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Long-term potentiation of synaptic transmission in the hippocampus induced by a bee venom peptide

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Several neurotoxins have been isolated from bee venom[1]. One of these, the mast cell degranulating peptide (MCD), releases histamine from mast cells and on central administration produces arousal at low concentrations and convulsions at higher doses[2,3]. These effects are mediated through specific high-affinity binding sites[4] which are concentrated in cortical structures, notably the hippocampus[3]. This structure appears to be the source of changes in the electrocorticogram that follow injections of MCD into the cerebral ventricle, and which induce a quasi-permanent hippocampal theta rhythm in the motionless rat alternating with epileptiform spike waves[3]. We report here that brief application of MCD to the CA1 region of hippocampal slices induces long-term potentiation, that is, a long-lasting increase in the efficacy of synaptic transmission. This potentiation seems to be indistinguishable from the classical LTP produced by trains of high-frequency electrical stimulation[5,6] and considered to be related in some way to memory. Using binding to synaptosomal membranes and radioimmunoassay techniques, we have also found an endogenous peptide equivalent of MCD in brain extracts. This raises the possibility that a MCD-like peptide may be important in long-term potentiation.

Conventional hippocampal slices were prepared from adult male wistar rats and maintained *in vitro* (details in ref. 7). Application of MCD (0.5-2 μM) produced a membrane depolarization, often associated with spike discharge (Fig. 1*b*). MCD (1 μM) produced a depolarization of 12.5 ± 1.28 mV, $n = 6$ (mean ± s.e.m.). The depolarization started 60 s after MCD application and reached a peak within a few minutes. The membrane potential returned to the control level within 3-5 min of washing with MCD-free solution. The depolarization was usually not associated with a significant change in input resistance; a slight (<10%) increase was observed in two cases. Both tetrodotoxin (TTX) (1 μM) and cobalt (2 mM) completely blocked the depolarization produced by MCD, implying that this effect is synaptically mediated.

MCD consistently produced a long-term potentiation (LTP) of synaptic transmission ($n = 6$). This was characterized by a progressive increase of the amplitude of the excitatory postsynaptic potential (e.p.s.p.) starting 2-5 min after the wash and reaching a maximum 10-20 min later. Often the facilitated

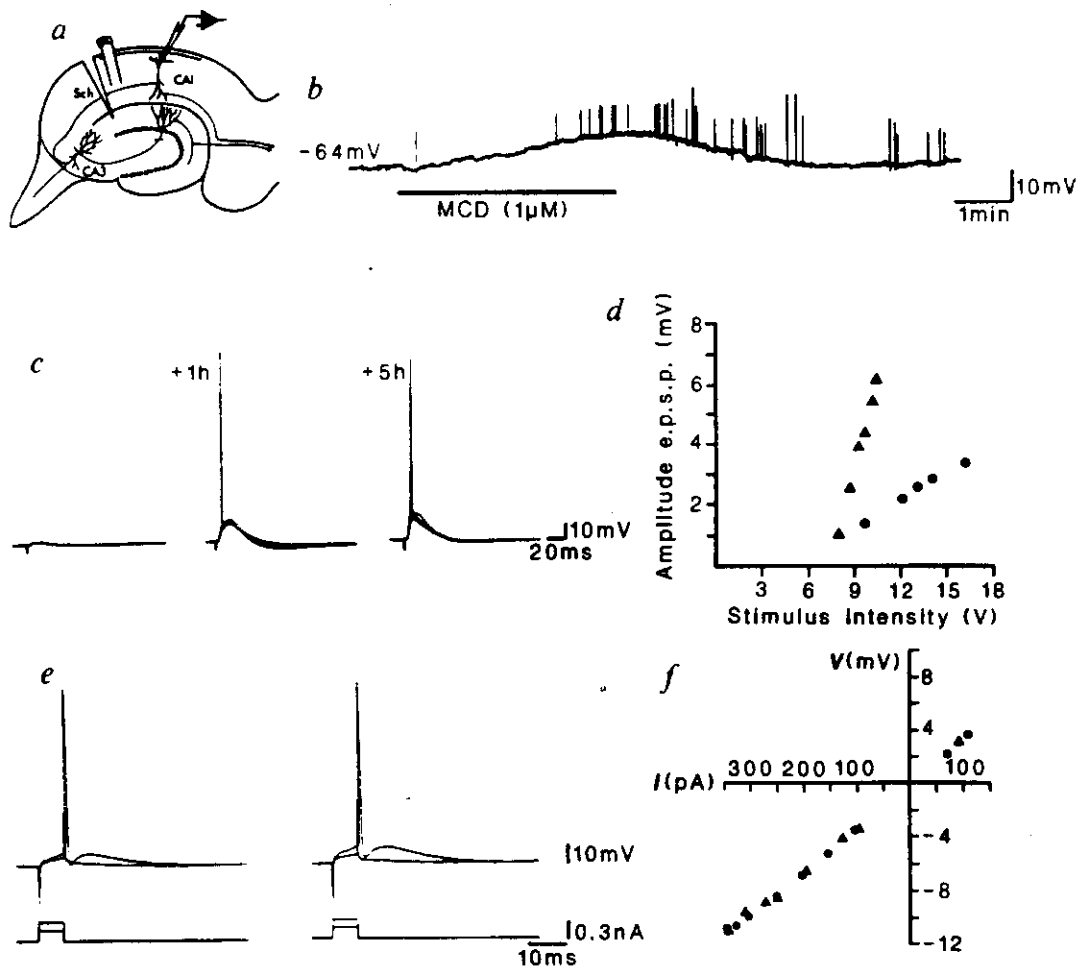

Fig. 1 MCD enhances synaptic transmission in the hippocampus. *a*, Experimental arrangement: intracellular recordings of the pyramidal cells of CA1 were made with potassium-acetate-containing electrodes; bipolar stimulating electrodes were positioned in stratum radiatum to activate the Schaffer collaterals. The CA1 region was isolated from CA3 by a knife cut to prevent spread of bursting activity from the latter region[20]. *b-e*, The effects of MCD in the same neuron. *b*, Depolarization and spike activity produced by bath application of 1 μm MCD (bar). *c*, Superimposed digitized chart records of monosynaptic e.p.s.p. before, 1 h and 5 h after MCD. *d*, Plot of the amplitude of the e.p.s.p. against stimulation intensity before (●) and after (▲) MCD. *e*, Action potentials directly evoked by intracellular depolarizing pulses before (left) and after (right) MCD. The excitability of the neuron was not evidently changed by the peptide. Upper trace, voltage recordings; lower trace, current recordings. *f*, *V/I* curve constructed before (●) and 1 h after (▲) application of MCD. In this and following figures the frequency of stimulation of the Schaffer collaterals was 0.05 Hz.

Methods. MCD was purified according to Taylor *et al.*[4], and the MCD II fraction was used throughout. The purity of the peptide was improved by an additional step of HPLC purification on C18 columns (10 × 250 mm, 7 μm particle size, Merck). Elution was performed with an acetonitrile water gradient containing 0.5% trifluoroacetic acid and 0.85% triethylamine. MCD was dissolved in water. Because the peptide is positively charged, plastic and glass tubes were treated with Sigmacoat (Sigma).

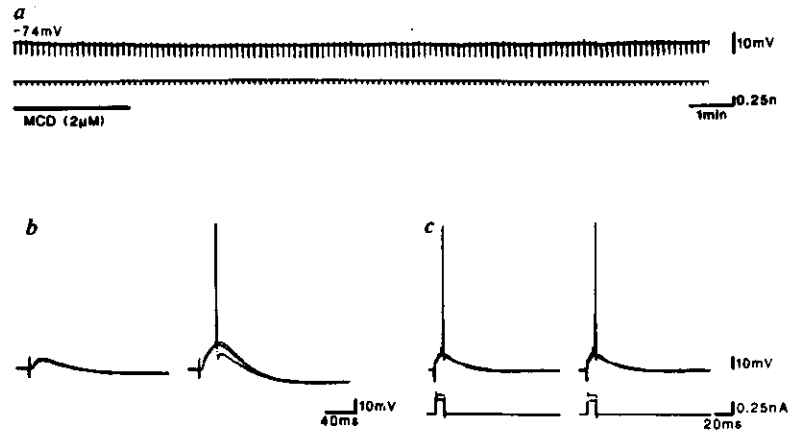

Fig. 2 The enhancement of synaptic transmission by MCD is independent of membrane depolarization. *a–c*, Same neuron. *a*, Slice bathed in a solution containing 6 mM Mg^{2+} and 4 mM Ca^{2+} to reduce excitability and bicuculline (30 μM) to block GABAergic inhibition. MCD (2 μM) was applied for 5 min, only the end of the period of superfusion is represented by the bar. The peptide induces neither a depolarization nor a change in input resistance (as tested by electrotonic potentials induced by hyperpolarizing constant current pulses through the recording electrode). Scale bars: (upper) 10 mV; (lower) 0.25 nV vertical, 1 min horizontal. *b*, Superimposed digitized chart records of e.p.s.p. evoked before (left) and 2 h after (right) application of MCD. Scale bar: 10 mV vertical; 40 ms horizontal. *c*, Action potentials evoked by depolarizing current pulses before (left) and 2 h after (right) application of MCD. Scale bars: (upper) 10 mV; (lower) 0.25 nV vertical, 20 ms horizontal.

e.p.s.p. reached the spike threshold (Fig. 1*c*). This enhanced e.p.s.p. lasted for up to 6 h (the longest duration of stable intracellular recordings which was obtained) and even partial recovery was not observed. The enhancement produced by 1 μM of MCD measured in three cases in which spikes were not produced was 259±33% (mean±s.e.m.). The relationship between the amplitude of the e.p.s.p. and the stimulation intensity was linear before and after application of MCD but the slope of the responses obtained after superfusion of the toxin was considerably increased (Fig. 1*d*). The minimum dose of MCD required to produce the potentiation was 500 nM, but higher concentrations (1–2 μM) produced larger and more rapid effects. The enhancement of the e.p.s.p. was not associated with changes in membrane resistance or cellular excitability as measured with intracellular current pulses (Fig. 1*e–f*). Therefore, the potentiation of synaptic transmission produced by MCD, like that induced by electrical stimulation[6], does not seem to involve long-lasting changes in postsynaptic cell excitability.

The long-lasting effects of MCD are not due to the continuous presence of the toxin in the tissue because the effects of MCD on membrane potential and resistance rapidly washed out and a brief (1–3 min) concomitant exposure to TTX or cobalt (2 mM) completely prevented the long-lasting effects of MCD ($n=2$). Interestingly, once the potentiation had been induced, brief application of either agent caused only a temporary block of the long-lasting effects ($n=3$). This suggests that the potentiating effect of MCD requires synaptic activity.

To examine whether the effects of MCD were due to a fragment of the peptide, we also tested the effects of MCD pretreated either with chymotrypsin, which does not destroy the MCD peptide, or with trypsin, which does. The long-lasting enhancement of the e.p.s.p. still occurred with the former treatment ($n=2$) but not with the latter ($n=3$) which confirms the need for the peptide to be intact for activity.

Blockade of Cl^--mediated GABAergic (γ-amino butyric acid) mediated inhibition does not prevent the development of LTP produced by trains of electrical stimulation[8]. We have therefore examined the effects of MCD in the presence of the GABA antagonist bicuculline. To suppress the synchronous firing which occurs in these conditions[9], high concentrations of divalent cations were used (6 mM Mg^{2+} instead of 1.3 mM, and 4 mM Ca^{2+} instead of 2 mM). MCD (2 μM) induced long-term potentiation of synaptic transmission but not the initial depolarization (Fig. 2; $n=2$). We conclude that GABAergic inhibition is not

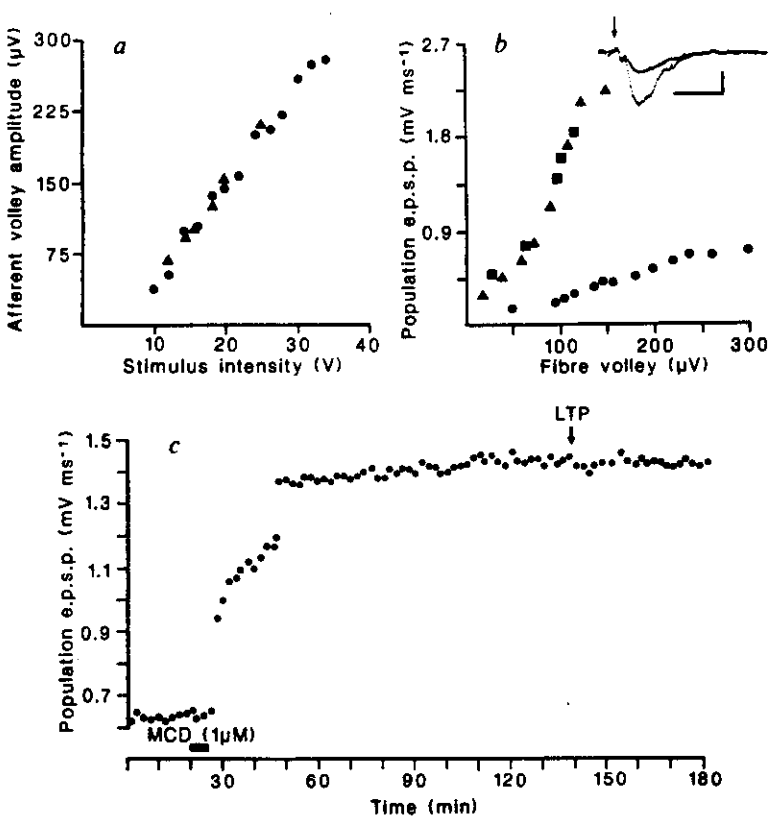

Fig. 3 *a*, The enhancement of the field e.p.s.p. produced by MCD is not associated with a change in the afferent volley. The amplitude of the afferent volley is plotted against the intensity of stimulation before (●) and 1 h after (▲) MCD (1 μM). *b*, Same slice as *a*. Input-output curve before (●) and 1 h after (▲) MCD. Electrical stimulation was repeated at this time and 30 min later (■). Each point is the average of five consecutive responses. Inset, typical digitized field e.p.s.p. and afferent volleys obtained in the control conditions and 1 h after MCD application (lower trace). Scale bar, 0.5 mV vertical, 10 ms horizontal. *c*, Time-course of the enhancement of the field e.p.s.p. produced by 1 μM MCD (bar) in another slice. Each point is the average of five consecutive responses. Electrical tetanization was induced 2 h after MCD (arrow). This did not produce a further potentiation of the synaptic response. In three other experiments LTP was first induced by trains of electrical stimulation and MCD applied 1 h later. The electrically potentiated response was further enhanced by the peptide (175±10%, mean±s.e.m.).
Methods. The initial slope of the field e.p.s.p. was measured as an indication of the amplitude of synaptic currents[6]. LTP was induced by two trains of 100-Hz stimulation at 30-s interval. Two experimental approaches have been used to study the interactions between MCD-induced synaptic potentiation and electrical LTP. In both cases the tetanus was induced once the MCD effects were maximal (usually 1 h after application). In the first case (see *b*) the strength of stimulation used to elicit the LTP was reduced by 50% to obtain a field e.p.s.p. similar to the control (pre-drug) conditions. In the second approach (in *c*) the strength of stimulation was kept constant throughout the experiment.

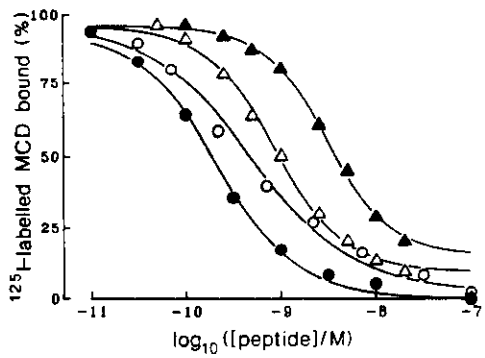

Fig. 4 Evidence for the existence of an endogenous brain substance with MCD-like activity. Effect of MCD (circles) and the MCD-like peptide (triangles) on binding of ^{125}I-MCD (4 pM) to synaptosomal membranes (0.2 mg protein ml^{-1}) in a radio-receptor assay (RRA) (filled symbols) and of the same proteins on ^{125}I-labelled MCD (8 pM) precipitation by rabbit anti-MCD immunoglobulin (final dilution 1:200,000), in a radio-immunoassay (RIA) (open symbols).

Methods. RRA was carried out as previously described[4]. Anti-MCD immunoglobulin was prepared from the serum of a rabbit immunized with MCD coupled to BSA and RIA for MCD was performed as previously described for the RIA for apamin[21]. Protamine (100 μg ml^{-1}) was added to the RIA incubation buffer to avoid nonspecific precipitation of ^{125}I-labelled MCD. The MCD-like peptide was extracted from 250 pig brains. The first steps of purification were carried out as previously described for the apamin-like substance[19]. Three HPLC steps were then used: one step on C_{18}-reverse phase eluted by an acetonitrile/water gradient containing 0.5% trifluoroacetic acid and 0.85% triethylamine, then two steps on TSK-IEX SP-5PW cationic exchanger eluted by a 500 mM to 1,500 mM ammonium acetate gradient and finally one step on TSK G-2000 SW gel filtration eluted by 300 mM ammonium acetate *p*H 4.5 to yield 60 pmol of the pure MCD-like peptide (relative molecular mass = 2,900).

important in the long-lasting enhancement of the e.p.s.p. induced by MCD.

Several mechanisms could account for the enhancement of the e.p.s.p., including an increase in the number of presynaptic fibres excited by the electrical stimulation. To test this possibility we have examined the effects of MCD upon the afferent volleys and the field e.p.s.ps. The curve relating the stimulation intensity and the amplitude of the afferent volley was identical in control conditions and following MCD application (Fig. 3*a*). On the other hand, there was a significant shift in the input–output curve obtained by plotting the amplitude of the afferent volley against the initial slope of the field e.p.s.p. (Fig. 3*b*). Clearly a given afferent volley produces a larger postsynaptic response after MCD, suggesting that the potentiation of synaptic transmission is not due to an enhancement of presynaptic axon excitability. A similar observation has been reported with electrically-induced LTP[6].

Therefore the potentiation produced by MCD is strikingly reminiscent of the LTP induced by trains of high-frequency electrical stimulation[5,6]. To examine this similarity further, trains of electrical stimulation which produce LTP in control slices were applied in MCD-pretreated slices. The trains were found to produce a frequency potentiation lasting 10–15 min (in 4 out of 6 tests) but did not produce a long-lasting enhancement of the e.p.s.p. (Fig. 3*c*). The fact that the chemically potentiated synapse cannot be further enhanced by electrical stimuli which otherwise produce LTP suggests that the enhancement of synaptic transmission by both procedures may have a common mechanism. To examine further the similarity between MCD-induced long-lasting enhancement of the synaptic transmission and LTP, we tested the effects of D(−)-2-amino-5-phosphonovaleric acid (APV), known to prevent LTP[10]. Application of APV (10–30 μM) before MCD suppressed the enhancement of the e.p.s.p. induced by MCD in three out of four cells tested.

The precise mechanism underlying the action of MCD in the hippocampus is unknown. One possibility is that, like phorbol esters, MCD activates protein kinase C (PKC). Following activation of this sort, K^+ channels could be phosphorylated and partly or completely blocked[11–13] and/or Ca^{2+} channels activated [14]. Recent studies suggest that phosphorylation of proteins by PKC is important in LTP[15] and that phorbol esters produce long-term potentiation in the hippocampus[16]. Another possibility is that MCD directly blocks or activates ionic channels involved in neurotransmitter release in the hippocampus. Another bee venom toxin, apamin, is a selective blocker of one type of neuronal Ca^{2+}-activated K^+ channel[17,18]. Similarly to apamin, for which there is an endogenous equivalent in the central nervous system[19], we have demonstrated the presence of an endogenous MCD-like activity in mammalian brain (Fig. 4). The endogenous peptide prevents ^{125}I-labelled MCD binding to its synaptosomal receptor and cross-reacts in a radioimmunoassay against MCD. The substance is a peptide that is destroyed by trypsin, like MCD itself. The endogenous substance may induce long-term potentiation in the hippocampus, a possibility that will be tested when larger amounts of the substance become available.

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