Domoic Acid Induces a Long-Lasting Enhancement of CA1 Field Responses and Impairs Tetanus-Induced Long-term Potentiation in Rat Hippocampal Slices

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Received April 23, 2009; accepted June 22, 2009

Domoic acid (DOM) is known to cause hippocampal neuronal damage and produces amnesic effects. We examined synaptic plasticity changes induced by DOM exposure in rat hippocampal CA1 region. Brief bath application of DOM to hippocampal slices produces a chemical form of long-term potentiation (LTP) of CA1 field synaptic potentials. The potentiation cannot be blocked by NMDA receptor antagonist MK-801 but can be blocked by the calcium-calmodulin–dependent protein kinase II (CaMKII) inhibitor KN-62 or cAMP-dependent protein kinase (PKA) inhibitor H-89. DOM-potentiated slices show decreased autophosphorylated CaMKII (p-Thr286), an effect that is also dependent on the activity of CaMKII and PKA. Increased phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR1 (p-Ser831) was seen in DOM-potentiated slices. Therefore, aberrant regulation of CaMKII and GluR1 phosphorylation occurs after DOM application. In addition, tetanus-induced LTP as well as the increase of phosphorylation of CaMKII (p-Thr286) were reduced in DOM-potentiated slices. Compared with brief exposure, slices recovering from prolonged exposure did not show potentiation or altered levels of CaMKII (p-Thr286) or GluR (p-Ser831). However, decreased phosphorylation of GluR1 at Ser845 was seen. These results describe a new chemical form of LTP and uncover novel molecular changes induced by DOM. The observed impairment of tetanus LTP and misregulation of CaMKII and GluR1 phosphorylation may partially account for DOM neurotoxicity and underlie the molecular basis for DOM-induced memory deficit.

Key Words: AMPA; kainate; amnesia; glutamate receptor; PKA; CaMKII.

The amnesic shellfish toxin, domoic acid (DOM), is one of the major marine neurotoxins and frequently causes poisoning accidents when transferred through the food chain (Gulland et al., 2002; Lefebvre et al., 1999; Perl et al., 1990). One of the fascinating features of this toxin observed in a poisoning accident of human beings is the severe memory impairment or anterograde amnesia (Perl et al., 1990; Teitelbaum et al., 1990). Human autopsy and animal studies have shown that hippocampus is one of the major targets of DOM (Qiu and Curras-Collazo, 2006; Scallet et al., 1993; Teitelbaum et al., 1990). Systemic administration of DOM produces seizures and extensive hippocampal neuronal degeneration in experimental animals (Dakshinamurti et al., 1993; Scallet et al., 1993; Strain and Tasker, 1991). As the hippocampus plays an important role in central nervous system plasticity and memory function, neuronal death after DOM exposure may well account for the observed anterograde amnesia (Teitelbaum et al., 1990) or compromised learning ability in animals (Kuhlmann and Guilarte, 1997; Sutherland et al., 1990). Interestingly, relatively low exposures that are subthreshold for seizures can still produce long-lasting memory deficits (Burt et al., 2008; Teitelbaum et al., 1990). Current knowledge on the acute hippocampal functional changes induced by DOM at sublethal concentrations to hippocampal neurons is limited. The synaptic correlates of hippocampal memory dysfunction after DOM exposure remain unidentified.

Hippocampal long-term potentiation (LTP), an activity-dependent strengthening of synaptic efficacy, is the leading synaptic mechanism that may underlie learning and memory (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). A major form of LTP in the hippocampal CA1 field is known to depend on NMDA-type glutamate receptor activation (Collingridge and Singer, 1990; Nicoll and Malenka, 1999). Ca$^{2+}$ influx through these receptors activates downstream protein kinases including calcium-calmodulin–dependent protein kinase II (CaMKII; Lisman et al., 1997; Soderling and Derkach, 2000) and cAMP-dependent protein kinase (PKA; Abel et al., 1997; Nayak et al., 1998), which phosphorylate a variety of cellular substrates including α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors (AMPARs; Barria et al., 1997; Lee et al., 1990) to achieve strengthened synaptic efficacy. In agreement with the notion that LTP is a putative cellular and molecular mechanism for memory formation, agents that affect LTP have been shown to induce deficits in learning ability and...
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memory acquisition (Brun et al., 2001; Davis et al., 1992; Highfield et al., 1996; Morris et al., 1986; Nihei and Guilarte, 2001). Seizure-like activity, which can be induced by kainate receptor (KAR) activation (Sarkisian et al., 1997) and is frequently found during DOM exposure conditions (Dakshinamurti et al., 1993; Sari and Kerr, 2001), has been shown to disrupt hippocampal LTP (Barr et al., 1997; Kerr et al., 2002; Stewart and Davies, 1996). However, the direct effects of DOM on hippocampal LTP and the associated molecular pathways leading to plasticity changes have not been determined.

Studying the effects of DOM on hippocampal synaptic transmission should help in understanding the possible mechanisms of DOM-induced memory deficits as well as in developing therapeutic strategies against this neurotoxin. Consequently, we have performed experiments aimed at clarifying DOM-induced acute changes of synaptic plasticity and the temporal profiles of such changes. Our results show that brief application of DOM causes long-lasting potentiation of CA1 field potentials and produces some distinct biochemical changes that prevent tetanus-induced LTP. The observed changes in hippocampal synaptic plasticity after DOM exposure could underlie the mechanisms of DOM-induced memory deficits.

MATERIALS AND METHODS

Slice preparation and electrophysiological recordings. Animal care and use procedures were in accordance with Guide and Care for the Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and were approved by the Institutional Animal Care and Use Committee of University of California, Riverside. Briefly, 3- to 4-week-old male Sprague-Dawley rats were anesthetized with Nembutal (50 mg/kg) and decapitated. The brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) (contains in mM: 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.2 MgCl2, 2.5 CaCl2, 1.25 KH2PO4, and 10 glucose, pH 7.3–7.4). The hippocampus was dissected out and transverse slices (300–400 μm) were cut using a slice chopper. Slices were immersed in ACSF and saturated with 95% O2 and 5% CO2 and kept in room temperature (RT) for at least 1 h before recording.

A single slice was transferred to the recording chamber, placed on the surface of a nylon net, and superfused with ACSF. The chamber consisted of a circular well of low volume (2–3 ml) and was perfused constantly at a rate of 2–3 ml/min at 32°C ± 0.5°C. Both stimulating and recording electrodes were placed under visual guidance with the aid of a dissecting microscope. Extracellular recording of field potentials were obtained from the CA1 region in response to biphasic stimulus pulses (100–900 μA; 250 μs duration; 0.033 Hz) delivered to fibers of the stratum radiatum near CA3 region. Potentials were recorded separately either in stratum radiatum layer for the field excitatory postsynaptic potentials (fEPSP) or in stratum pyramidale layer for synchronized population spike (PS) responses using glass micropipettes filled with 3 M NaCl and with tip diameters about 1 μm (5–10 Ω) electrical resistance. Stimulating electrodes were constructed from twisted insulated stainless steel wire, and stimulation was controlled by a constant current stimulator (Grass Instruments, Quincey, MA). Recorded electrical signals were amplified, filtered at 1 kHz, and digitized at 5 kHz through a PowerLab A/D interface and stored on a Macintosh PowerPC 7200 computer under control of Scope software (AD Instruments, Colorado Springs, CO), which is also used for part of the off-line data analysis. Pulses of stimulus intensity that gave 30–40% of the maximal fEPSP or PS amplitude were used throughout the experiments. Baseline synaptic responses were recorded for at least 30 min before drug application or delivering tetanus stimulation. The strength of synaptic transmission was quantified by measuring the slope of fEPSP or PS amplitude. Slope of fEPSPs was measured using a time window of the initial 1 ms of the rising segment after fiber volley. PS amplitudes were measured from the peak of negativity to a tangent line drawn between the first and the second maximum positivities. The fiber volley was measured using the peak amplitude where discernable on the fEPSP recordings. Paired-pulse facilitation (PPF) of PS was measured as the ratio of amplitudes of a second PS over the first. For tetanus-induced LTP, two 1-s trains of 100 Hz stimulus (same intensity during the baseline recording) were delivered with an interval of 20 s, which is known to cause NMDA receptor–dependent LTP in CA1. Before application of DOM and other chemicals, the average of response during a 10-min period was taken as the baseline. All values reported were converted into percent changes in fEPSP slope or PS amplitude.

All drugs were applied by dissolving them to the desired final concentrations in ACSF and by switching the perfusion from control ACSF to drug-containing ACSF. KN-62 and H89 were dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C as stock solutions. To test the effects of these drugs on DOM-induced potentiation, slices were preincubated with these drugs for 30 min before being switched into DOM-containing ACSF. The concentration of DMSO in the recording chamber medium was 0.1% during drug application, which alone had no effect on the induction of LTP in rat CA1 (Hsu and Huang, 1997). Other drugs were dissolved in ACSF. DOM was purchased from Tocris Cookson (Ellisville, MO). KN-62 and H89 were obtained from Sigma-Aldrich (St Louis, MO).

Western blot analysis. After recording was completed, slices were quickly frozen on dry ice and CA1 region was dissected under a dissecting microscope. Three to four CA1 tissue slices receiving same treatment were pooled together to obtain sufficient amount of protein. CA1 tissue was then sonicated in 150 μl protease inhibitor cocktail solution containing 50mM Tris-HCl, pH 7.4, 10mM EDTA, 100μM leupeptin, 1 μg/ml aprotinin, 10 μg/ml bacitracin, 100μg/ml phenylmethylsulfonyl fluoride, and 1mM sodium orthovanadate. Protein contents of homogenates were determined using a bicinchoninic acid assay (Pierce, Rockford, IL). The sample was then mixed with equal volume of 2× loading buffer (containing 10mM Tris-TH, pH 6.8, 10% (vol/vol) β-mercaptoethanol, 0.1% bromphenol blue, 30% (vol/vol) of 20% SDS, and 40% glycerol). Ten micrograms of total protein was resolved on 10% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Immobilon-P; Fisher Scientific, Waltham, MA). The membranes were transferred to a blocking solution containing 5% nonfat dry milk and 1% bovine serum albumin for 2 h at RT before incubation with primary antibodies in 0.01M PBS and 0.05% Tween 20 for 2 h at RT. Monoclonal CaMKII (1:4000 dilution) and α-tubulin (1:5000) primary antibodies or polyclonal primary antibodies targeting GluR1 (1:1000) were purchased from Millipore (Billerica, MA), p-Ser845 GluR1 antibody and p-Thr286 CaMKII antibody (1:1000) were obtained from Millipore and Promega (Madison, WI), respectively. After 2-h incubation with primary antibodies at RT, the membranes were then washed in 0.01M PBS-Tween and incubated for 1 h at RT with horseradish peroxidase-conjugated synthetic polypeptide secondary antibodies that recognize rabbit or mouse species (Dakocytomation, Carpinteria, CA). The membranes were then washed in 0.01M PBS-Tween and developed using the enhanced chemiluminescence method (Amersham Pharmacia, Arlington Heights, IL). The optical density of bands of each Western blot was quantified by densitometry using ImagePro software (Media Cybernetics, Carlsbad, CA) and normalized with α-tubulin immunoreactivity to compensate for loading errors.

Data analysis. Electrophysiological data analysis was performed using Scope software (AD Instruments). Statistical analysis and graphing were carried out using GraphPad Prism software (GraphPad Inc., San Diego, CA). All data for electrophysiology experiment were normalized to the baseline responses; normality and equal variance tests were performed to select appropriate statistical tests for comparisons. Student’s paired or unpaired t-test.
was employed for paired or group comparisons with data that passed normality and/or equal variance test. Otherwise nonparametric Wilcoxon and Mann-Whitney tests were performed for paired versus group comparison, respectively. Friedman repeated measures ANOVA on ranks followed by Dunn's tests were performed for multiple comparisons when the normality tests failed. One-way ANOVA analysis with post-hoc Student-Newman-Keuls test was used for multiple comparisons when data passed normality and equal-variance tests. Significance was assessed at \( p < 0.05 \) using two-tailed Student's t-test or \( p < 0.01 \) when using nonparametric tests.

RESULTS

DOM preferentially activates KARs at submicromolar concentrations (Hampson and Manalo, 1998). KARs are known to play a role in epileptiform activity, particularly in CA3 region of hippocampus (Khalilov et al., 1999; Meier and Dudek, 1996). Therefore, to mimic the in vivo conditions during DOM exposure, we preserved the intact CA3 input to CA1 without sectioning the Schaffer collateral pathway as other studies have done (Frerking et al., 2001; Sari and Kerr, 2001), thus allowing potential effects of CA3 somatodendritic KARs to be seen. CA1 field potential responses were affected by DOM across a ranges of doses under our experimental settings (Supplementary Fig. 1). Application of DOM at subthreshold concentration for producing spontaneous CA1 epileptiform activity (400nM) rapidly depressed fEPSP response within minutes (Fig. 1A). The depression of fEPSP is most likely due to AMPA/KA receptor–induced soma depolarization and thus loss of driving force for cations, an effect also seen with other excitatory amino acids application in hippocampal slices (Broutman and Baudry, 2001). After washout of DOM, fEPSP slope gradually recovered and continued to increase and surpassed the baseline by an average of 33.1% at 1 h following washout. The potentiated response stabilized thereafter and lasted throughout the remaining 1-h duration of the experiment (Fig. 1A). This long-lasting potentiation induced by a brief DOM application is hereafter referred to as DOM-LTP. DOM-LTP did not change CA1 input fiber excitability throughout the time course (Fig. 1B). Increased response/stimulus ratio was observed, with same fiber volley amplitude producing larger fEPSP response during DOM-LTP (Fig. 1C). PPF of PS amplitude was also seen at shorter interpulse intervals (40 and 60 ms) during which control slices showed paired-pulse depression (Fig. 1D).

We next tested tetanus-induced LTP property in slices manifesting DOM-LTP. Following development of DOM-LTP, tetanus-induced LTP of fEPSP slope was dramatically reduced to 112.6 ± 7.4% of baseline at 1 h after tetanus
compared with 171 ± 7.1% in control slices under the same conditions ($p < 0.0001, n = 6, t$-test; Fig. 2). To assess whether the reduced LTP can be attributed to a previously saturated response (prepotentiation) during DOM-LTP, stimulus strength was reduced to size-match fEPSP with baseline values before DOM application. In this testing condition, tetanus LTP was also dramatically lowered (128.1 ± 7.9%, $n = 6, p < 0.0001$; Fig. 2) compared with that of control slices at 1 h after tetanus. Therefore, in slices that were potentiated by brief exposure to DOM, tetanus-induced LTP was impaired compared with control slices receiving no DOM exposure.

We then examined whether prolonged application of DOM had similar effects compared with brief exposure to DOM. In slices superfused with 400nM DOM for 1 h, fEPSP remained depressed in the presence of DOM (Fig. 3A). fEPSP slope gradually recovered during the next 30 to 50 min after bathing solution was replaced with normal ACSF and reached 93.3 ± 8.9% ($n = 7$) of the baseline after 1 h wash time, a value not significantly different from that of pre-DOM baseline at this time ($p > 0.05$). Thus, DOM-LTP was not induced with prolonged 1-h DOM exposure. No change of fiber volley amplitude was seen as a result of prolonged DOM exposure (Fig. 3B). The stimulus-response curve was not changed either at this time compared with that generated during basal recording (Fig. 3C, $p > 0.05$; Mann-Whitney test), which argues against the possibility of reduced viability of CA1 neurons during the experimental period. We further tested tetanus-induced LTP property in these slices. In slices with prior 1-h DOM exposure and 1-h recovery time, LTP was established but at much lower amplitude (122.6 ± 9.4% of baseline at 1-h post tetanus) compared with control group LTP (164.3 ± 10.1% baseline at 1-h after tetanus, $n = 7, p < 0.0001$; Student’s $t$-test; Fig. 3D). Therefore, after prolonged exposure to DOM, tetanus LTP can be established but at greatly reduced amplitude.

The observation of impaired tetanus LTP following DOM application prompted us to test for the possible mechanisms underlying DOM-LTP. It is well known that induction and maintenance of tetanus-induced CA1 LTP is dependent on activation of NMDA receptors (Collingridge and Singer, 1990; Nicoll and Malenka, 1995) and requires interacting signaling components of CaMKII (Lledo et al., 1995; Malinow et al., 1989) and PKA (Abel et al., 1997; Nayak et al., 1998; Otmakhova et al., 2000). We adopted a tetanus LTP protocol that is known to be dependent on NMDA receptors, and the downstream biochemical cascade is relatively well known. We hypothesized that the impaired LTP property is the result of a perturbation of the signal transduction cascade required for the induction or maintenance of LTP. Compared with NMDAR-dependent LTP, NMDAR-independent LTP has been demonstrated to require Ca$^{2+}$-calmodulin–sensitive adenylyl cyclase–dependent PKA in CA3 (Weisskopf et al., 1994). Surprisingly, we found that DOM-LTP did not require activation of NMDA receptors since DOM effects on fEPSP persisted with identical time course (Fig. 4A, $p > 0.05, n = 5$; Wilcoxon test) when DOM was given after 30-min preapplication and in the continued presence of MK–801 (5μM), an activity-dependent, noncompetitive NMDA receptor blocker. The same results were obtained when another competitive NMDAR blocker D-AP5 (100μM) was used (data not shown). However, DOM-LTP was abolished when slices were pretreated with either H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide, 10μM), a PKA-specific inhibitor (Fig. 4B), or KN-62 (10μM), a CaMKII-specific inhibitor (Fig. 4C). Neither KN-62 nor H-89 applied alone at 10μM changed baseline fEPSP responses during the 30-min preincubation period or during 1 h following washout compared with control slices receiving vehicle treatment (data not shown).

Given the importance of CaMKII and PKA in CA1 LTP, we next measured the level of autophosphorylated CaMKII (Thr286) during 400nM DOM application and following washout. CaMKII, which plays a central role in learning and memory (Giese et al., 1998; Weeber et al., 2003), is autophosphorylated at Thr286 of alpha subunit to achieve a persistent active form during tetanus LTP (Barria et al., 1997; Giese et al., 1998).
CaMKII activation also increases AMPAR-mediated responses by phosphorylation of GluR1 at Ser831 (Derkach et al., 1999; Mammen et al., 1997). In contrast, phosphorylation of another GluR1 site, Ser845, is dynamically regulated by PKA during plasticity changes (Banke et al., 2000; Lee et al., 2000; Roche et al., 1996; Tavalin et al., 2002). A recent study has shown that phosphorylation of GluR1 is required for synaptic plasticity and retention of memory (Lee et al., 2003). Thus, the temporal profiles of CaMKII, CaMKII (p-Thr286), GluR1, and GluR1 (p-Ser831 or p-Ser845) were examined in homogenates CA1 slices with or without DOM exposure. We tested the levels of these proteins in different groups of slices receiving either 400nM DOM for 10 min or 1 h, 400nM DOM for 10 min and followed by 1-h washout (during DOM-LTP), or 1-h DOM followed by 1-h washout when basic responses were restored without DOM-LTP. Our results (Fig. 5A) showed that in slices with 10-min or 1-h exposure to DOM, p-Thr286 immunoreactivity was 154.2 ± 11.1% (n = 6, p < 0.01) and 107.5 ± 14.3% (n = 5, p > 0.05) of control slices level, respectively. Therefore, a brief (10 min) exposure to DOM transiently increases autophosphorylation of CaMKII (p-Thr286). Remarkably, p-CaMKII immunoreactivity was significantly reduced to 33.4 ± 12.5% (n = 7, p < 0.001) of control level during the phase of DOM-LTP. No change was detected in slices receiving 1-h exposure to 400nM DOM and followed by 1-h washout (89.3 ± 11.5% of control, n = 5, p > 0.05). Notably, total amount of CaMKII was not changed (Fig. 5A).

It is well established that p-Thr286 of CaMKII is increased during tetanus-induced CA1 LTP (Fukunaga et al., 1995; Giese et al., 1998; Ouyang et al., 1999). Since tetanus-induced LTP in DOM-LTP slices or slices with prolonged DOM application is reduced, we tested whether these slices show similar tetanus-induced increase of p-Thr286 as control slices do (Fig. 5B). Our results show that in control, slices tetanus stimulus increased p-Thr286 by 44.8 ± 7.8% (n = 5) when measured at 30 min after tetanus, while in DOM-LTP slices and slices with prolonged 1-h exposure and 1-h recovery, tetanus stimulus increased p-Thr286 by 12.2 ± 7.1% (n = 6, p < 0.05 compared with control slices LTP). Thus, in slices with prior DOM application, the ability for CaMKII autophosphorylation in response to tetanus is reduced.

We next examined the phosphorylation levels at two GluR1 sites for DOM-treated slices at different time points during the experimental period. Exposing slices to 400nM DOM for
10 min or 1 h marginally increased p-GluR1 (Ser831) to 114.0 ± 9.3% and 112.0 ± 11.8% (n = 6, p > 0.05 for both) of control levels, respectively (Fig. 6). In comparison, in slices with 10-min and 1-h exposure and both followed by 1-h washout, GluR1 (p-Ser831) immunoreactivity was 142 ± 9.7% (n = 6, p < 0.01) and 115.0 ± 13.3% (n = 5, p > 0.05) compared with control slices. Phosphorylation level of GluR1 (p-Ser845) was not significantly different for DOM-LTP slices (93.2 ± 10.1%, n = 5, p > 0.05). In slices with 1-h prolonged exposure and 1-h exposure followed by 1-h washout, the level of GluR1 (p-Ser845) was reduced to 71.3 ± 10.4% and 74.4 ± 7.9% (n = 5, p < 0.05 for both), respectively. Therefore, like the levels of autophosphorylated CaMKII, phosphorylation of GluR1 is also determined by the duration of DOM exposure and the recovery time after DOM removal.

In view of the fact that both PKA inhibitor H-89 and CaMKII inhibitor KN-62, but not NMDAR inhibitor MK-801, blocked DOM-LTP, we examined the level of CaMKII (p-Thr286) in slices pretreated with these drugs (Fig. 7). We found that MK-801 did not change the level of p-Thr286 in slices showing DOM-LTP (n = 5, p > 0.05), while both KN-62 and H-89 reversed the reduction of p-Thr286 by DOM-LTP (n = 4, p < 0.05 compared with DOM-LTP slices and p > 0.05 compared with control levels). Application of these drugs alone did not change the level of p-Thr286. These biochemical data further substantiate the involvement of CaMKII and PKA in DOM-induced changes of synaptic plasticity and impairment of LTP.

FIG. 4. DOM-LTP is dependent on protein kinase activation. (A) DOM-LTP is not blocked by NMDAR antagonist MK-801. The time course of fEPSP slope for 80 min following DOM exposure (10 min, 400nM) was not changed by preincubation of slices with MK-801 (5μM) (n = 5, p > 0.5; Wilcoxon test). (B) DOM-LTP can be blocked by preincubating slices with 10μM H-89. Compared with fEPSP slope of DOM exposed slices showing potentiation over washout period, the fEPSP slope barely exceeded the baseline level in slices preincubated with H-89 and was significantly reduced compared with control slices (p < 0.0001, n = 6; Wilcoxon test). (C) Preincubation with 10μM KN-62 also significantly reduced DOM-LTP compared with control slices (p < 0.000, n = 6; Wilcoxon test). Data were normalized as percentage of baseline values. Note that MK-801, KN-62, and H-89 did not change the basal response during the 30-min preincubation period.

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DISCUSSION

We have shown that DOM exposure can produce a chemical form of synaptic potentiation and impair CA1 tetanus LTP property in rat hippocampal slices. In view of the hippocampal plasticity changes induced by DOM exposure, our findings may have implication in DOM-induced memory deficits. Acute application of DOM changes field potential responses and has long-lasting effects on hippocampal LTP even after DOM is removed. It is well known that hippocampal LTP is subject to synaptic modifications. For example, cytokines (Barrientos et al., 2002), hormones (Gureviciene et al., 2003), factors influencing glutamate release (for a review, see Malenka and Nicoll, 1993), and recent history of synaptic activity (Hsu et al., 2000; Huang et al., 1992; Lee et al., 2000) all have been shown to influence hippocampal synaptic plasticity. Application of high concentrations of K⁺ (Frerking et al., 2001; Semyanov et al., 2000), NMDA (Broutman and Baudry, 2001), or taurine (del Olmo et al., 2003) also leads to potentiated response of CA1 field potentials. This study shows for the first time that hippocampal slices with prior exposure to DOM exhibit compromised tetanus-induced LTP. Since agents that affect LTP have been shown to produce deficits in learning ability and memory acquisition (Brun et al., 2001; Highfield et al., 1996; Nihei and Guilarte, 2001), our findings may have implications for DOM-induced memory deficit. Indeed, based on a well-documented human poisoning accident (Perl et al., 1990; Teitelbaum et al., 1990), people showing memory impairment had a median consumption amount of 200 mg DOM, which could reach peak serum levels of DOM at lower
micromolar ranges. Even with the existence of a blood-brain barrier, in vivo hippocampal DOM concentrations may reach levels comparable to those observed in this study to cause impairment of LTP in rat hippocampal slices.

Additionally, we have shown that DOM-induced LTP does not require NMDAR but requires both CaMKII and PKA. This NMDAR-independent synaptic potentiation can be produced with brief bath application of DOM. However, prolonged 1-h exposure followed by washout failed to produce similar potentiated responses, which indicates that brief application of DOM induces CA1 plasticity changes, whereas prolonged activation may employ different mechanisms to suppress or offset these effects. The potentiated slices show an increased

![FIG. 5. DOM application alters autophosphorylation of CaMKII (p-Thr286). (A) Temporal profiles of CaMKII (p-Thr286) and CaMKII during or after DOM application. T1, T3, CaMKII (p-Thr286) levels detected after 10-min and 1-h DOM application, respectively. T2, DOM-LTP slices; T4, 1-h DOM followed by 1-h recovery. C1–C4 control for T1–T4, respectively. Application of DOM does not change total amount of CaMKI in all groups. p-Thr286 significantly increases after 10 min DOM and then decreased when DOM-LTP is shown. (B) Reduced increment of tetanus-induced CaMKII (p-Thr286) in DOM-LTP slices and slices with prolonged 1-h exposure and 1-h washout. Immunoreactive densities of p-Thr286 were standardized to α-tubulin levels and plotted as percent of control values. (B) In control slices and DOM-LTP slices, tetanus induced an average of 44.8 and 12.2% increase of p-Thr286 immunoreactivity when measured at 30 min after tetanus, respectively. *p < 0.05, n = 6; two-way ANOVA test.](image1)

![FIG. 6. Temporal profiles of changes of GluR1 phosphorylation by DOM application. (A) DOM-LTP slices show increased p-Ser831, while prolonged application of DOM reduces p-Ser845. T1, T3, 10-min and 1-h DOM application, respectively. T2, DOM-LTP slices; T4, 1-h DOM followed by 1-h recovery. C1–C4 in-group control for T1–T4, respectively. Application of DOM does not change total amount of GluR1. (B) DOM-LTP slices showed increased phosphorylation of GluR1 at Ser831 without changing phosphorylation of Ser845. However, in slices with prolonged 1-h exposure, phosphorylation of Ser845 was significantly reduced. Plotted bar graph represents mean ± SEM for six to eight experiments. *p < 0.05 compared with control bands, using Student’s t-test.](image2)

![FIG. 7. KN-62 and H-89 but not MK-801 blocked the reduced autophosphorylation of CaMKII (p-Thr286) during DOM-LTP. Lane 1, control; lane 2, DOM-LTP slices; lane 3, DOM-LTP slice with 5μM MK-801 preapplied for 30 min; lane 4, slices in which DOM-LTP was blocked by 10μM KN-62; lane 5, slices in which DOM-LTP was blocked by 10μM H-89; lane 6–8, slices receiving MK-801, KN-62, and H-89 treatment alone, respectively. Immunoreactivity of each band was normalized with α-tubulin level of the same lane and plotted as percent of control value, n = 5, *p < 0.05 compared with lane 1; #p < 0.05 compared with lane 2 (one-way ANOVA with Student-Newman-Keuls test).](image3)
response to the same stimulus and an increased PPF ratio, which indicates that presynaptic mechanisms are likely involved in this potentiation, consistent with a presynaptic role of KARs (Ferking et al., 2001). We further explored the molecular basis for this chemical form of LTP. It is known that NMDAR-mediated Ca\(^{2+}\) influx is required in many forms of CA1 LTP (Bliss and Collingridge, 1993). However, increasing postsynaptic Ca\(^{2+}\) without associated NMDAR-mediated processes is also effectively coupled to downstream biochemical pathways leading to changes of synaptic plasticity (Neveu and Zucker, 1996). For example, NMDAR-independent LTP can be induced at CA1 by very–high frequency (> 200 Hz) stimulations and activation of voltage-sensitive calcium channels (Bauer et al., 2002; Grover and Teyler, 1990). In addition, AMPAR-mediated Ca\(^{2+}\) influx can induce NMDA-independent LTP as well (Feldmeyer et al., 1999).

Activation of AMPA/KARs by DOM can lead to de-polarization and facilitate Ca\(^{2+}\) influx through NMDAR, voltage-gated calcium channels or some Ca\(^{2+}\) permeable AMPARs, which have been shown to activate downstream protein kinases and lead to either strengthening or weakening of synaptic strength depending on the dynamics of Ca\(^{2+}\) change (D’Alcantara et al., 2003; Lee et al., 2000). Based on the pivotal role of CaMKII and PKA in tetanus LTP (Barria et al., 1997; Giese et al., 1998; Nayak et al., 1998), we hypothesize that Ca\(^{2+}\)-mediated biochemical pathways involving protein kinases CaMKII and PKA are important in DOM-LTP. CaMKII is centrally located in the biochemical cascade during tetanus LTP (Fukunaga et al., 1993; Lledo et al., 1995; Miller et al., 2002) and is crucial for memory formation (Giese et al., 1998; Weeber et al., 2003). Autophosphorylation of CaMKII at Thr286 prolongs the enzyme function beyond the transient rise in intracellular Ca\(^{2+}\) concentration and it happens during tetanus LTP (Barria et al., 1997; Giese et al., 1998). In agreement with our hypothesis, we found that CaMKII (p-Thr286) was transiently increased after 10-min DOM exposure, indicating that DOM affects CaMKII activity by altering autophosphorylation of CaMKII. However, in striking contrast with tetanus LTP-induced increased autophosphorylation (Barria et al., 1997; Giese et al., 1998; Ouyang et al., 1999), CaMKII (p-Thr286) was decreased when DOM-LTP is shown. This dynamic changes of CaMKII (p-Thr286) may reflect effects of integrated biochemical events downstream to Ca\(^{2+}\) signal, as Ca\(^{2+}\)-controlled regulation of plasticity can either favor CaMKII phosphorylation or dephosphorylation through activation of protein phosphatases (Blitzer et al., 1998; D’Alcantara et al., 2003; Mulkey et al., 1994; Winder and Sweatt, 2001). Consistent with the pivotal role of CaMKII in LTP and the dynamic changes of autophosphorylation of CaMKII caused by DOM application, we found that KN-62, a specific CaMKII inhibitor, significantly blocked DOM-LTP, as it did during tetanus LTP. This further supports that activity of CaMKII is required in DOM-LTP. Surprisingly, preapplication of KN-62, but not MK-801, also blocked the reduction of CaMKII (p-Thr286) level during DOM-LTP, suggesting that the dephosphorylation of CaMKII (p-Thr286) levels by phosphatases (D’Alcantara et al., 2003; Malleret et al., 2001; Mulkey et al., 1994) may require the activity of CaMKII itself.

The NMDAR-independent DOM-LTP is equally blocked by PKA inhibitor, H-89, indicating cAMP-mediated signaling pathway is involved. Ca\(^{2+}\)-triggered biochemical events involving PKA are required in both early (Otmakhova et al., 2000) and late-phase CA1 LTP (Huang et al., 2000, 2004; Nayak et al., 1998) elicited by tetanus stimulation or by exogenous application of chemicals (del Olmo et al., 2003; Huang et al., 1995). Our results demonstrate that PKA activity is also required for expression of DOM-LTP since it was blocked by H-89. Taken together, our findings suggest that two interacting kinase pathways, one involving CaMKII and the other PKA, are both required in developing DOM-LTP. It has been shown that increased Thr286 autophosphorylation and Ca\(^{2+}\)-independent CaMKII activity occurs in a cAMP-dependent manner, and protein phosphatase I is used by the cAMP pathway to “gate” CaMKII signaling during LTP (Blitzer et al., 1998). Interestingly, the reduced autophosphorylation of CaMKII during DOM-LTP was also reversed when PKA activity is inhibited by H-89, which supports a possible interaction between PKA and CaMKII during a chemical form of LTP as well. It is likely that biochemical events downstream to PKA are also required for an increased activity of protein phosphatases, which could dephosphorylate p-Thr286 during DOM-LTP.

The increased field potential responses during DOM-LTP may be related at least in part to the change of glutamatergic responses mediated by AMPARs because of increased phosphorylation of Ser831 of GluR1 during DOM-LTP, although redistribution of AMPAR to synapse (Esteban et al., 2003; Hayashi et al., 2000; Lisman and Zhabotinsky, 2001) may contribute to the increased synaptic responses. It is known that phosphorylation AMPAR subunit GluR1 at distinct sites is dynamically regulated during synaptic plasticity. Phosphorylation of Ser831 increases AMPAR conductance (Banke et al., 2000; Derkach et al., 1999) and has been shown to occur during tetanus LTP (Barria et al., 1997; Lisman et al., 1997). Phosphorylation of the Ser845 site of GluR1 increases channel open probability and is regulated by PKA during bidirectional synaptic plasticity (Lee et al., 2000). In striking contrast to brief DOM application, prolonged application of DOM does not increase p-Ser831. This could be the result of increased Ca\(^{2+}\)-dependent phosphatase activities leading to reduced phosphorylation of Ser831. However, prolonged application of DOM significantly reduces phosphorylation of Ser845, which was not seen in DOM-LTP slices induced by brief DOM application. The PKA dependency of DOM-LTP and the decreased p-Ser845 after prolonged exposure all demonstrate involvement of Ca\(^{2+}\)/cAMP-dependent pathway during DOM-induced plasticity changes.
In slices exhibiting DOM-LTP, tetanus LTP was remarkably decreased, which cannot be attributed to prepotentiation of synaptic transmission. When the stimulus intensity was adjusted to rule out this possible “ceiling” effect, tetanus LTP is still dramatically reduced. In addition, slices recovered from prolonged exposure also had impaired tetanus LTP, although basal response to fixed stimuli, stimulus-response relationship curves, and CaMKII (p-Thr286) and GluR1 (p-Ser831) levels were not changed. Although the exact mechanisms underlying impaired tetanus LTP in DOM-LTP slices remains to be determined, this study nevertheless demonstrates that these two forms of plasticity recruit some common biochemical machinery while yet exhibiting distinct characteristics. Considering the prominent role of CaMKII in LTP (Giese et al., 1998; Lledo et al., 1995), it is likely that severely decreased CaMKII (p-Thr286) levels may account for the compromised LTP property. In addition, although the basal level is low in DOM-LTP slices, the increase of CaMKII (p-Thr286) levels was minimal after tetanus stimulation, which suggests that antagonizing mechanisms (e.g., phosphatase activities) for CaMKII autophosphorylation prevails at this time and prevents further synaptic potentiation.

CONCLUSIONS

Brief application of DOM followed by recovery induces a chemical form of LTP that is accompanied by altered levels of autophosphorylated CaMKII and phosphorylation of the AMPAR subunit GluR1. DOM-LTP is not dependent on NMDAR but requires activity of both CaMKII and PKA. In DOM-LTP slices, impaired tetanus-induced LTP is seen. Our results suggest that DOM-induced plasticity changes share some distinct biochemical mechanisms with tetanus LTP. These findings are significant in that they provide better understanding of spatial and temporal effects of DOM in the hippocampus and reveal the first evidence of DOM-induced changes of plasticity that impairs CA1 LTP property. The observed plasticity changes have implications in DOM-induced memory deficit resulting from poisoning accidents.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

FUNDING

University of California Toxic Substances Research and Teaching Program as a predoctoral fellowship to S.Q.

ACKNOWLEDGMENTS

We thank Dr R. Dingledine for his critical review of this manuscript.

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