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Epileptogenic properties of the mast cell degranulating peptide in CA3 hippocampal neurones

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The epileptogenic properties of the mast cell degranulating peptide (MCD) have been investigated in the CA3 region of the hippocampal slice preparation. Brief (3–5 min) bath application of MCD (0.5–2 μM) to CA3 hippocampal neurones produced an enhancement of the spontaneous synaptic activity and the appearance of spontaneous bursts that persisted for several hours. These bursts were network driven and the underlying paroxysmal depolarizing shift met the criteria for a giant excitatory postsynaptic potential (EPSP), with a reversal potential close to 0 mV. Furthermore following the application of MCD, stimulation of the mossy fibres, commissural or temporo-ammonic pathway evoked an EPSP followed by an evoked network burst. The bursts which could be elicited for several hours were reversibly blocked by a brief application of tetrodotoxin (TTX; 1 μM) or cobalt (2 mM). In contrast, prior and concomitant treatment with TTX or cobalt prevented the occurrence of the bursts induced by MCD. The effects of MCD were not due to a blockade of GABAergic inhibition since the toxin did not reduce the fast and slow IPSP. Furthermore, the *N*-methyl-D-aspartate (NMDA) antagonists D-2-amino-phosphonovalerate (D-APV; 30 μM) or DL-amino-phosphoheptanoic acid (AP-7, 30 μM) did not block the action of MCD, suggesting that the activation of NMDA receptors are neither necessary nor sufficient for MCD-induced bursts. It is concluded that MCD induces in the CA3 region long-lasting changes in the synaptic responses which may be mediated through a presynaptic mechanism.

INTRODUCTION

Several peptides with neurotoxic action have been isolated from bee venom[15], notably apamin, a blocker of the calcium-dependent potassium channel[21] and a closely related 22 amino acid peptide, the mast cell degranulating peptide (MCD). MCD releases histamine from mast cells[7,19,22] and upon intracerebroventricular administration induces hippocampal theta rhythm, paroxysmal activity and convulsions followed by death of the animal[5,16]. These effects are mediated through the activation of a high affinity receptor[35], localized mainly in cortical structures and hippocampus[5]. Understanding the mechanisms of action of MCD is of general interest since, as for apamin[12], the brain contains an endogenous MCD-like peptide[8]. In a recent in vitro study, it was found that brief application of MCD induces a long-term potentiation (LTP) of the synaptic responses evoked by Schaffer collateral stimulation in the CA1 region of the hippocampus. This potentiation was indistinguishable from the classical LTP produced by a train of high frequency stimulation[8]; thus the long-lasting effect was not associated with significant changes in postsynaptic cell excitability, suggesting that the toxin acts at a presynaptic level to release a factor(s) which produces LTP.

The principal aim of the present study was to examine the epileptogenic actions of MCD in the CA3 region of the hippocampus, which is well known for its pacemaker properties[17].

MATERIALS AND METHODS

Adult male Wistar rats (150–200 g) were anaesthetized with ether and killed by a heavy blow to the

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chest. Transverse 450 μm thick hippocampai slices were prepared and maintained in vitro following the technique fully described previously[13]. The slices were superfused (2 ml/min) with artificial cerebrospinal fluid containing (mM): NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, glucose 11, $NaHCO_3$ 25, at 36 °C, gassed with a mixture of O_2 95% and CO_2 5% (pH = 7.3). Intracellular recordings were made with glass micropipettes filled with 3 M KCl, 4 M potassium acetate or 2 M potassium methylsulphate (resistance 50–100 MΩ). Concomitant extracellular recordings were made with NaCl-filled electrodes (resistance 5–10 MΩ). The extracellular electrodes were usually positioned in the mossy fibre terminal region (i.e. stratum lucidum). Bridge balance was checked repeatedly during the course of the impalement. Capacitative transients with the electrode tip outside the neurone were reduced to a minimum by negative capacity compensation. The mossy fibre, the commissural or the temporo-ammonic pathways were stimulated by means of bipolar nickel–chrome electrodes (50 μm diameter). Stimuli consisted of 0.01–0.02 ms pulses (10–50 V) delivered at a frequency of 0.05 Hz. The stimulus intensity was adjusted to evoke an EPSP subthreshold for spike generation. Individual action potentials, electrotonic potentials, spontaneous or evoked bursts and postsynaptic potentials were digitized and displayed on a digital oscilloscope and on a computer-driven chart recorder.

Drugs were dissolved in the artificial cerebrospinal fluid and bath applied via a 3-way tap system; effects

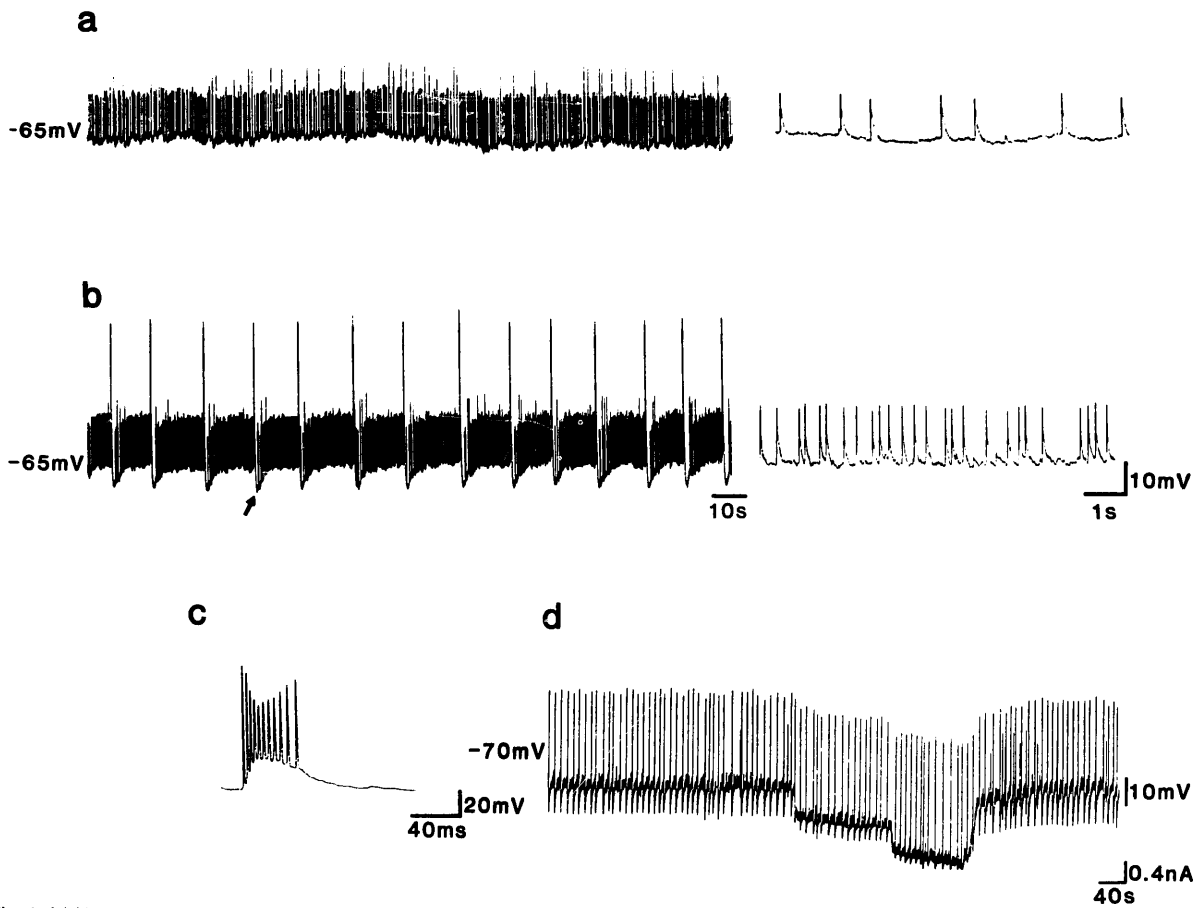

Fig. 1. MCD induces spontaneous network bursts. Spontaneous activity before (a) and 1 h after MCD (b). MCD (1 μM) was applied to the bath for 5 min. Spontaneous bursts started to develop after 3 min of drug application. c: faster display of burst shown in b (arrow). d: the frequency of the bursts produced in another neurone by MCD (1 μM) was not changed by membrane hyperpolarization. Note that at more hyperpolarized potentials, the afterhyperpolarization following the burst was almost completely abolished. In a, b and c, the neurone has been recorded with KCl-filled microelectrode; in d and the following figures with potassium acetate electrodes.

of different drugs were observed within 20–30 s of the solution entering the bath and equilibrium was apparently reached within 3 min. MCD was purified according to Taylor et al.[35]. The MCD II fraction was used throughout (see for further details on purification ref. 8). MCD was dissolved in water. Since the peptide is positively charged, plastic and glass tubes were treated with Sigmacoat (Sigma). Tetrodotoxin (TTX) and bicuculline were purchased from Sigma and D-2-amino-phosphonovalerate (D-APV) was from Cambridge Research Biochemicals. DL-Amino-phosphoheptanoic acid (AP-7) was a gift of Dr. Herrling (Sandoz, Berne). Naloxone was obtained from Endo.

RESULTS

MCD induces spontaneous network bursts (SNB)

Long-lasting intracellular recordings were made from 50 CA3 pyramidal neurones. All the neurones fulfilled the following criteria: resting membrane potential greater than −58 mV, average input resistance 48 ± 6 MΩ ($\bar{x}$ ± S.E.M., n = 8) and action potential amplitude greater than 85 mV. MCD (0.5–2 μM) was applied in the bath for 3–5 min. The application rapidly (2–5 min) induced an increase in spontaneous synaptic activity (Fig. 1). These were often grouped in clusters and led to an increased cell discharge. In 45 out of 50 neurones, MCD induced spontaneous epileptiform bursts, within 3–5 min of its application. These usually developed in the absence of any change in membrane potential or resistance and consisted of a brief high frequency spike discharge riding on a paroxysmal depolarizing shift (PDS)[25]. Each burst was followed by a long-lasting hyperpolarization. The burst frequency was variable from cell to cell, ranging between 5 and 22 bursts/min. Typically the highest frequency was observed during the first minutes of wash. Bursting continued at the same rate for 3–4 h and then slowly declined but a complete wash was never achieved (Fig. 9). Synchronous interictal events were recorded with the extracellular electrode (Fig. 2). These consisted of multiple population spikes riding on a positive field potential when the electrode was located in the pyramidal layer. In keeping with the observations made

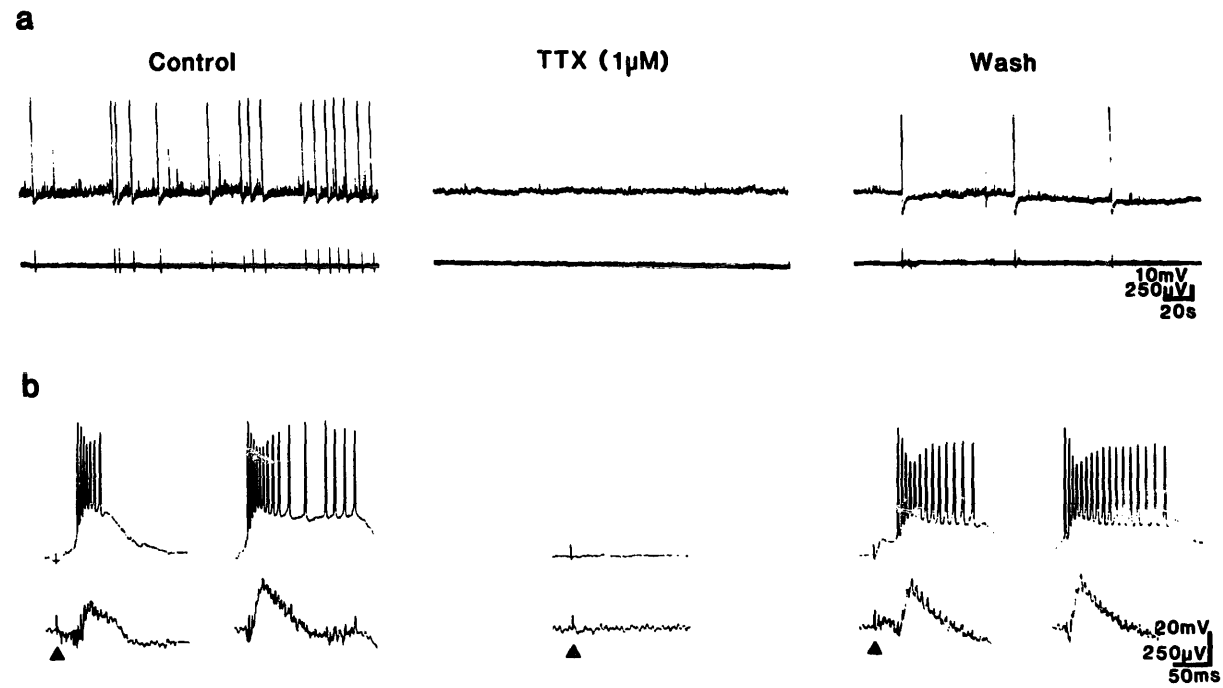

Fig. 2. Spontaneous and evoked bursts induced by MCD are blocked by TTX. In a and b the upper traces are intracellular recordings, the lower traces are extracellular recordings. In a, while recording from the same neuron, MCD was applied and induced bursts (control); 20 min later, TTX (1 μM) completely blocked the bursts. The bursts reappeared 40 min after TTX was washed out (Wash). In b, faster display of the same records to depict spontaneous and mossy fibre evoked bursts (triangles); note that TTX (middle trace) blocked the bursts which, however, reappeared 40 min after TTX was washed out. Resting membrane potential −68 mV.

with other convulsants[34], negative field potentials corresponding to the site of generation of the PDS were recorded in the distal apical dendrites of the CA3 region. The following observations suggest that the bursts were network-driven events and not endogenous pacemakers[23]: (i) they were synchronous in intracellular and extracellular recordings; (ii) their frequency was independent of the membrane potential (Fig. 1); (iii) their amplitude was a linear function of the membrane potential; (iv) the extrapolated reversal potential was near 0 mV (not shown), suggesting that the PDS was a giant EPSP (n = 2). In keeping with these observations, the SNB were abolished by agents which block spike propagation or synaptic activity, notably TTX, 1 μM (Fig. 2) or cobalt, 2 mM (Fig. 3). During washout the spontaneous bursts reappeared; however, their frequency was reduced.

Treatment with TTX or cobalt (n = 4) prior and during perfusion with MCD prevented the occurrence of bursts, suggesting that synaptic activity is required to produce the SNB (Fig. 4). As shown in Fig. 4, while recording from the same neurone, bursts developed during a second application of MCD, once TTX or cobalt had completely been washed out (Fig. 4). This observation eliminates the possibility that the long-lasting occurrence of the spontaneous bursts is somehow dependent on the continous presence of the toxin in the tissue. It bears stressing that in the presence of TTX, MCD produced an increase in spontaneous synaptic events raising the possibility

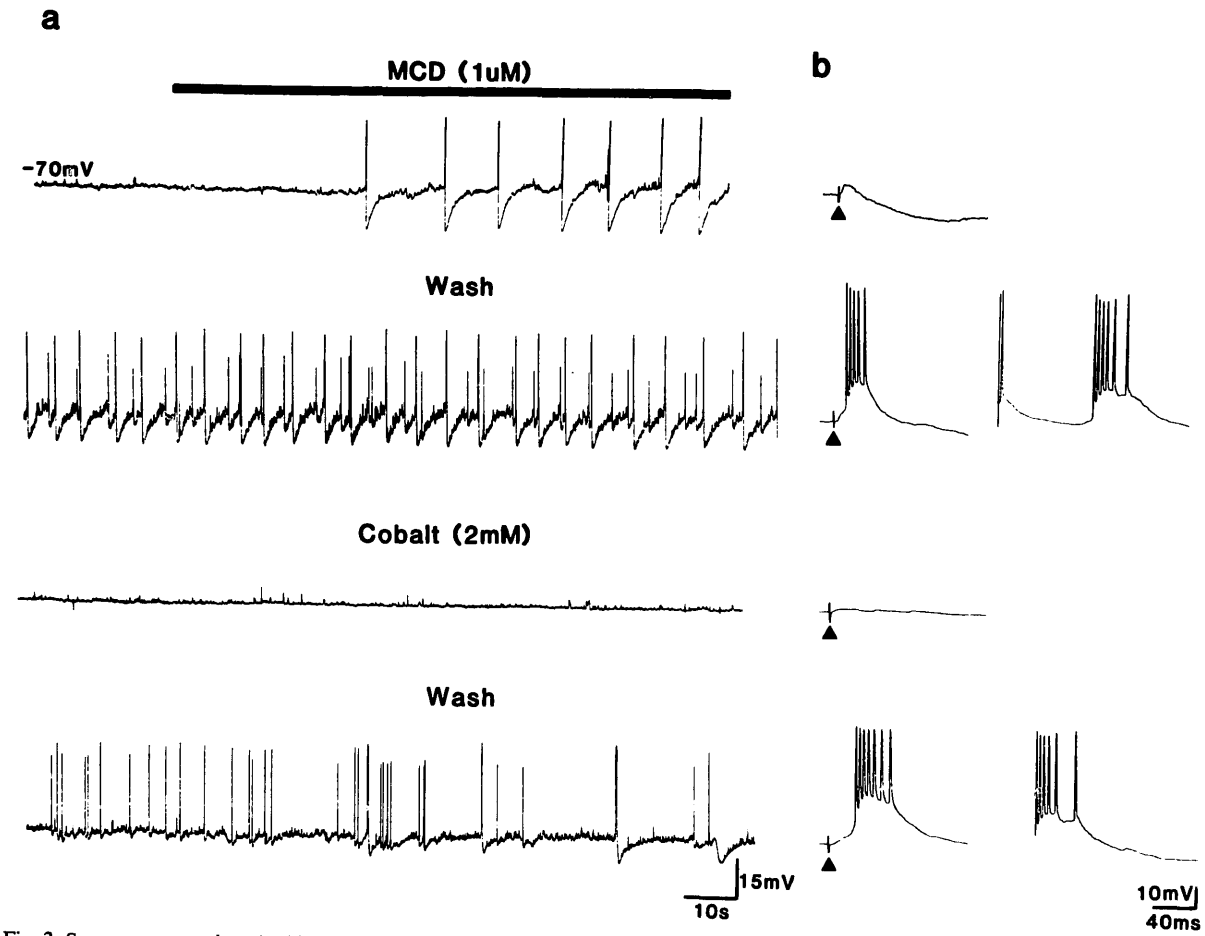

Fig. 3. Spontaneous and evoked bursts induced by MCD are temporarily blocked by cobalt. While recording from the same neurone MCD and cobalt were successively applied. Spontaneous and evoked bursts (triangles) are shown at a faster sweep on the right side of the figure (b). Before MCD, stimulation of the mossy fibres elicited an EPSP–IPSP sequence (b, top trace). MCD induced spontaneous and evoked bursts which persisted for a prolonged period after the drug was washed out (Wash). Cobalt applied 40 min after MCD, completely blocked the bursts. These, however, reappeared 25 min after cobalt was washed out (lowest trace). Note that the EPSP is apparent as a small inflection on the rising phase of the PDS in the evoked responses.

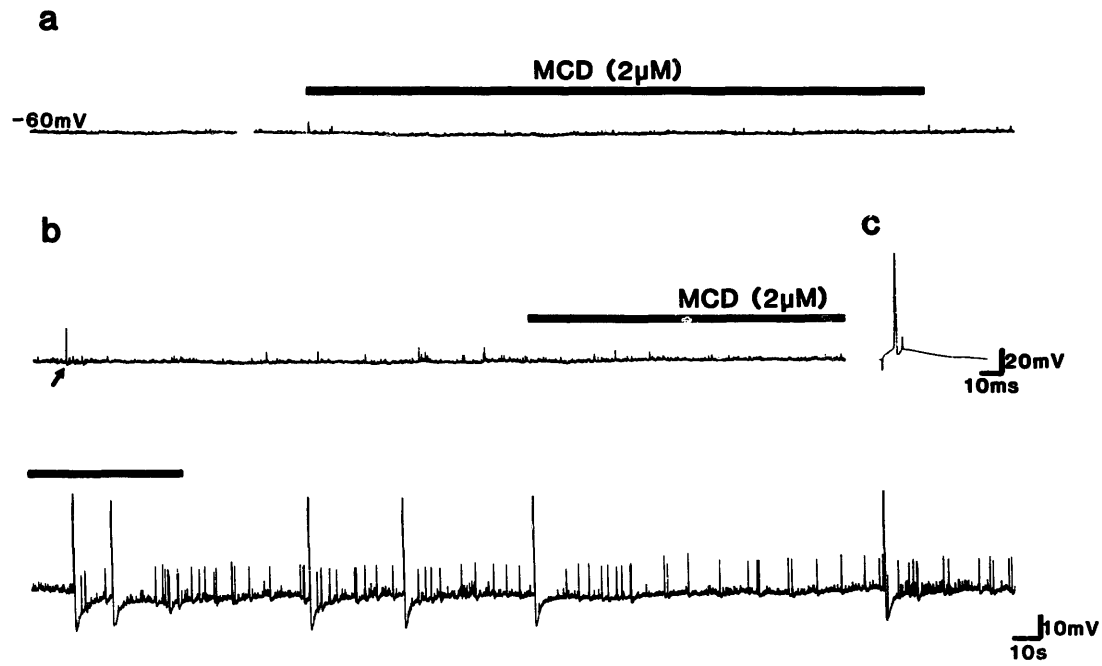

Fig. 4. Prior administration of TTX prevents the effects of MCD. While recording from the same neuron, TTX was first applied for 10 min to completely block the sodium spikes. In a, application of MCD with TTX failed to elicit bursts. b: 1 h and 40 min after TTX was washed out, an action potential could be elicited by intracellular current injection at the arrow (action potential shown in c). At this time, a second application of MCD induced bursting activity. In b, the upper and lower traces are continuous.

that the peptide acts directly at the terminal level to release endogenous transmitters. We cannot exclude, however, the possibility that TTX and cobalt block specific membrane conductances possibly activated by MCD and which contribute to burst generation.

In the remaining 5 neurones, MCD produced an increase in spontaneous synaptic activity and spike

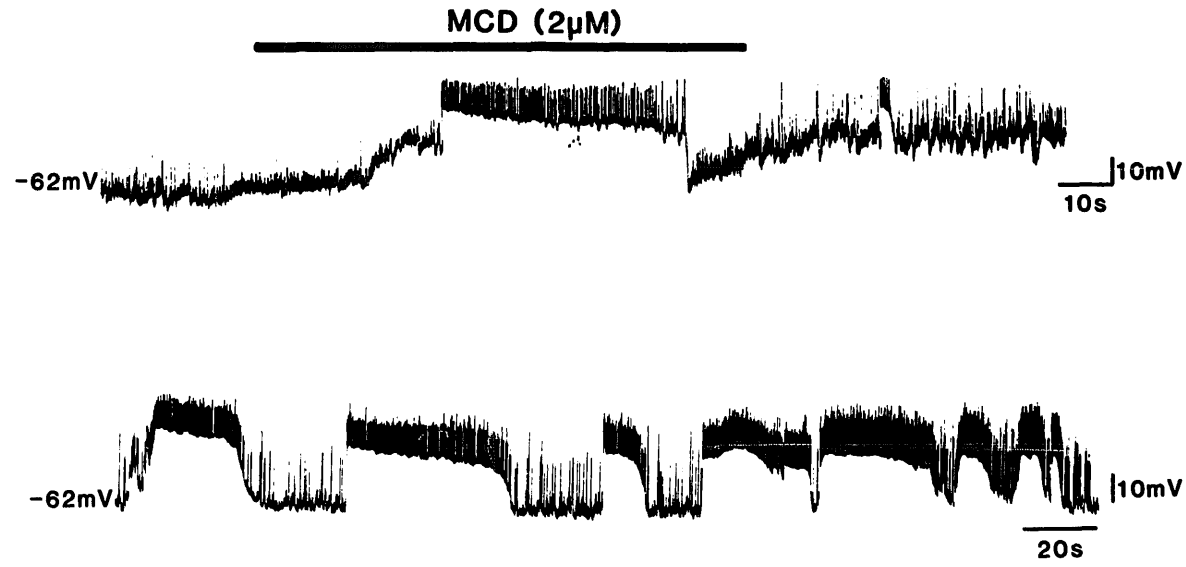

Fig. 5. Bistable behaviour induced by MCD in a CA3 neurone. The abrupt depolarization and repolarization of the membrane potential and increased firing lasted for 12 min, then the effects of MCD completely washed out. In this neurone MCD did not induce spontaneous and evoked network bursts.

discharges but did not induce spontaneous bursts. In two of these cells the peptide induced a bistable behaviour, consisting of consecutive abrupt depolarizations of the membrane leading to a plateau followed by abrupt repolarizations (Fig. 5). Similar plateau potentials have been described following NMDA application[11]. In the remaining 3 neurones, MCD produced a membrane depolarization of 11.6 ± 2 mV ($\bar{x}$ ± S.E.M.) that in two cases was associated with a small increase in membrane input resistance (<10%). The actual values were 38 and 52 MΩ before MCD and 40 and 56 MΩ during MCD-induced depolarization. The depolarization started 1–2 min after MCD application and reached a peak within a few minutes. The membrane potential returned to control values 8–15 min after wash. The MCD-induced depolarization was probably synaptically mediated since it was completely blocked by superfusion of TTX (1 μM) or cobalt (2 mM). All the effects observed in these 5 neurones were transient as they disappeared within 15 min of washing MCD.

MCD induces evoked network bursts (ENB)

Superfusion with MCD also led to the appearance of evoked network bursts. Thus, as shown in Fig. 3, whereas in control medium, stimulation of the mossy fibres evoked an EPSP followed by an IPSP, after perfusion with MCD this response was replaced by an EPSP followed by a burst of action potentials and an afterhyperpolarization. These evoked bursts shared a number of similarities with the spontaneous network bursts: (i) they were synchronous in the intracellular and extracellular records suggesting that the evoked bursts are of the network type (Fig. 2); (ii) like the SNB, the extracellular field consisted of a positive or negative wave in the stratum pyramidale or radiatum respectively; (iii) the amplitude of the intracellular PDS was a linear function of the membrane potential, depending only on the driving force of this giant EPSP and the extrapolated reversal potential was near 0 mV (n = 3), not shown. Furthermore, like the SNB, the ENB were only temporarily blocked by superfusion of TTX (1 μM) and cobalt (2

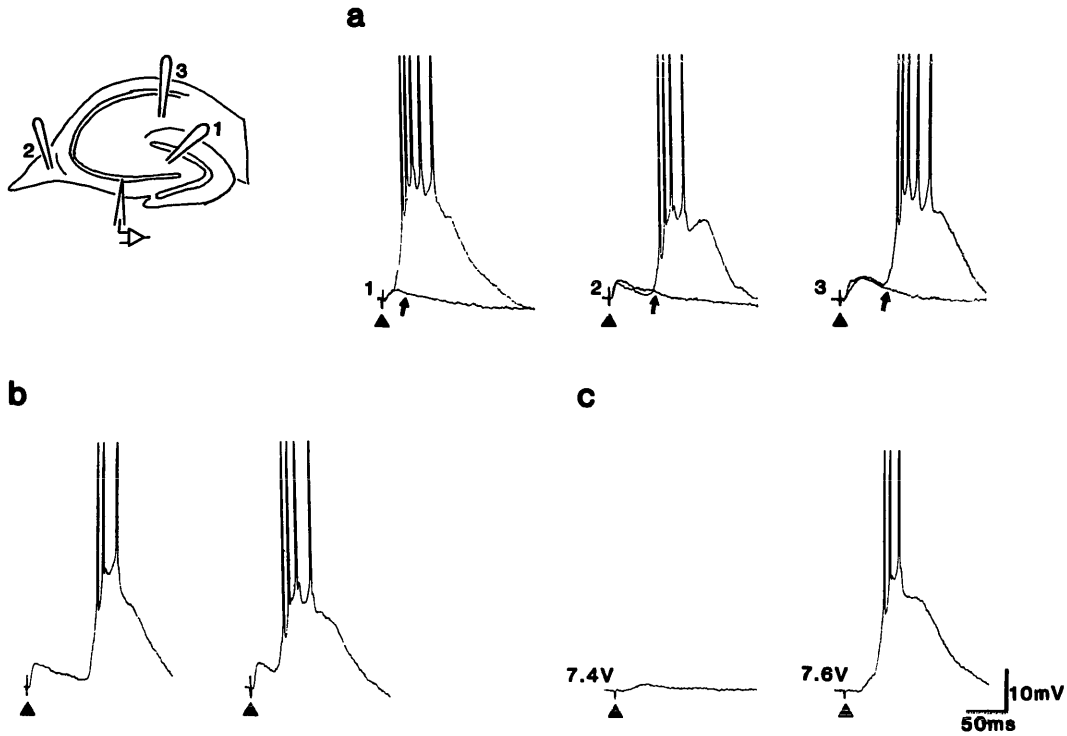

Fig. 6. Heterosynaptic convergence and the all-or-none characteristic of the evoked network burst induced by MCD. As shown in the diagram, stimulating electrodes were positioned to activate the mossy fiber (1), the commissural pathway (2) and the temporo-ammonic pathway (3). After MCD application, stimulation of the 3 pathways elicited network bursts. a: reflects the convergence of these inputs at the circuitry generating the bursts; the arrows indicate the synaptic responses before application of MCD. b: the ENB elicited by commissural pathway stimulation after MCD had a variable latency. c: the bursts evoked by the mossy fibres stimulation after MCD were elicited in an all-or-none manner.

mM), see Figs. 2 and 3. However, during washout the delay between the monosynaptic EPSP and the burst was increased, suggesting the involvement of a polysynaptic pathway in the generation of these bursts.

Other observations reflect the involvement of a polysynaptic circuitry in generating the ENB. Thus, the bursts were evoked by the electrical stimulation in an all-or-none manner and with a variable latency (Fig. 6). Occasionally the preceding EPSP was only apparent as a small inflection in the rising phase of the PDS. The ENB were elicited by the stimulation of afferent input to the CA3 pyramidal neurones, notably the mossy fibres, the commissural or the temporo-ammonic pathway (Fig. 6). This suggests that there is a considerable convergence of inputs on the polysynaptic circuitry generating the ENB. Fig. 6 illustrates another important feature of the action of MCD, i.e. the long-lasting changes in the synaptic responses. ENB were readily evoked for up to 4 h after a brief application of the toxin (longest stable intracellular record performed).

Effects of various transmitter antagonists on the bursts induced by MCD

The long-term induction of bursts by MCD may result from a prolonged blockade of the GABAergic inhibition. We have therefore examined the effects of MCD on the IPSP ($n = 2$). To prevent the occurrence of the bursts, the slices were perfused with a medium containing elevated concentrations of calcium (5 mM) and magnesium (5 mM). Under these circumstances MCD failed to produce spontaneous and evoked bursts. As shown in Fig. 7, the IPSP was not reduced by MCD. After return to a normal medium, spontaneous and evoked network bursts did develop as a consequence of MCD's action (Fig. 7). In additional experiments ($n = 4$), the GABA antagonist, bicuculline, was applied at a concentration which completely blocks the Cl^--mediated GABAergic inhibition (30 μM) and in the presence of a high divalent cation concentration (4 mM calcium and 6 mM magnesium), to prevent burst firing[27]. In these conditions, addition of MCD readily evoked burst discharge (Fig. 8), raising the possibility of a direct ef-

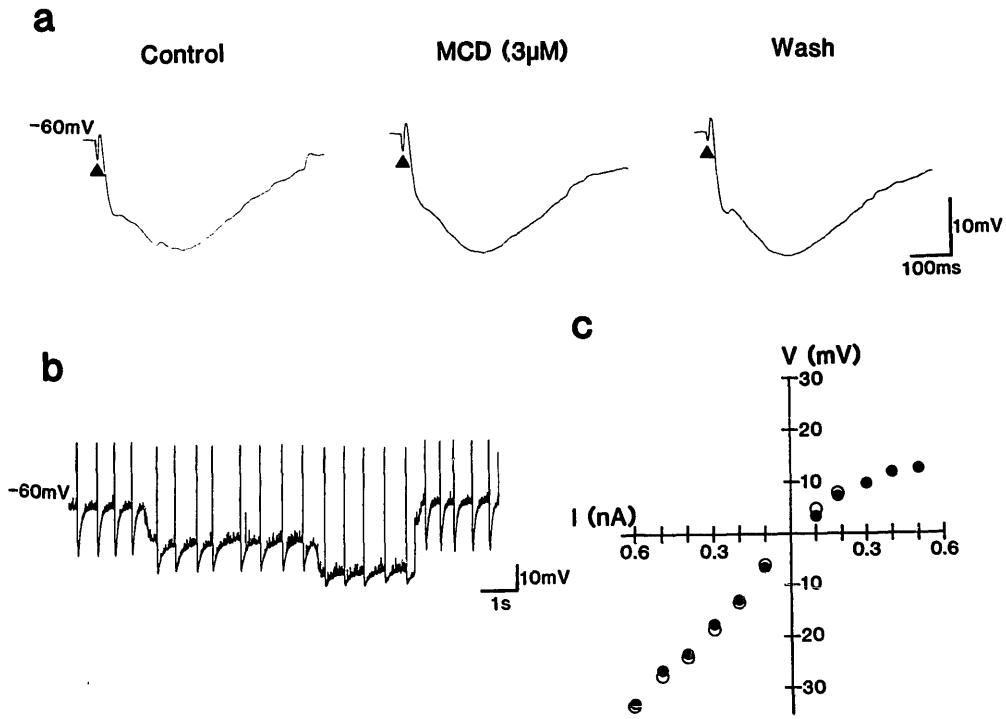

Fig. 7. The fast and slow IPSP are not reduced by MCD. a: EPSP followed by a fast and a slow IPSP evoked by mossy fibre stimulation (triangles) in a medium containing 5 mM calcium and 5 mM magnesium. Note that during and after MCD application the EPSP and the IPSP were slightly increased. Potassium methylsulphate containing electrode. b: after return to a medium containing 2 mM calcium and 1.3 mM magnesium, spontaneous bursts developed. They were network bursts since their frequency was not changed by hyperpolarizing the membrane to -75 and -90 mV. c: V/I curve before (○) and during (●) MCD application.

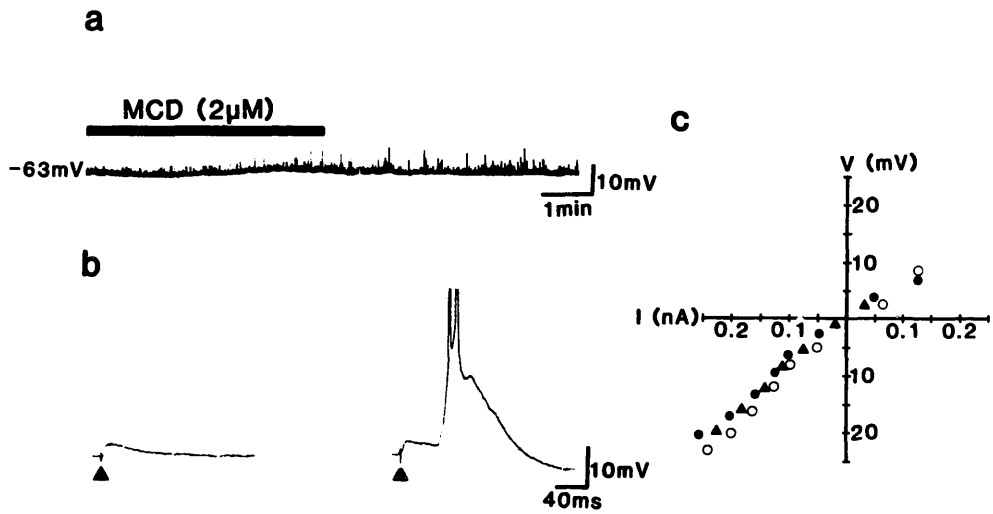

Fig. 8. The ENB induced by MCD are not due to the blockade of the Cl^--dependent GABAergic inhibition. a: in a medium containing higher concentrations of divalent cation and bicuculline (30 μM), MCD failed to induce spontaneous bursts; it caused only a small depolarization of the membrane potential. However, as shown in b, in the same neurone, the mossy fiber EPSP (left side) was followed after application of MCD (right side) by a typical ENB. c: V/I curve before (○), 10 min (●) and 50 min (▲) after MCD.

fect on excitatory transmission. Another possibility is that MCD acts presynaptically releasing other peptides or amino acids which might produce the spontaneous and evoked network bursts. The endogenous opioid peptides, known to induce paroxysmal activity in the hippocampus[20], are present in the mossy fibres[26]. Interictal bursts also develop in CA3 neurones in the absence of magnesium presumably as a consequence of the removal of the magnesium block of the *N*-methyl-D-aspartate (NMDA) receptor-activated channel[1,31]. We have therefore tested the effects of the opioid antagonist naloxone (1 μM) and the NMDA antagonists D-APV and AP-7 (30 μM) on the spontaneous and evoked network bursts induced by MCD.

Prior or subsequent to exposure of the slices to naloxone ($n = 2$) or D-APV and AP-7 ($n = 4$) did not prevent the occurrence of SNB and ENB (Fig. 9), ruling out the possibility that the bursts induced by MCD were mediated through the activation of an opioid or an NMDA type of receptor.

DISCUSSION

The major finding reported in the present study is that MCD produces in CA3 hippocampal neurones spontaneous and evoked network bursts that persist for prolonged periods of time after washout of the toxin. The effects of MCD are mediated through specific, high affinity receptors[35] which are localized in the hippocampus and other cortical structures[5]. Moreover, similarly to the MCD analogue, apamin, for which there is an endogenous equivalent in the CNS[12], binding and radioimmunoassay techniques

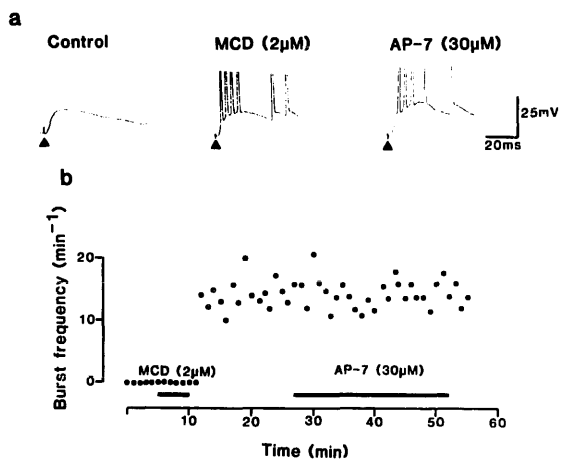

Fig. 9. Bursts induced by MCD are not antagonized by AP-7. a: stimulation of the mossy fibres (triangles) evoked before MCD application an EPSP (control). Following MCD, the same stimulus evoked a burst. This was not blocked by AP-7. In b, the frequency of the spontaneous burst vs time is plotted in the same neurone. Spontaneous bursts only developed following wash out of MCD. Period of MCD application is indicated by solid bar. Note that the frequency of the bursts remained stable despite superfusion of AP-7 for 25 min.

suggest the presence of an endogenous MCD like peptide in the brain[8]. This raises the possibility that an endogenous ligand plays a role in generating paroxysmal interictal events.

It bears stressing that the bursts produced by MCD are very similar to those produced by several convulsants, including pentylenetetrazol[6], penicillin and bicuculline[37], high potassium[29], (+)-tubocurarine and kainate[2] or produced by electrical stimulation of afferent pathways to CA3[32]. These agents produce paroxysmal interictal events which are generated in a polysynaptic circuitry[2,37]. MCD also probably operates through a polysynaptic circuitry since the bursts induced by the peptide are readily blocked by a medium containing high divalent cation concentrations, known to reduce polysynaptic transmission[4]. Although the exact anatomical substrate for the synchronous activity has not been completely elucidated, the existence of recurrent excitatory collaterals between the pyramidal neurones of CA3 appears to play an important role in producing the network burst[27].

Several mechanisms may account for the MCD-induced epileptogenic activity. Thus interictal bursts may develop as a consequence of a reduction of the GABA-mediated synaptic inhibition as it has been shown in the case of bicuculline[37], penicillin[9], kainate[10] or repetitive stimulation of afferents[3,33]. However this possibility is very unlikely since: (i) the IPSP is not reduced during and after application of MCD; (ii) MCD still induces bursts when the Cl^--mediated GABAergic inhibition is blocked by bicuculline.

Another possibility is that MCD acts at a presynaptic site enhancing the release of neurotransmitters. The enhancement of the frequency of the spontaneous postsynaptic potentials by MCD, even after blockade of spike discharge and synaptic transmission by TTX, is suggestive of such a mechanism. In this respect MCD may act like the potent protein kinase C (PKC) activator, phorbol ester[24]. Like phorbol ester[24], MCD produces in CA1 a long-term potentiation of the EPSP[8]. It is therefore possible that like phorbol derivates, MCD enhances transmitter release through the activation of PKC which is localized on the presynaptic nerve terminals[14]. In favour of a possible presynaptic site of action is also the lack of clear postsynaptic effects on the membrane potential, resistance and cell excitability, particularly when measured in the absence of spontaneous interictal bursts during superfusion with a medium containing high divalent cation concentrations. Alternatively MCD, like apamin, a selective blocker of one type of Ca-activated potassium channel[21] may block or directly activate ionic channels involved in neurotransmitter release. It has been recently shown that MCD interacts with dendrotoxin, inhibiting its binding to solubilized extracts of synaptosomes[28]. Like this toxin[18], MCD causes in CA1 hippocampal neurones the generation of an anode break action potential consistent with a blockade of a transient K^+-current (I_A)[28]. This effect may contribute to the enhancement of transmitter release in the same way as 4-aminopyridine[30,36].

The nature of the transmitter or factor released by the toxin is not known; however, on the basis of pharmacological experiments we can exclude that NMDA and/or an opioid peptide play a major role in the SNB or ENB elicited by MCD. In conclusion, MCD produces a long-term potentiation of the EPSP in CA1[8] and long-lasting induction of SNB and ENB in CA3 region. It is possible that in both cases, the toxin acts presynaptically to release factor(s) which produce the long-lasting changes in the synaptic responses.

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