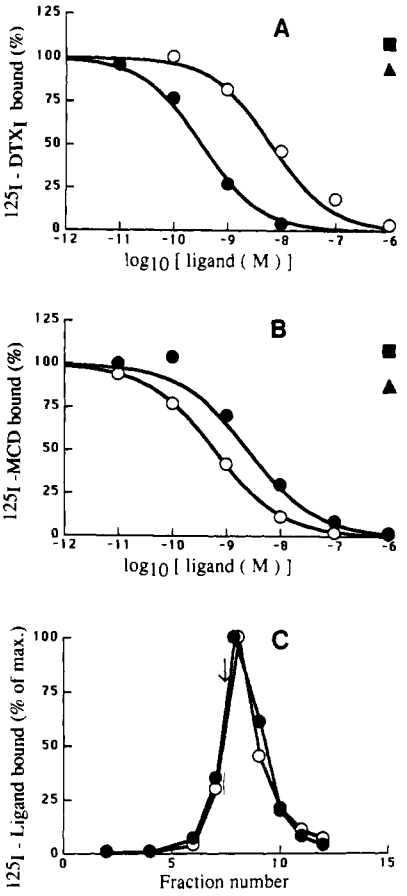

FIGURE 2: (A and B) Effects of MCD and DTX$_I$ on peptide binding specific of ^{125}I-DTX$_I$ and ^{125}I-MCD to detergent extract from brain membranes. Binding of ^{125}I-DTX$_I$ (84 pM) (A) or ^{125}I-MCD peptide (79 pM) (B) to detergent extract (270 μg/mL of protein) in the absence (control) or presence of DTX$_I$ (●), MCD peptide (○), apamin (■), and ATX$_{II}$ (▲). (C) Sucrose gradient [3–20% (w/v)] centrifugation of solubilized ^{125}I-DTX$_I$ (●) and ^{125}I-MCD (○) binding components. The arrow marks the position of catalase.

Anemonia sulcata toxin II, an effector of the voltage-dependent Na^+ channel (Vincent et al., 1980), were without effect on the binding of both ^{125}I-DTX$_I$ and ^{125}I-MCD at concentrations as high as 1 μM (Figure 2). The mast cell degranulating agent, compound 48/80 (50 μg/mL) was also without effect on binding of the two iodinated toxins. DTX$_I$ is a more potent inhibitor of ^{125}I-MCD binding to synaptosomal membranes and brain sections than MCD itself (Bidard et al., 1987b). The order of potency is reversed after solubilization, suggesting that the allosteric interaction between DTX$_I$ and MCD binding sites is slightly different in the solubilized state.

Receptors for ^{125}I-DTX$_I$ and ^{125}I-MCD Have Similar Stability and Similar Sedimentation Behaviors and Copurify. Solubilization of both ^{125}I-DTX$_I$ and ^{125}I-MCD receptors in an active conformation requires the presence of K^+ and Rb^+ ions (Table I). Table I shows that conditions which stabilize one of the toxin receptors stabilize the other one to the same extent. The exchange of K^+ for Li^+, Na^+, or Cs^+ ions in the solubilization procedure leads to disappearance of the binding activity for both ligands in parallel.

Figure 2C shows that the solubilized ^{125}I-DTX$_I$ and ^{125}I-MCD binding sites could not be separated in sucrose gradient centrifugation, indicating similar sedimentation coefficients. A reduction of the glycerol concentration in the gradient from 10 to 4% resulted in a higher migration speed. However, again no separation of the ^{125}I-DTX$_I$ and ^{125}I-MCD binding sites was observed (not shown).

Table I: Effects of Various Solubilization Conditions on the ^{125}I-DTX$_I$ and ^{125}I-MCD Peptide Binding Activity of Detergent Extracts of Rat Brain Membranes[a]

ion conditions during solubilization	^{125}I-DTX bound (% of control)	^{125}I-MCD bound (% of control)
control (120 mM KCl)	100 ± 2.0	100 ± 7.0
40 mM KCl	58 ± 2.6	68 ± 14.0
120 mM LiCl	<1	<1
120 mM NaCl	<1	4 ± 2.4
120 mM RbCl	62 ± 3.0	73 ± 6.3
120 mM CsCl	2 ± 0.6	<1
120 mM KCl, 1 mM EDTA	115 ± 3.6	116 ± 10.4

[a] Equal amounts of rat brain membranes were extracted with Triton X-100 in buffer A containing the indicated ions instead of 120 mM KCl (as described under Experimental Procedures). When EDTA (1 mM) was added, $CaCl_2$ and $MgSO_4$ were omitted. The binding activity of the extracts was then determined in buffer A. The data are given as mean ± SD from three experiments.

Although the complete purification of either ^{125}I-DTX$_I$ or ^{125}I-MCD binding components has not yet been carried out, a partial purification was obtained. Table II shows that ^{125}I-DTX$_I$ and ^{125}I-MCD binding sites copurify in two (independent) purification steps. Both sites seem to be located on acidic glycoproteins since they are retained on DEAE-Affi-Gel and on a WGA column.

Labeling of ^{125}I-MCD and ^{125}I-DTX$_I$ Binding Sites Using Cross-Linking Experiments. Cross-linking experiments with the two ^{125}I-labeled toxins were carried out with DMS (Figures 3 and 4). Cross-linking experiments with ^{125}I-MCD in the presence of different DMS concentrations identified a single polypeptide component of M_r 80000 (Figure 3, track h; Figure 4A, track a). Similar experiments with ^{125}I-DTX$_I$ revealed the specific labeling of a single component of M_r 83000 (Figure 3, track k; Figure 4B, track a). At 2.5 mg/mL DMS, several minor components were labeled in addition to the main component at M_r 83000 (Figure 3, track l). However, at this cross-linker concentration, the electrophoretic pattern of membrane proteins was changed (not shown). The DMS concentration that was routinely used (0.5 mg/mL) did not produce detectable changes in the electrophoretic pattern of membrane proteins. No labeling was detected with denatured membranes (Figure 3, tracks a–d) or in conditions where membranes were omitted (Figure 3, tracks e–i).

Experiments shown in parts A and B of Figure 4 demonstrate the specificity of the covalent labeling obtained with ^{125}I-MCD and ^{125}I-DTX$_I$. Incubation of brain membranes with ^{125}I-MCD (Figure 4A) in the presence of increasing concentrations of unlabeled MCD (tracks a–c) or DTX$_I$ (tracks d–f) resulted in a concentration-dependent protection against the labeling of the M_r 80000 band. Covalent labeling of the band was abolished with 100 nM MCD (track c) or 10 nM DTX$_I$ (track e). Likewise, for ^{125}I-DTX$_I$ cross-linking experiments (Figure 4B), increasing concentrations of unlabeled DTX$_I$ (tracks a–f) or MCD (tracks g–i) gave a concentration-dependent protection against the labeling of the M_r 83000 band.

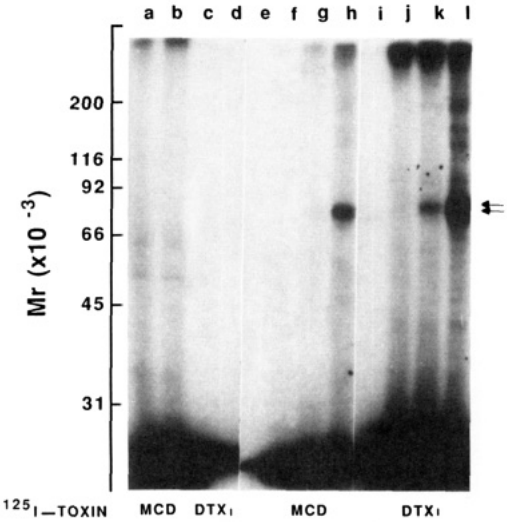

FIGURE 3: Cross-linking of ^{125}I-MCD or ^{125}I-DTX$_I$ to rat brain membranes. Cross-linking experiments carried out with heat-denatured membranes (0.5 mg of protein boiled for 10 min) (tracks a and c, ^{125}I-toxin alone; tracks b and d, ^{125}I-toxin in the presence of the unlabeled toxin). Cross-linking under condition of absence of membranes (tracks e and i, ^{125}I-toxin alone). In all these experiments, DMS was at 0.5 mg/mL. Tracks f–h (^{125}I-MCD) and j–l (^{125}I-DTX$_I$) correspond to the labeling of intact membranes obtained with ^{125}I-toxins in the presence of different DMS concentrations: 0 mg/mL (tracks f and j), 0.1 mg/mL (tracks g and k), and 2.5 mg/mL (tracks h and l).

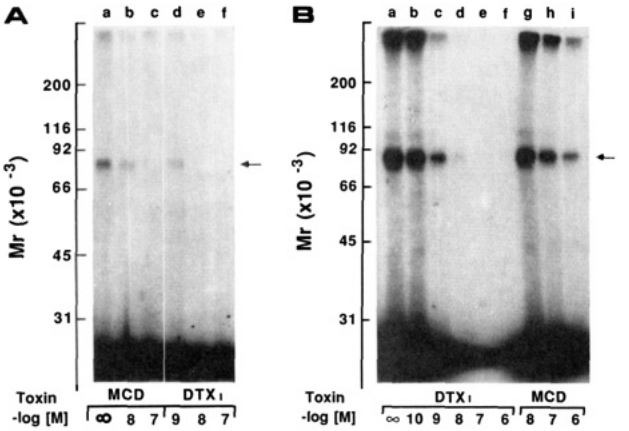

FIGURE 4: Protection against cross-linking (0.5 mg/mL DMS) of ^{125}I-MCD (panel A) or ^{125}I-DTX$_I$ (panel B) to synaptic membranes by unlabeled MCD and DTX$_I$. Membranes (0.5 mg of protein) were incubated with the ^{125}I-labeled toxin in the presence of the indicated concentrations of unlabeled MCD or DTX$_I$. The specifically labeled bands are indicated by arrows.

Cross-linking experiments with ^{125}I-MCD were also carried out with disuccinimidyl suberate instead of DMS (data not shown). This treatment also incorporated ^{125}I-MCD into a M_r 80000 band.

Discussion

Behavioral and electrocorticographic responses following intracerebroventricular injections of various doses of MCD

Table II: Copurification of ^{125}I-DTX and ^{125}I-MCD Peptide Binding Sites[a]

		^{125}I-DTX binding sites			^{125}I-MCD peptide binding sites		
	total protein (mg)	sp act.[b]	recovery (%)	purification (x-fold)	sp act.[b]	recovery (%)	purification (x-fold)
detergent extract	115.6	64	100	1	37	100	1
eluate from DEAE-Affi-Gel blue	3.16	1115	47.4	17.3	831	60.7	22.2
eluate from WGA column	0.60	2327	18.8	36.1	992	16.3	31.5

[a] Binding assays with 6.5 fmol of ^{125}I-DTX/assay and 7 fmol of ^{125}I-MCD peptide/assay and column chromatographies performed as described under Experimental Procedures. [b] Specific activity is given in femtomoles per milligram of iodinated peptide bound.

have shown that the peptide produces a quasi-permanent hippocampal θ rhythm associated with an increase in the level of wakefulness before epileptic discharges are induced (Bidard et al., 1987a). The long-lasting quasi-permanent presence of θ rhythm induced by MCD in the quiet and motionless animal seems to be unique to this bee venom toxin. This electrocorticographic response appears to be closely associated with the observation that a brief application of MCD to the CA_1 region of hippocampal slices induces long-term potentiation (Cherubini et al., 1987), i.e., a long-lasting increase in the efficacy of synaptic transmission considered to be related in some way to memory. For all these reasons, and also because MCD appears to have an endogeneous equivalent in the brain (Cherubini et al., 1987), it appeared that it would be useful to have more information about the receptor site of this interesting bee venom peptide. The function of the MCD receptor is not well identified at the molecular level although preliminary information has been reported indicating that the peptide blocks voltage-dependent K^+ channels in hippocampal slices and nodose ganglia (Dolly et al., 1987).

More information is available concerning another family of excitatory polypeptide toxins extracted from the venom of snakes of the *Dendroaspis* species: dendrotoxins. Dendrotoxins facilitate transmitter release and induce repetitive firing (Harvey & Karlsson, 1982; Harvey & Anderson, 1985). The physiological effects of this type of toxin seem to be due to its potency to block voltage-dependent K^+ channels (Weller et al., 1985; Benoit & Dubois, 1986; Halliwell et al., 1986; Penner et al., 1986).

The observation has been made previously in this laboratory that DTX_I inhibits ^{125}I-MCD binding both to brain membranes and to brain slices (Bidard et al., 1987b). This allosteric inhibition could have involved different receptor molecules with their mutual interaction necessitating the integrity of the membrane. This paper shows (i) that conditions of solubilization can be found with which one can preserve the binding activity of both ^{125}I-DTX_I and ^{125}I-MCD with a high affinity and (ii) that inhibition of ^{125}I-MCD binding by DTX_I persists in the solubilized state and that one can also observe in parallel inhibition by MCD of ^{125}I-DTX_I binding. The latter observation suggests that both toxins bind to the same target that, considering the well-known action of DTX_I, is associated with K^+ channel functioning. The conclusion that a single protein assembly is responsible for the binding of both ^{125}I-MCD and ^{125}I-DTX_I is strengthened by a series of observations which have shown (i) that the activity of receptors for both toxins disappears in parallel in solubilization conditions in which K^+ or Rb^+ ions are replaced by Na^+, Li^+, or Cs^+ ions, (ii) that MCD and DTX_I receptors have a similar sedimentation behavior in a sucrose gradient centrifugation, and (iii) that MCD and DTX_I receptors copurify in a partial purification protocol.

Labeling of the MCD receptor has been achieved in cross-linking experiments with suberimidate derivatives. A specifically labeled polypeptide was identified with a M_r of 80 000 ± 2000. Assuming the binding of one MCD (M_r 2600) molecule per receptor, one then finds a M_r of 77 000 ± 2000 for the MCD receptor.

Dendrotoxin from *Dendroaspis angusticeps*, a less active analogue of DTX_I, has been previously cross-linked to its protein receptor in chick synaptic membranes, and a complex with a M_r of 82 000 was observed (Black & Dolly, 1986). Results found in the present work with DTX_I lead to the identification of a specifically labeled polypeptide chain of M_r 83 000 ± 2000 in rat brain membranes. Again assuming a 1:1 relationship for the toxin (M_r 7100)–receptor association, one finds that the polypeptide chain that binds DTX_I has a M_r of 76 000 ± 2000.

Obviously there is a high similarity in molecular weights for the two polypeptides that serve as receptors for MCD and DTX_I, respectively. Moreover, and as expected, cross-linking of ^{125}I-MCD to its receptor was prevented by DTX_I, and cross-linking of ^{125}I-DTX_I to its receptor was prevented by MCD. A first, simple explanation of these results would be that the same polypeptide chain of M_r 76 000–77 000 contains binding sites for both MCD and DTX_I. However, the possibility that the bee venom and the snake venom toxins bind to two different but sufficiently homologous polypeptide chains to have similar molecular weights cannot and should not be eliminated especially if there are two DTX_I binding sites for one MCD binding site. The latter interpretation would imply that the two polypeptides are associated within the same protein complex to explain the allosteric interactions between MCD and DTX_I receptors.

β-Bungarotoxin is another snake toxin active on neurosecretion (Howard & Gundersen, 1980) that appears to block K^+ channels (Dreyer & Penner, 1987). Allosteric relationships between β-bungarotoxin, DTX_I, and MCD binding sites have been observed (Schmidt et al., 1988), and the β-bungarotoxin binding site also needs K^+ for maintaining the binding conformation in the solubilized state (Rehm & Betz, 1984). It will be interesting to see, once the K^+ channel has been purified, how many different polypeptide chains are necessary to bind these three toxins.

The important aspect of the analysis of the properties of these toxins is their relationship with voltage-dependent K^+ channels. It is interesting to observe that a voltage-dependent K^+ channel from *Drosophila* has recently been cloned and sequenced. It corresponds to an inactivating A-type K^+ channel (Salkoff & Wyman, 1981; Papazian et al., 1987). The main constitutive chain of this channel has a M_r of 70 200, which is a molecular weight similar to that found for the DTX_I/MCD binding component. It should be noted, however, that the *Drosophila* K^+ channel protein may undergo glycosylation. Also the functional K^+ channel may be a multimere of this polypeptide. It is not yet known whether MCD has any effect on A-type K^+ channels, but it has been previously demonstrated that dendrotoxin blocks A-type K^+ channels in CA_1 hippocampal neurons (Halliwell et al., 1986) and it is in hippocampus that the interaction between DTX_I and MCD receptors is particularly potent (Bidard et al., 1987b).

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Articles

Site-Directed Mutagenesis of the T4 Endonuclease V Gene: Role of Lysine-130†

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ABSTRACT: The DNA sequence of the bacteriophage T4 *den*V gene which encodes the DNA repair enzyme endonuclease V was previously constructed behind the hybrid λ promoter O_LP_R in a plasmid vector. The O_LP_R-*den*V sequence was subcloned in M13mp18 and used as template to construct site-specific mutations in the *den*V structural gene in order to investigate structure/function relationships between the primary structure of the protein and its various DNA binding and catalytic activities. The Lys-130 residue of the wild-type endonuclease V has been postulated to be associated with its apurinic endonuclease (AP-endonuclease) activity. The codon for Lys-130 was changed to His-130 or Gly-130, and each *den*V sequence was subcloned into a pEMBL expression vector. These plasmids were transformed into repair-deficient *Escherichia coli* (*uvr*A *rec*A), and the following parameters were examined for cells or cell extracts: expression and accumulation of endonuclease V protein (K-130, H-130, or G-130); survival after UV irradiation; dimer-specific DNA binding; and kinetics of phosphodiester bond scission at pyrimidine dimer sites, dimer-specific *N*-glycosylase activity, and AP-endonuclease activity. The enzyme's intracellular accumulation was significantly decreased for G-130 and slightly decreased for H-130 despite normal levels of *den*V-specific mRNA for each mutant. On a molar basis, the endonuclease V gene products generally gave parallel levels of each of the catalytic and binding functions with K-130 > H-130 > G-130 ≫ control *den*V⁻. A surprising exception to this trend was that G-130, while low in dimer-specific binding capacity, demonstrated an AP-endonuclease function several times as efficient as that of the wild-type enzyme. Overall, these results suggest that the alterations of Lys-130 chiefly compromise the ability of the mutant enzymes to bind DNA at dimer sites. Dimer-specific binding, however, appears not to be required for the AP-endonuclease activity.

The product of the bacteriophage T4 *den*V gene is a DNA repair enzyme, endonuclease V, which functions in the removal of UV-induced pyrimidine dimer photoproducts. The enzyme performs the initial strand scission in the repair of these lesions by mechanisms which involve four sequential actions: (1) a processive scanning process which monitors DNA for the presence of dimers; (2) a DNA binding function specific for dimer sites; (3) a pyrimidine dimer–DNA glycosylase (PD–glycosylase)[1] activity which cleaves the *N*-glycosyl bond be-

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[1] Abbreviations: PD–glycosylase, pyrimidine dimer–DNA glycosylase; AP, apurinic or apyrimidinic; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; RF, replicative form; kDa, kilodalton(s).

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